AQUATIC-TERRESTRIAL TRANSFER OF CURRENT-USE PESTICIDES BY EMERGING AQUATIC INSECTS AND POTENTIAL FOR DIETARY EXPOSURE OF TERRESTRIAL INSECTIVORES

by

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3 Roodt, A.P., Huszarik, M., Entling, M.H., Schulz, R. (2023) Aquatic-terrestrial transfer of neonicotinoid insecticides in riparian food webs. *J. Hazard. Mater.* 455, 131635.

Contents

Abbreviation List

AZO – Azoxystrobin

BAF – Bioaccumulation Factor

BCF – Bioconcentration Factor

BOS – Boscalid

BWAF – Biota-water accumulation factor

CF – Concentration Factor

DDE – Dichlorodiphenyldichloroethylene

DDD – Dichlorodiphenyldichloroethane

DDT – Dichlorodiphenyltrichloroethane

DW – Dry weight

CYF – Cyflufenamid

FLU – Fluopyram

HOP – Halogenated organic pollutant

IND – Indoxacarb

K_{ow} – Octanol/water partition coefficient

LOD – Limit of detection

LOQ – Limit of quantification

NAP – Napropamide

OC – Organic contaminant

PAH – Polycyclic aromatic hydrocarbon

PCB – Polychlorinated biphenyl

PFAS – Per- and polyfluorinated alkyl substances

PIR – Pirimicarb

PRO – Propyzamide

PYR – Pyrimethanil

RCF – Relative concentration factor

RSD – Relative standard deviation

TEB – Tebuconazole

THI - Thiacloprid

TRI – Trifloxystrobin

TWAC – Time-weighted average concentration

Abstract

Adult emerging aquatic insects can transfer micropollutants, accumulated during their aquatic development, from aquatic to terrestrial ecosystems. This process depends on both contaminant- and organism-specific properties and processes. The transfer of contaminants can result in the dietary exposure of terrestrial insectivores at the aquatic-terrestrial ecosystem boundary. It is, however, unknown whether this route of contaminant transfer is relevant for current-use pesticides, despite their ubiquity in freshwater ecosystems globally. Furthermore, empirical investigation of pesticides in terrestrial insectivores which consume emerging aquatic insects (e.g. riparian spiders) is lacking. In the present work, two laboratory batch-scale studies and a field study were conducted to investigate the transfer of current-use pesticides by emerging aquatic insects and the dietary exposure of riparian spiders preying on emerging insects. In the two laboratory studies, larvae of the model organism, *Chironomus riparius*, were exposed, either chronically to seven fungicides and two herbicides, or acutely (24-hours) to three individual insecticides during their development. The pesticides were all small organic molecules, selected to cover a low to moderate lipophilicity range ($\log K_{ow}$ 1.2 – 4.7). Exposure took place at three environmentally relevant concentrations for the fungicides and herbicides (1.2 – 2.5, 17.5 – 35.0 or 50.0 – 100.0 ng/mL) and two for the insecticides (0.1 and either 4 or 16 ng/mL). Eight of the nine fungicides and herbicides, as well as one of the three insecticides were detected in the adult insects after metamorphosis. Concentrations of the pesticides decreased over metamorphosis. However, the transfer of individual pesticides was not well predicted using published models which are based on contaminant lipophilicity and were developed using other contaminant classes. In the present work, pesticide-specific differences in bioaccumulation by the larvae, retention through metamorphosis and sex-specific bioamplification and elimination over the course of the terrestrial life stage were observed. The neonicotinoid, thiacloprid, was the only insecticide retained by the emerging insects, due to its slow elimination by the larvae. Thiacloprid also decreased insect emergence success. An approximate 30% higher survival to emergence in the low exposure level (0.1 ng/mL), however, resulted in a relatively higher insecticide flux, from the aquatic to the terrestrial environment compared to the higher exposure (4 ng/mL). For the field study, a method for the analysis of 82 current-use pesticides by high-performance liquid chromatography tandem to triple quadrupole mass spectrometry using small volumes (30 mg) of insect material was validated and applied to samples of emerging insects and *Tetragnatha* spp. spiders which were collected from stream sites impacted by agricultural activities. Emerging aquatic insects from three orders (Diptera, Ephemeroptera and Trichoptera) contained 27 pesticides whereas 49 pesticides were found in the aquatic environment (water, sediment and aquatic leaf litter). This included mixtures of up to four neonicotinoid insecticides in the insects, with concentrations up to 12300 times greater than were found in the water. Furthermore, the web-building riparian spiders contained 29 pesticides, generally at low concentrations, however concentrations of three neonicotinoids and one herbicide were biomagnified compared to the emerging

insects. The three studies included in this thesis thus reveal that the aquatic-terrestrial transfer of current-use pesticides occurs, even at very low environmentally relevant exposure concentrations. Furthermore, new knowledge was generated on the diverse interactions between current-use pesticides and organisms over their entire lifecycles, affecting the propensities for individual pesticides to be transferred via insect emergence. A wide range of pesticides were found to be dietarily bioavailable to riparian spiders, and likely many other riparian insectivores. The neonicotinoid insecticides stood out for their potential to negatively impact adjacent terrestrial food webs through negative impacts on aquatic insect emergence (i.e. biomass flux), while still having a high propensity to be transferred by emerging insects and bioaccumulated in riparian spiders.

1 Introduction

1.1 The transfer of micropollutants from aquatic to terrestrial ecosystems by emerging aquatic insects

Freshwater ecosystems globally are contaminated with micropollutants originating from a variety of anthropogenic sources, such as industry, agriculture and waste-water effluents. This contamination includes a wide variety of small organic molecules, for example, pharmaceuticals and pesticides, as well as trace heavy metals (Kumar et al., 2019; Stehle et al., 2018; Wilkinson et al., 2022). Contamination of aquatic ecosystems results in the exposure of the aquatic developmental stages of emerging insects which, after completing metamorphosis, spend the reproductive phase of their lifecycle in the adjacent terrestrial ecosystem. Micropollutants which are accumulated and retained by emerging aquatic insects are thus transferred across the aquatic-terrestrial ecosystem boundary, potentially providing a route for these contaminants into terrestrial food webs (Bundschuh et al., 2022; Kraus et al., 2014, 2020; Schulz et al., 2015). Recent studies have shown this route of transfer for micropollutants from a wide range of chemical classes, including metals (Chételat et al., 2008; Walters et al., 2020; Wesner et al., 2017), metal-based nanoparticles (Bundschuh et al., 2019), polychlorinated biphenyls (PCBs) (Walters et al., 2008), per- and polyfluorinated alkyl substances (PFAS) (Koch et al., 2021), halogenated organic pollutants (Y. Liu et al., 2018), pharmaceuticals (Previšić et al., 2021) and pesticides (Derr & Zabik, 1972; Harkey & Klaine, 1992; Kraus et al., 2021; Reinhold et al., 1999). However, despite hundreds of pesticides contaminating freshwaters globally (Stehle et al., 2023; Wolfram et al., 2018, 2021; Zubrod et al., 2019), their transfer via emerging aquatic insects is greatly understudied. In fact, the full pathway of pesticide uptake by aquatic larvae through metamorphosis to adult insects has only been studied for the legacy organochlorine insecticides, DDD and DDE (metabolites of DDT), trans-chlordane and hexachlorocyclohexane (Derr & Zabik, 1972; Harkey & Klaine, 1992; Reinhold et al., 1999). More recently, six pesticides and metabolites, including imidacloprid, clothianidin and bifenthrin, from currently used, neonicotinoid and pyrethroid, insecticide classes, were detected in adult insects emerging from wetlands impacted by agriculture (Kraus et al., 2021).

1.2 Pesticide- and organism-specific properties affecting contaminant transfer by emerging aquatic insects

The aquatic-terrestrial transfer of micropollutants by emerging aquatic insects is influenced by both physicochemical properties of the contaminants and biological parameters related to the organisms. The physicochemical properties affect the distribution and movement of micropollutants. For organic contaminants (OCs), this is most often characterised by their octanol-water partition coefficient (K_{ow}), which is used to estimate their lipid-solubility (or lipophilicity). The lipophilicity along with other properties, such as molecular size and number of proton donor and acceptor groups can influence the ability for OCs to partition across biological membranes (Lipinski et al., 1997).

Bioaccumulation of contaminants by aquatic insects refers to the process of assimilation of these substances from the environment into the organism, both

passively and through dietary exposure (Arnot & Gobas, 2006). For moderately to highly lipophilic ($\log K_{ow}$ 6 – 8) OCs, bioaccumulation and trophic magnification potential are related to their lipophilicity (Walters et al., 2016). This is true for aquatic insect larvae (*Chironomus* spp.) under laboratory conditions, where bioconcentration factors (BCFs) of diverse pesticides ($\log K_{ow}$ 2.4 – 8.1) have a weak (r^2 = 0.48), but positively increasing correlation with pesticide lipophilicity (Katagi & Tanaka, 2016). This correlation however resulted from BCFs for a single genus (*Chironomus*), and no correlation was found when including multiple orders of emerging insects.

The bioaccumulation potential of OCs is furthermore influenced by processes of metabolism and elimination by organisms (Walters et al., 2016). Moreover, metamorphosis can reduce or increase OC concentrations in emerging aquatic insects (Kraus et al., 2014). This process has similarly been related to contaminant lipophilicity, with distinct patterns for OCs above and below a $\log K_{ow}$ value of approximately five units (Kraus et al., 2014) The retention of more lipophilic OCs ($\log K_{ow}$ 5 – 8) correlates non-linearly with increasing lipophilicity, and potentially reflects biomagnification potential in food webs (Kraus et al., 2014). On the other hand, OCs which are less lipophilic ($\log K_{ow}$ 3 – 5) show a decreasing tendency to be retained through metamorphosis with increasing lipophilicity, and it is unclear whether this relationship reflects the potential for trophic transfer within food webs (Kraus et al., 2014). These observations have, however, been based on a limited number of chemicals and chemical classes, which included only three organochlorine pesticides. Evaluations of a larger number of pesticides, many of which fall into the low lipophilicity range ($\log K_{ow}$ < 5), and how their physicochemical properties correlate with the prevalence and concentrations in adult emerging insects is lacking.

Furthermore, reduction in organism weight during metamorphosis and over the adult life stage of emerging insects can concentrate contaminants in the insects, i.e. bioamplification (Daley et al., 2014). This process can be affected by sex-specific differences during insect development and life cycle (e.g. through weight or contaminant loss due to oviposition in females), although the effects of these differences on contaminant transfer by emerging insects is not often studied (Kraus et al., 2014). Sex-specific differences in contaminant concentrations have however been observed for PCBs and zinc in adults of several mayfly species, as well as for halogenated organic pollutants (HOPs) in a terrestrial species of Lepidoptera (Daley et al., 2011; Huang et al., 2020; Wesner et al., 2017). In the case of emerging Chironomidae, which make a large contribution to emerging insect biomass (Raitif et al., 2018), bioamplification can potentially impact concentrations of contaminants in the organisms. Male midges (*C. riparius*), for example, lose more body weight during metamorphosis, and can weigh half as much as females directly after emergence (Day et al., 1994), potentially concentrating contaminants during the process.

1.3 Implications for terrestrial insectivores

Pesticide contamination of freshwaters has been linked to negative impacts on communities and populations of terrestrial insectivores, such as birds and riparian spiders (Graf et al., 2019; Hallmann et al., 2014). Emerging aquatic insects serve as prey for a variety of terrestrial insectivores and are a source of essential fatty acids which are not readily substituted by terrestrial insect prey (Raitif et al., 2019; Twining et al., 2016). Emerging insect prey can, for example, drive breeding success in some birds (Twining et al., 2018). However, emerging insects also serve as vectors of contaminant transfer from aquatic ecosystems, resulting in the dietary exposure of terrestrial insectivores. For example, web-building riparian spiders, which consume emerging aquatic insects as a large proportion of their diet (Wieczorek et al., 2015), can accumulate a wide range of aquatic contaminants, such as PCBs (Walters et al., 2008), pharmaceuticals (Previšić et al., 2021; Richmond et al., 2018) or PFAS (Koch et al., 2021). The mechanisms by which micropollutants contaminating aquatic environments can have negative impacts on terrestrial consumers were recently formulated in a heuristic model (Kraus, 2019), and further elucidated in a recent review article (Bundschuh et al., 2022). In the heuristic model, the interplay of emerging insect biomass- and insect mediated contaminant-fluxes as mechanisms impacting terrestrial insectivores are developed. These two mechanisms contrast the potential for contaminant bioaccumulation and transfer against the toxicity of the contaminants to developmental stages of emerging insects (Kraus, 2019). Contaminants, such as heavy metals, which are toxic to aquatic developmental stages but generally not well retained through emergence, negatively impact terrestrial insectivores through reductions in the flux of emerging insect biomass. On the other hand, contaminants with low toxicity and high bioaccumulation potential, such as PCBs, negatively impact terrestrial insectivores by direct dietary exposure after consuming contaminated insects. There is, however, a lack of information on the role of each mechanism for current-use pesticides from diverse classes (Bundschuh et al., 2022; Kraus, 2019). Newer classes of neonicotinoid insecticides, for example, have negative impacts on aquatic insect abundance and emergence (Sánchez-Bayo et al., 2016). This highly hydrophilic insecticide class is considered to have a low bioaccumulative potential compared to legacy insecticides (Tooker & Pearsons, 2021), yet the bioaccumulation of some neonicotinoids has recently been reported in aquatic macroinvertebrates in field studies (Crayton et al., 2020; Lauper et al., 2022) and adult emerging insects (Kraus et al., 2021). Furthermore, the dietary exposure of terrestrial predators to pesticides through consumption of emerging insects has been limited to calculations based on published consumption rates in combination with pesticide concentrations in emerging insects for a very limited number of pesticides (Kraus et al., 2021), but empirical knowledge combining the detection of pesticides in both emerging aquatic insects and riparian predators is lacking.

2 Research objectives and thesis outline

The overall goal of this thesis was to contribute to the understanding of anthropogenic stresses propagating across aquatic-terrestrial ecosystem boundaries by investigating the emerging insect mediated transfer of current-use pesticides. The introduction provides an overview of the existing knowledge on classes of micropollutants which cross this ecosystem boundary via insect emergence and highlights the knowledge gaps around current-use pesticides, despite their ubiquity in freshwaters worldwide. **Establishing the aquatic-terrestrial link for current-use pesticides thus becomes the first objective of this thesis.** Furthermore, existing knowledge on physicochemical and biological parameters potentially affecting the transfer of pesticides are also discussed in the introduction. **The empirical investigation of contributions from both physicochemical and biological parameters for different classes of pesticides is the second objective of this thesis.** Finally, an argument for the relevance of surface water contamination with pesticides negatively affecting terrestrial insectivores at a large scale was introduced. Additionally, potential mechanisms of negative impacts cascading to terrestrial consumers via emerging insects based on data available for other contaminant classes was discussed. **Contributing to the understanding of the dietary exposure of terrestrial insectivores to current-use pesticides transferred from the aquatic environment by emerging insects is therefore the third objective of this thesis.** The approach to addressing these objectives combined both controlled laboratory studies and field sampling, with the measurement of a large number of pesticides in small volumes of biological samples (Fig. 1). In Chapter 4 the results from all three thesis manuscripts, as listed in Appendix I to III, are discussed and interpreted in an integrated way, and not separately for each manuscript.

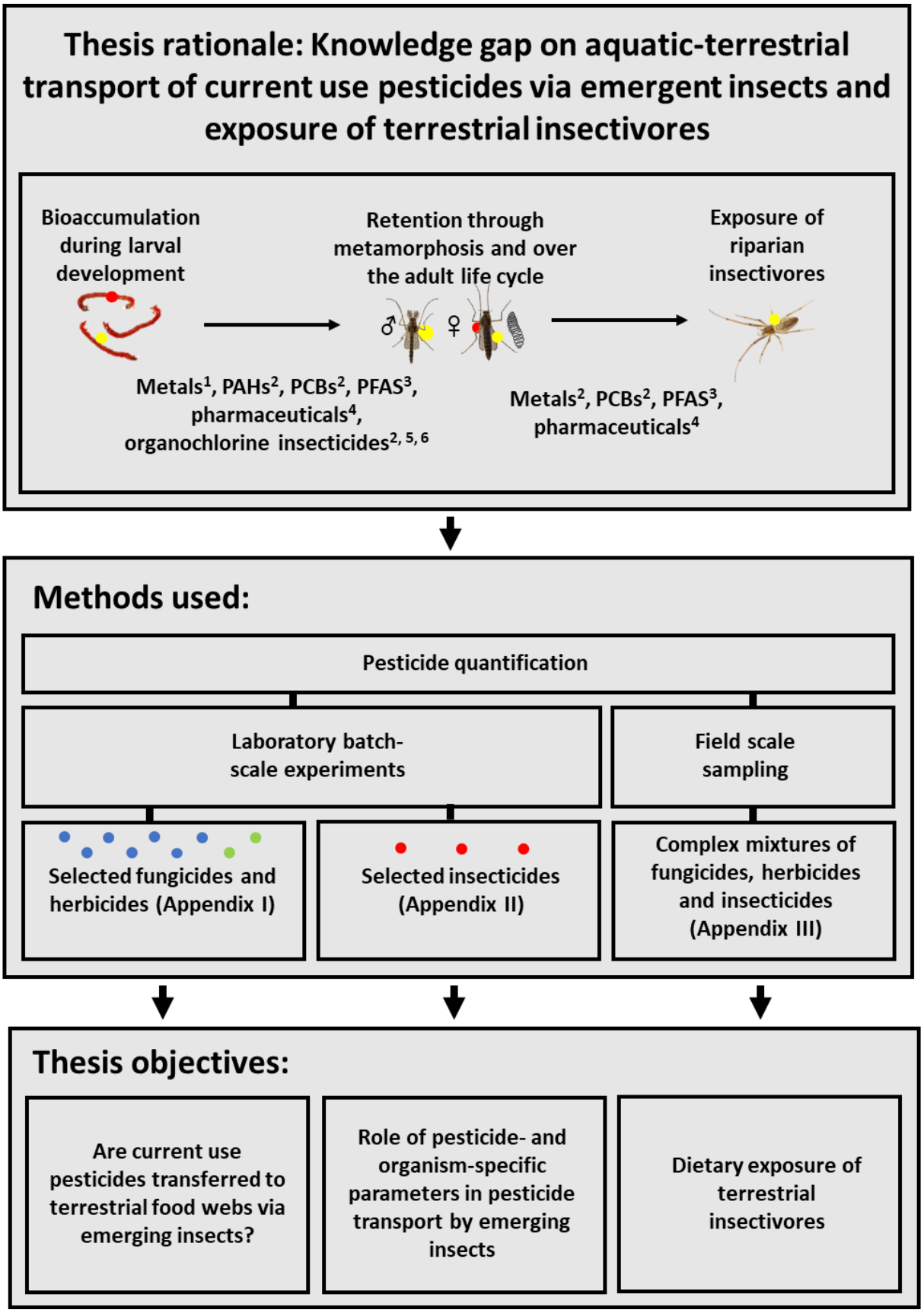

Figure 1. Overview of thesis rationale, methods and objectives. Literature cited in the rationale: [1](Chételat et al., 2008), [2](Reinhold et al., 1999),[3](Koch et al., 2021),[4](Previšić et al., 2021),[5](Derr & Zabik, 1972),[6](Harkey & Klaine, 1992). Coloured dots represent contaminant classes, namely insecticides (red), fungicides (blue), herbicides (green), other contaminant classes (yellow).

3 Methods

This chapter provides a summarised overview of the methods used in the individual studies submitted in this thesis. Detailed information pertaining to each of the three studies can be found in the Appendices I – III.

3.1 Pesticide analyses

Pesticide concentrations in all three studies (Appendices I – III) were measured by high-performance liquid chromatography coupled to a triple quadrupole mass spectrometer by electrospray ionization (HPLC-ESI-MS/MS). Sample extraction and targeted data acquisition methods were developed and validated for each study based on the International Conference on Harmonisation, ICH Harmonised Tripartite Guideline (International Conference on Harmonisation, 2005). The method applied for field samples (Appendix III) of emerging aquatic insects and riparian spiders was validated for 82 current-use fungicides, herbicides and insecticides.

3.2 Laboratory batch-scale experiments

Two studies in this thesis, i.e. Appendices I and II (Roodt et al., 2022, 2023), were conducted under laboratory conditions which were designed to reflect field relevant exposure scenarios. These studies were based on modified OECD 219 Sediment-Water Chironomid Toxicity Tests (OECD, 2004). Briefly, larvae of the holometabolous midge, *Chironomus riparius,* were raised in aquaria containing artificial sediment and aqueous test medium (Borgmann, 1996) under climate-controlled conditions.

In the first laboratory batch-scale study (Appendix I), midge larvae were exposed over their full development to a pulse exposure of a mixture of nine current-use fungicides and herbicides. The fungicides were azoxystrobin (AZO), boscalid (BOS), cyflufenamid (CYF), fluopyram (FLU), pyrimethanil (PYR), tebuconazole (TEB), and trifloxystrobin (TRI). The two herbicides were napropamide (NAP) and propyzamide (PRO). Exposure took place at one of three concentration levels, with concentrations ranging between 1.2 – 100 ng/mL for individual pesticides. The exposure concentrations were similar to what has been found in agricultural streams during peak runoff events (7 – 83.4 ng/mL, Appendix I). The individual fungicides and herbicides selected for this study were all small (< 500 Da) organic molecules with few proton donor and acceptor groups. They were selected to cover a range of low to moderate lipophilicities ($\log K_{ow}$: 2.5 – 4.7). Eight of the nine pesticides were applied as formulation products as opposed to pure substances, increasing the field relevance of the study. The pesticides concentrations were not renewed during the study. Water samples were thus taken at three time points to monitor the decrease in individual pesticide concentrations over the exposure periods of larvae and adult insects separately. These concentrations were used to calculate time-weighted average exposure concentrations (TWACs) for the larvae and adults (Appendix I).

Midge larvae were introduced to the aquaria at the start of the experiment shortly after hatching (< 3 days old). Larvae samples were collected and frozen for pesticide analysis after 14 days of development, which was shortly before adult insects began to emerge. Adult insects were collected daily after emergence and counted. Approximately 35% of individuals from each sex were frozen directly after collection,

in preparation for pesticide analysis. The remaining individuals were transferred to a separate enclosure, where mating and egg laying could take place. Adult insects were not fed during this period and after completing their terrestrial life cycle, the insects died naturally. The dead adults were also collected daily from the floors of the mating cages and frozen. Dry weights (dw) were determined for all samples using an analytical balance (d=0.001).

Concentration factors (CFs) for concentrations of individual pesticides (P) were calculated for larvae and pooled adult samples with equation 1.

$$\text{Equation 1: } \mathrm{CF} = \frac{\mathrm{P}}{\mathrm{TWAC}}$$

The term CF was used instead of bioaccumulation factor (BAF) because a steady state exposure concentration was not maintained throughout the course of the experiment. Between 4 to 11 days passed between the date of larvae sampling and the date of peak adult emergence, for the pesticide treated replicates. Comparison of larval and adult CFs was therefore performed in order to mitigate the effect of pesticide degradation on the exposure over the different durations taken to reach each life stage. Pesticide relative concentration factors (RCFs) were thus calculated for each pesticide to evaluate the effect of metamorphosis on pesticide concentrations in the organisms using equation 2. The average of the adult male and female CFs from each replicate were used in this calculation.

$$\text{Equation 2: } \mathrm{RCF} = -\frac{CF_{(larvae)}}{CF(average\ adults)}$$

The second laboratory batch-scale study (Appendix II), used a similar design to the first. However, in this study midge larvae were exposed to individual insecticides for only 24-hours. The exposure took place after approximately 10-days of development in a clean aquarium. The three insecticides used were thiacloprid (THI), pirimicarb (PIR) and indoxacarb (IND). Exposure took place at one of two concentrations in aqueous medium without the presence of sediment. The low treatment level was the same for all three insecticides, namely 0.1 ng/g. The high treatment level was 16 ng/g for pirimicarb and indoxacarb, but 4 ng/g for thiacloprid, due to its higher toxicity for the larvae. After the 24-hour exposure, surviving larvae were counted, a sub sample was frozen for pesticide analysis and the remaining larvae were returned to uncontaminated aquaria containing sediment and water. After a further 72-hour depuration period, survival was determined once more and a further subsample of larvae was frozen for pesticide analysis. Emerging adult insects were collected daily, counted and frozen for pesticide analysis. Insect-mediated insecticide flux was calculated as the product of the average insecticide concentrations and total average dry weights of successfully emerged adult insects divided by the time taken for 50% of individuals to emerge (EmT_{50}) when calculated for total emergence of both sexes.

3.3 Field study

Sampling took place at ten stream sites in the upper Rhine valley of the Palatinate region of South-West Germany over two years (2020 and 2021). In 2020, weekly water grab samples and emerging insects, caught in floating emergence traps, were collected from two streams, namely the Modenbach (MB, 49°16'50.4"N 8°16'53.0"E) and Spiegelbach (SPI, 49°11'13.6"N 8°18'44.6"E). In 2021, these two sites and a further eight, namely the Katzenbach (KB, 49°16'12.0"N 7°57'58.0"E), Eusserbach (EB, 49°14'20.1"N 7°58'34.4"E), Ranschbach (RB, 49°11'57.0"N 8°04'55.0"E), Queich Site 1 (QS1, 49°12'01.0"N 8°05'40.0"E), Queich Site 2 (QS2, 49°12'04.7"N 8°08'16.1"E), Queich Site 3 (QS3, 49°12'19.1"N 8°11'32.0"E), Queich Site 4 (QS4, 49°12'39.0"N 8°13'43.0"E) and Queich Site 5 (QS5, 49°13'19.0"N 8°16'12.2"E) were sampled twice during the peak summer pesticide application period. During both sampling campaigns in 2021, aquatic sediment and leaf litter, as well as adult riparian spiders (*Tetragnatha* spp.) were collected. Additionally, daily water grab samples were collected at QS2 for 47 days over the same period.

Two of these sites, namely KB and EB are located within a forested region with very limited agricultural activities and were therefore considered the least impacted by pesticides. The remaining eight sampling sites lie on streams which flow from West to East through a region which is characterised by intensive agriculture. The sampling sites were therefore carefully selected to be sheltered from agricultural activities by areas of dense natural vegetation in order to minimise the potential impacts of deposition as a result of spray drift. RB, which was separated by approximately 60 m of forest from the nearest agriculturally used land, was the site nearest to agricultural activities among all ten sampling sites. Potential atmospheric deposition resulting from rainfall was, however unavoidable. Therefore, rain water samples were also collected from two sites during 2021, rainwater sampler 1 was located at a site within the forest (Eusserthal Ecosystem Research Station, 49°15'15.2"N 7°57'42.3"E) and rainwater sampler 2 was located within the agricultural landscape (QS2, 49°12'04.7"N 8°08'16.1"E).

Emerging aquatic insects were identified and pooled based on order (Diptera, Ephemeroptera or Trichoptera) in order to obtain samples with sufficient biomass for pesticide measurements. This resulted in a total of 15 samples (seven Diptera and four each for Ephemeroptera and Trichoptera) for pesticide analysis. Order-specific weekly pesticide fluxes were calculated by multiplying the average weekly emergence biomass flux by the respective average total pesticide concentrations. Biota-water accumulation factors (BWAF) were calculated for each pesticide in insect samples as the concentration in the sample divided by the median water concentration at the sampling site.

After collection, the live spiders were kept individually in plastic containers for 72-hours before being frozen. During this period spiders cleared their gut content, which allowed for pesticide measurements to reflect dietarily bioavailable pesticides. Spiders were pooled by sex in order to achieve adequate biomass for pesticide analysis. This resulted in a total of 45 and 34 samples of female and male spiders, respectively. Overall, between six and eleven spider samples were obtained for each of the ten sampling sites.

3.4 Statistical tests and data analysis

Due to the small numbers of samples available in all three studies (Appendices I – III), non-parametric statistical tests were used throughout. A Mann-Whitney U or Kruskal-Wallis H test was used to test for differences in independent samples. This was followed by a post hoc Dunn's test with Bonferroni correction when treatment effects were detected. A Wilcoxon signed-rank test was used to test paired samples and Spearman analyses were performed to test for correlations. The significance level, α, was set at 0.05 for all tests.

In the field study (Appendix III), a principal component analysis was performed on pesticides which had been categorised according to their frequency of detection in abiotic (sediment, aquatic leaf-litter and water) and biotic compartments (Emerging insects and spiders). Pesticides were categorised as either "transferred" or "not-transferred". The categorisation was performed using frequency data for sediment, leaf litter and spider samples. Pesticides which were frequently detected in the abiotic compartments (>70% detection frequency in at least one compartment), but had no detections in spider samples, or emerging insects, in the case of MB and SB, were categorised as "not transferred". Similarly, pesticides which satisfied these criteria, but were consistently detected in spider samples (and emerging insects at MB and SB) were categorised as "transferred". This process yielded eleven pesticides which were categorised as "transferred" and seven as "not-transferred" (Appendix III). The principal component analysis was performed on values representing physicochemical properties, toxicity and environmental persistence of the categorised pesticides (Table S3). This included the logarithmically transformed values for the Henry's law constant (HLC), aqueous solubility (S), topological polar surface area (TPSA), monoisotopic mass (MIM) octanol-water partition coefficient (K_{ow}), first dissociation constant (pKa_1), water-phase half-life (DT_{50}) and the chronic 28-day no observed effects concentration for *Chironomus riparius* (MidgeNOEC28). For neutral pesticides, the pK_{a1} was assigned the value 14. Additionally, for pesticides for which no appropriate NOEC was available, the proxy value of 100 mg/L was used. Statistical analyses were performed in R (R Core Team, 2021).

4 Results and discussion

4.1 The aquatic-terrestrial transfer of current-use pesticides by emerging insects

In the laboratory, bioaccumulation of twelve tested pesticides, including seven fungicides, two herbicides and three insecticides, was observed in the larvae of the emerging midge, *C. riparius* (Appendix I and II). Of these twelve pesticides, six fungicides, two herbicides and one insecticide were observed to be retained by adult insects after metamorphosis, and thus potentially transferred from aquatic to terrestrial ecosystems via emerging insects. The lowest aqueous concentrations which the organisms were exposed to in these two studies (1.2 – 2.5 ng/mL for fungicides and herbicides or 0,1 ng/mL for insecticides) were, however, up to three orders of magnitude greater than the corresponding median concentrations measured in field collected water samples (Fig. 2, Appendix III).

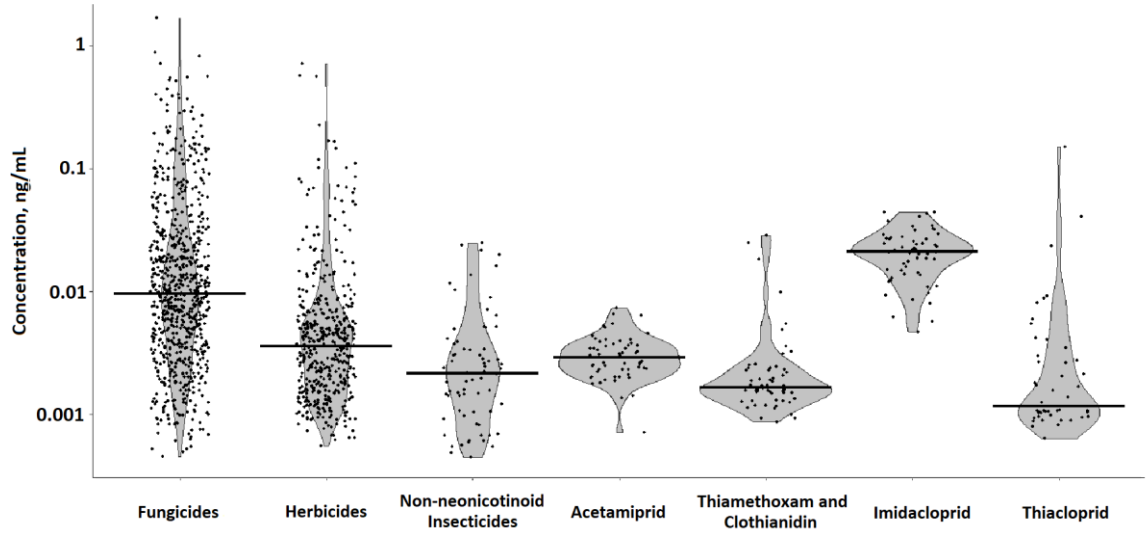

Figure 2. Pesticide concentrations in daily and weekly water samples collected from three stream sites; QS2, SB and MB (n=71 pooled from all three sites). Horizontal lines indicate the median concentrations (Source: Appendix III).

Despite this, of the 45 individual pesticides detected in field collected water samples, 27 were detected in at least one sample of adult emerging insects collected from the same sites (n = 15, Fig. 3, Appendix III). This included eleven fungicides, eight herbicides and eight insecticides. Four neonicotinoid insecticide were among the most frequently detected pesticides (acetamiprid and thiacloprid were found in 90 - 100% of insect samples, Appendix III), only two of which had previously been reported in insects emerging from wetlands in the USA (Kraus et al., 2021). The range of pesticide concentrations in the emerging insects (0.02 – 23.2 ng/g dw) were similar to the range of concentrations reported for individual pharmaceuticals and endocrine disrupting chemicals (approximately 0.01 – 100 ng/g dw) measured in the insects emerging from Croatian streams (Previšić et al., 2021), but lower than for total PFAS (average concentration of 700 ng/g dw) in insects emerging from Swedish surface waters (Koch et al., 2021). Neonicotinoid insecticides had the highest concentrations out of the pesticides measured in the insect samples (Fig. 3; up to 23.2 and 6.7 ng/g for

thiacloprid and imidacloprid, respectively), despite the median water concentrations being up to one order of magnitude lower than in the laboratory study (Appendix II). In fact, both the 50th and 90th percentiles of the individual neonicotinoid concentrations measured in the field collected water were lower than what has been recently reported (Stehle et al., 2023) for global surface water concentrations (Appendix III).

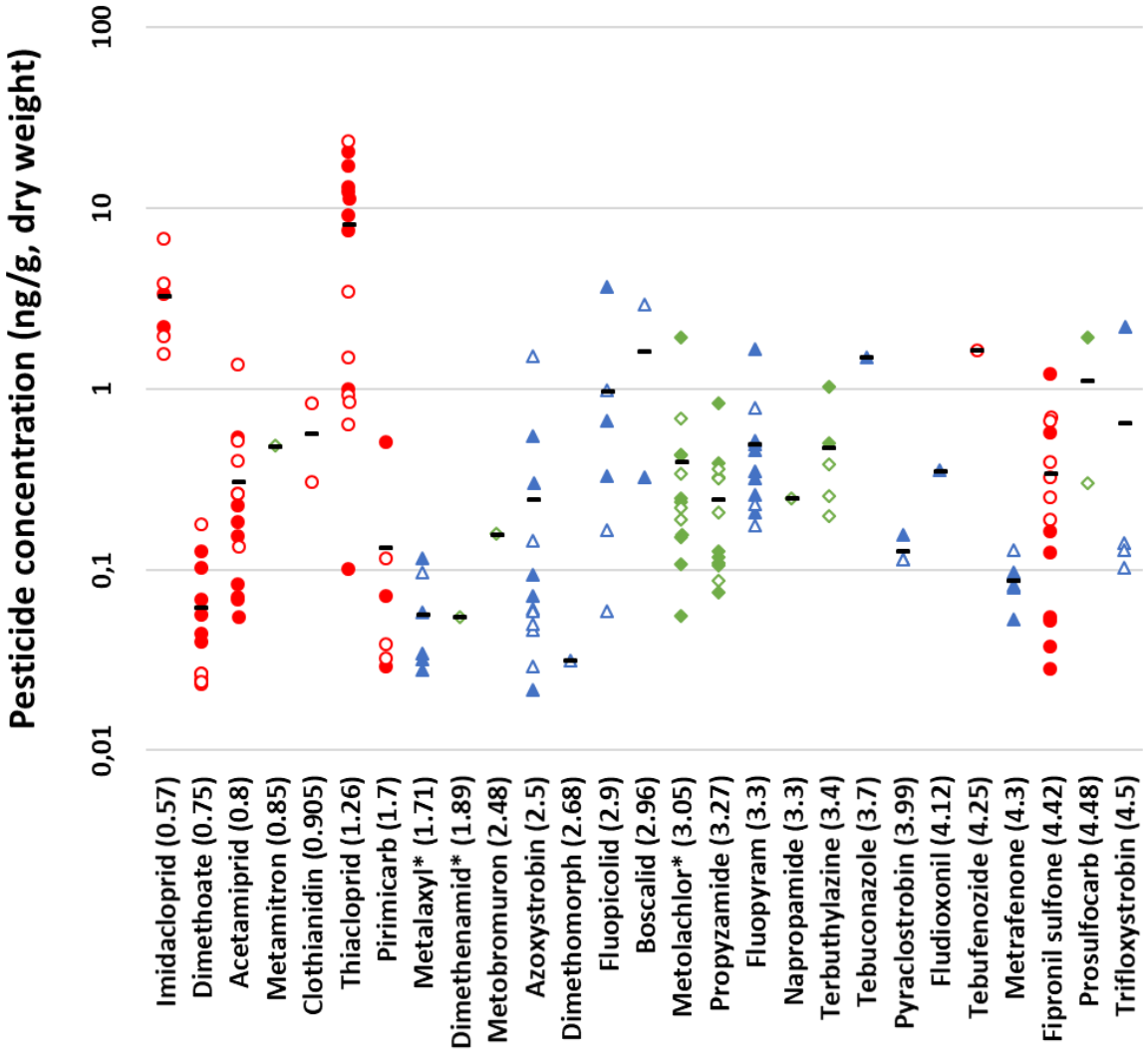

Figure 3. Pesticide concentrations in emerging aquatic insects from two sampling sites impacted by agriculture, namely the Modenbach (MB, solid shapes) and the Spiegelbach (SB, outlined shapes). Concentrations of fungicides (blue triangles), herbicides (green diamond) and insecticides or insecticide metabolite (red circles) in samples of emerging insects (including Diptera, Ephemeroptera and Trichoptera). Pesticides are arranged from left to right in order of increasing lipophilicity (logK_{ow} values are provided in brackets). Overall average concentrations for both sampling sites are indicated by black dashes. *Concentrations are reported for the sum of isomers (Source: Appendix III).

The results of the three studies (Appendices I – III) therefore confirmed that emerging insect mediated transfer of many low-lipophilicity (logK_{ow} 0.6 – 4.5) current-use organic pesticides does occur (further discussion of physicochemical properties and biological parameters affecting pesticide transfer is found in Section 4.2). Furthermore, the transfer of current-use pesticides occurs, even when exposure takes place at very low, globally relevant, exposure concentrations in the aquatic environment. These results, thus support the real-world relevance of this pathway of contaminant transfer and potential dietary exposure of terrestrial insectivores hunting at the aquatic-terrestrial

ecosystem boundary (further discussion of the dietary exposure of terrestrial insectivores is found in Section 4.3). Overall, the current-use pesticides found to be transferred by emerging insects in the presented studies (Appendices I – III) add to the list of aquatic contaminants which have been found to be transferred by emerging insects, including pharmaceuticals, endocrine disrupting chemicals, metals, metal-based nanoparticles, PAHs, PCBs, HOPs, PFAS, plasticizers and organophosphorus flame retardants (Bundschuh et al., 2022).

4.2 Evaluation of the contributions of compound- and organism-specific parameters to pesticide transfer

4.2.1 Laboratory batch-scale study – Chronic exposure to fungicides and herbicides

In the laboratory, an interplay between compound- and organism specific-parameters was found to influence the aquatic-terrestrial transfer of pesticides by the midge, *C. riparius* (Appendix I). Accumulation of nine fungicides and herbicides was observed in midge larvae when chronically exposed over their development period (14 days), regardless of the exposure concentration (Appendix I). However, no relationship between the larval CFs and the respective $\log K_{ow}$ values was observed (Fig. 4, Spearman's rank correlation: $\rho = 0.1$, $p > 0.05$). This was in contrast to a strong positive linear relationship ($R^2 = 0.98$) which has been reported for the BCFs of a series of increasingly halogenated chlorobenzenes in midge larvae, within the same range of $\log K_{ow}$ values as the fungicides and herbicides (Knezovich & Florence, 1988). Furthermore, a review of published BCFs in *Chironomus* spp. found a weak, yet still positive, linear correlation ($R^2 = 0.5$) for 14 structurally unrelated pesticides with $\log K_{ow}$ values between 2.4 and 8.1 (Katagi & Tanaka, 2016). The differences in CFs of pesticides with very similar lipophilicities (differences in $\log K_{ow} < 0.5$) suggests the important role of pesticide metabolism in determining the potential for pesticide-specific accumulation in the larvae (Walters et al., 2016). This is further supported by the decreasing tendency for a correlation between contaminant lipophilicity and accumulation potential with increasing diversity of contaminant molecular structure, as observed in the literature and the laboratory study.

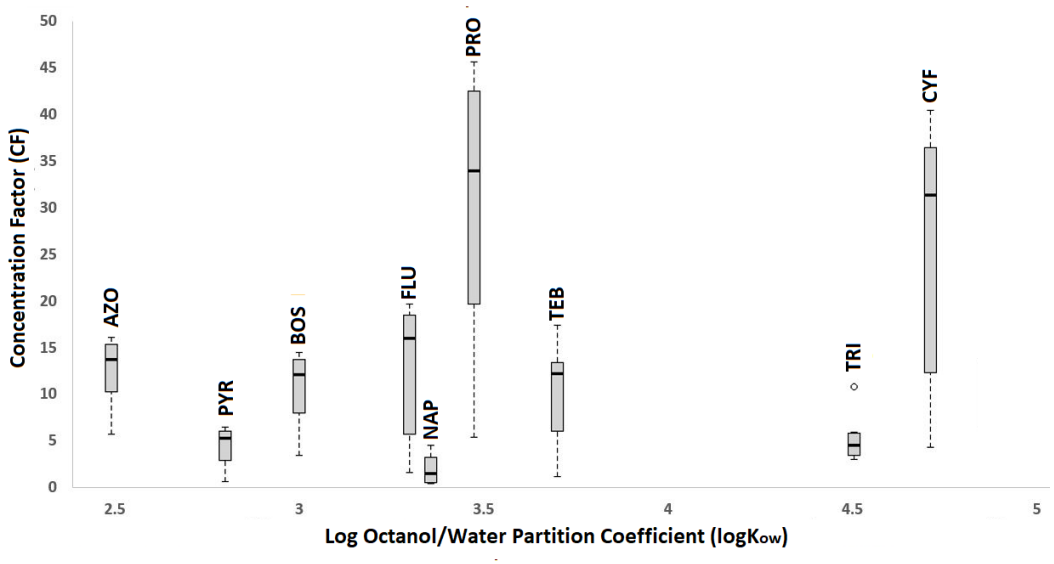

Figure 4. Pooled (n = 8) medium (7 – 23 ng/mL TWAC) and high (22 – 93 ng/mL TWAC) treatment-level concentration factors (CFs) of nine pesticides with increasing $logK_{ow}$ in midge larvae. CFs, which are larger than 1.5 times the interquartile ranges, are depicted as open circles. AZO = azoxystrobin, BOS = boscalid, FLU = fluopyram, NAP = napropamide, PRO = propyzamide, TEB = tebuconazole, TRI = trifloxystrobin and CYF = cyflufenamid (Source: Appendix I).

In the adult insects, eight of nine fungicides and herbicides were measured after emergence (< 24 hours) from the two highest treatment levels (TWACs: 7.2 – 93.3 ng/mL, Appendix I). Five of the nine pesticides were measured in the adults from the low treatment level (TWACs: 0.5 – 1.8 ng/mL). By comparing CFs between larvae and adult insects, metamorphosis was found to significantly lower concentrations of seven of the eight pesticides compared to the larvae by approximately 20 – 100 % (Mann-Whitney U test, $p < 0.05$, pooled medium and high treatment levels). Propyzamide, which had approximately equal average CFs for larvae and adults, was the only exception (Fig. 5). The two strobilurins, azoxystrobin and trifloxystrobin, had very similar propensity to be retained by the adults despite two orders of magnitude difference in lipophilicity between them, with average CFs which were approximately forty and thirty percent of the value in the larvae respectively. Overall, a weak negative trend (Spearman's rank correlation: $\rho = 0.33$, $p > 0.05$) between the RCFs and the lipophilicities of the tested pesticides was observed (Fig. 5). This result is consistent with the strong negative correlation ($R^2 = 0.96$) which has been reported for contaminants with $logK_{ow}$ values ranging from 3 – 5 (Kraus et al., 2014). The stronger correlation reported by these authors is predominantly based on the retention of PAHs, which is a more structurally uniform class of chemicals in comparison to the pesticides used in the microcosm study. The structural uniformity of the PAHs may result in a uniform rate of metabolism for these contaminants, which along with lipophilicity predicts bioaccumulation potential (Walters et al., 2016). The diverse pesticides, on the other hand may be metabolised at different rates. For example, relatively slower compound-specific metabolism could explain the similar propensity for retention of the two strobilurins, azoxystrobin and trifloxystrobin, which deviate from a linear trend with lipophilicity in our results, but have similar molecular structures and contain chemical moieties which are unique in the group of pesticides tested (Fig. 5).

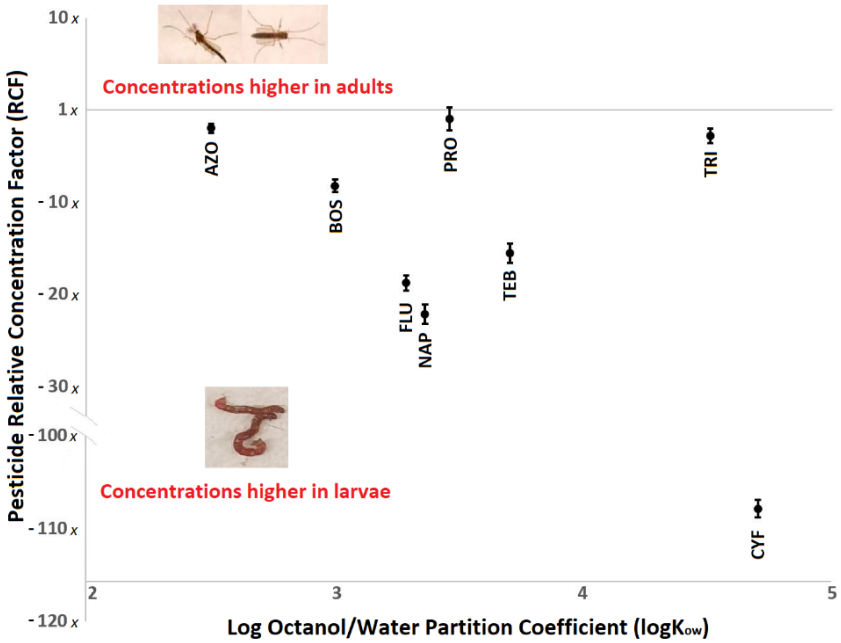

Figure 5. The average relative pesticide concentration factors (RCFs) for pooled medium (7 – 23 ng/mL TWAC) and high (22 – 93 ng/mL TWAC) treatment levels (n = 8). Values below the unity line indicate larger average CF for the larvae compared to the adults. Error bars represent the relative standard deviations. The Y-axis is broken between 30 and 100x in the larval direction. AZO = azoxystrobin, BOS = boscalid, FLU = fluopyram, NAP = napropamide, PRO = propyzamide, TEB = tebuconazole, TRI = trifloxystrobin and CYF = cyflufenamid (Source: Appendix I).

Comparison of pesticide concentrations between sexes of adult insects directly after emergence revealed greater concentrations of boscalid, napropamide, cyflufenamid and propyzamide in females (Fig. 6). The sex-specific difference was between a factor of 2 – 6 for the first three pesticides and the most pronounced for propyzamide, for which the females contained approximately 270 times greater concentrations than the males (Appendix I). The bioamplification of propyzamide in females offset the very low retention in males, resulting in the equality of average RCFs in both sexes (Fig. 5). Azoxystrobin and trifloxystrobin concentrations, on the other hand, were up to a factor 5 greater in males than in females (Fig. 6).

The effect of emerging aquatic insect sex on contaminant retention through metamorphosis has not been well documented for a wide range of contaminants. Bioamplification of contaminant concentrations which results from weight loss during pupation could be greater in male insects when compared to females (Day et al., 1994). This hypothesis is supported by the retention of PCBs, for which biota-sediment accumulation factors (BSAFs) are greater in male *Chironomus* spp. (Maul et al., 2006). Similarly, males of a terrestrial lepidopteran had greater concentrations of HOPs after dietary exposure as larvae (Huang et al., 2020). The results for pesticides, however, reveal a more complex pattern, indicating that sexual dimorphism during larval development and pupation, i.e. reduction in adult weight relative to the final larvae stage (Day et al., 1994), cannot fully explain the sex-specific differences in pesticide concentrations of emergent adults. Additional factors could therefore be considered, for example, a longer duration of larval development for females may result in increased exposure and correspondingly higher uptake of certain pesticides (Goedkoop et al., 2010). Additionally, sex-specific differences in pesticide metabolism may further modify concentrations of accumulated pesticides (Navarro-Roldán et al., 2020).

Over the course of the terrestrial life stage, pesticide concentrations in females tended to decrease while those in males tended to persist. Concentrations of propyzamide in female midges greatly decreased, by a factor of 8 – 12, but simultaneously increased in male insects (Fig. 6, Paired Wilcoxon signed-rank test, $p < 0.05$). Females also lost boscalid and cyflufenamid resulting in no detectable concentrations of these pesticides in dead females. In the males, boscalid concentrations tended to decrease, while concentrations of cyflufenamid persisted. Fluopyram concentrations in females showed a tendency to decrease in insects from the medium treatment level, but persisted in insects from the low treatment level. Concentrations of azoxystrobin and napropamide were similar between life stages for both sexes. Tebuconazole concentrations increased in females and trifloxystrobin tended to increase in both sexes.

Pesticide concentration increases can be explained by bioamplification arising due to body weight loss coupled with limited pesticide elimination over the course of the

terrestrial life stage. Adult midges which were transferred to mating cages after emergence lived for approximately 6 – 7 days, during which time the average dry weights of males and females decreased by approximately 28 and 55%, respectively. The general tendency towards decreases in pesticide concentrations in female insects suggests the potential for sex- and compound-specific loss through depuration, metabolism or oviposition over the course of the terrestrial lifespan. Across all treatments 0.9 ± 0.3 egg masses per female (n = 876) were collected, implying that the majority of females had successfully oviposited during the terrestrial life stage. Similar maternal transfers of organic contaminants via egg masses have been found in terrestrial moths (Huang et al., 2020). This route of contaminant loss is furthermore supported considering the general tendency towards retention or bioamplification of the same pesticides in males.

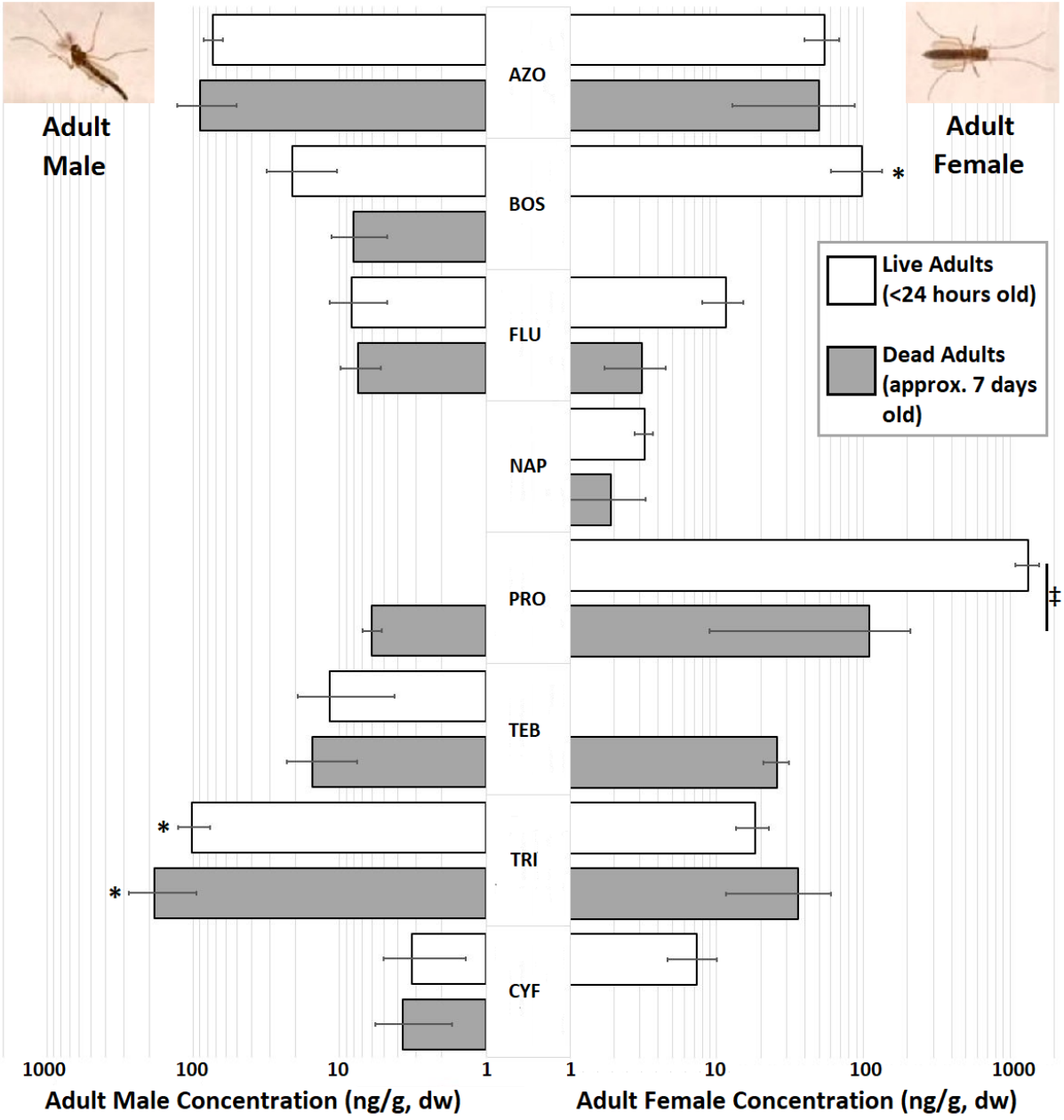

Figure 6. Average (n = 4) sex-specific pesticide concentrations in live (white bars) and dead (grey bars) collected adult male and female midges that emerged after exposure as larvae to the medium pesticide

treatment level. Error bars indicate the standard deviation. Asterisks indicate a sex-specific significant difference between the adults from the same life stage (Mann-Whitney U Test, $p < 0.05$). The symbol, ‡, indicates significant differences between life stages of the same sex (Paired Wilcoxon signed-rank test, $p < 0.05$). Pesticides are arranged from top to bottom in order of increasing lipophilicity. AZO = azoxystrobin, BOS = boscalid, FLU = fluopyram, NAP = napropamide, PRO = propyzamide, TEB = tebuconazole, TRI = trifloxystrobin and CYF = cyflufenamid (Source: Appendix I).

4.2.2 Laboratory batch-scale study – pulse exposure to single insecticides

Compound- and organism specific- parameters affecting the transfer of pesticides by emerging aquatic insects was further explored in a subsequent laboratory study involving the pulse (24-hour) exposure of midge larvae to three individual insecticides (Appendix II). Directly after the 24-hour exposure, all three insecticides were measured in the midge larvae (Fig. 7 A, B and C), but compound-specific elimination rates were observed in the 72-hour post exposure period. For the hydrophilic insecticide, pirimicarb ($logK_{ow}$ = 1.7) concentrations were measurable in larvae from the 16 ng/mL treatment level, but not the 0.1 ng/mL treatment level (Fig. 7 A). The average (n = 4, ± standard deviation) concentration in larvae after exposure at 16 ng/mL was 31.0 ± 8.1 ng/g. This concentration decreased to 0.4 ± 0.2 ng/g over the 72-hours post exposure period, corresponding to an approximate 99% reduction. Indoxacarb, the most lipophilic of the three insecticides ($logK_{ow}$ = 4.65), had greater average larval indoxacarb concentrations directly after the 24-hour exposure period, namely, 415.0 ± 90.5 ng/g and 3837.9 ± 1142.1 ng/g in the 0.1 and 16 ng/mL treatment levels, respectively. Over the subsequent 72-hour post exposure period, the larval concentrations were no longer detectable in the 0.1 ng/mL treatment level, but were still measurable, at 224.7 ± 175.4 ng/g, in the 16 ng/ml treatment level, thus decreasing by at least 94% (Fig. 7 B). The most hydrophilic insecticide in the study, thiacloprid ($logK_{ow}$ = 1.26) was found to have average concentrations of 125.2 ± 18.3 ng/g and 287.2 ± 90.8 ng/g in the larvae after 24-hours exposure to the 0.1 and 4 ng/mL treatment levels, respectively (Fig. 7 C). In contrast to the first two insecticides, these concentrations decreased by only 30 – 50% over the 72-hours post exposure period, to 58.2 ± 14.1 and 198.0 ± 33.7 ng/g.

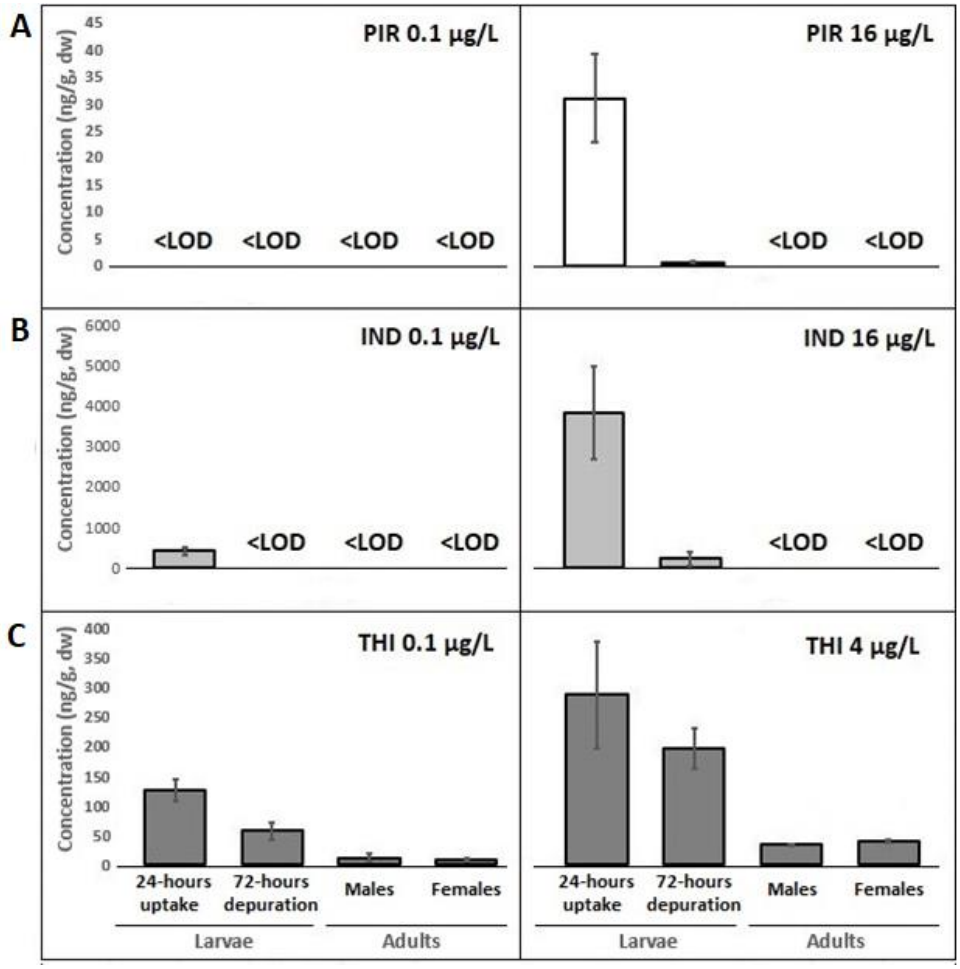

Figure 7. Average (n = 4 for all treatments, except THI 4 where n = 3; ± standard deviation) concentrations of pirimicarb (Panel A, PIR, white bars), indoxacarb (Panel B, IND, light grey bars) and thiacloprid (Panel C, THI, dark grey bars) in midge larvae sampled after 24-hours exposure or 72-hours depuration and in adult insects. Concentrations are based on the sample dry weights (dw). LOD = Limit of Detection. Y-Axis scales are different for each insecticide (Source: Appendix II).

Indoxacarb had the highest concentrations in the larvae and pirimicarb the lowest (Fig. 7 A and B), thus generally correlating with their lipophilicities (i.e. greater bioaccumulation for the more lipophilic insecticide). Thiacloprid, however, did not fit this generalisation and despite having the lowest lipophilicity of the insecticides, was more accumulative than pirimicarb but less than indoxacarb. A similar underestimation of bioconcentration potential based on the $\log K_{ow}$ has been reported for the neonicotinoid, imidacloprid, in an aquatic oligochaete (Contardo-jara & Gessner, 2020). Additionally, underestimation of the bioconcentration potential of neonicotinoids in amphipods has been reported in the literature, and some evidence exists attributing this to binding of the insecticide to large biomolecules of the organisms (Chen & Kuo, 2018; Lauper et al., 2022; Li et al., 2021).

Thiacloprid was not only the slowest to be eliminated by the aquatic larvae, but was also the only of the three insecticides to be retained though metamorphosis, which took place approximately 6 – 7 days after the exposure (Fig. 7 C, Appendix II). Directly after insect emergence (< 24-hours), thiacloprid had an average concentration approximately 20% of that measured in the larvae 72-hours post-exposure, or

approximately 10 – 15% of the concentration in the larvae directly after the pulse exposure. No differences were found between male and female insects (Fig. 7 C).

When considering only passive uptake of insecticides from the surrounding water, metabolism by relevant enzymes is an important factor determining the resulting concentrations in aquatic organisms (Katagi, 2010). Insecticide toxicity, in turn, is related to the concentrations present in the organism in combination with the toxic mode of action (Katagi & Tanaka, 2016). The observed rates of insecticide elimination by the larvae therefore correlated with the insecticides' relative toxicities (i.e., 28-day NOECs: pirimicarb >> indoxacarb > thiacloprid, Appendix II). The results from this laboratory study thus indicate a potential positive correlation between insecticide toxicity and the potential for aquatic-terrestrial transfer, due to the rate of insecticide metabolism.

4.2.3 Field study – Pesticides in emerging insects and spiders from agricultural streams

In a field study, the contributions of three orders of emerging insects to pesticide transfer were investigated. Emerging dipterans made the greatest contribution to the flux of insect biomass (70 – 90%, 238 – 763 mg/m^2·week) at the two stream sites (Appendix III). Aquatic insect communities are known to shift towards dominance by more tolerant Diptera in streams impacted by agriculture, while abundances of other more sensitive taxa decrease (Raitif et al., 2018; Stenroth et al., 2015). Dipterans however, also had greater BWAFs for some pesticides compared to other orders, which was significant for two insecticides, dimethoate and thiacloprid compared to Trichoptera (Fig. 8). Emerging Diptera thus contributed the greatest to the pesticide flux, accounting for 94 – 96% (9.6 – 10.5 ng/m^2·week), at both sites. Average total pesticide concentrations were the lowest in trichopterans at both sites compared to the other two orders (Appendix III). Thus, despite contributing approximately 10 – 25% to the emerging biomass, they contributed similarly (0.3 – 0.4 ng/m^2·week) to the weekly pesticide flux as the ephemeropterans (0.1 – 0.3 ng/m^2·week), which contributed only approximately 1 – 5% of the total biomass.

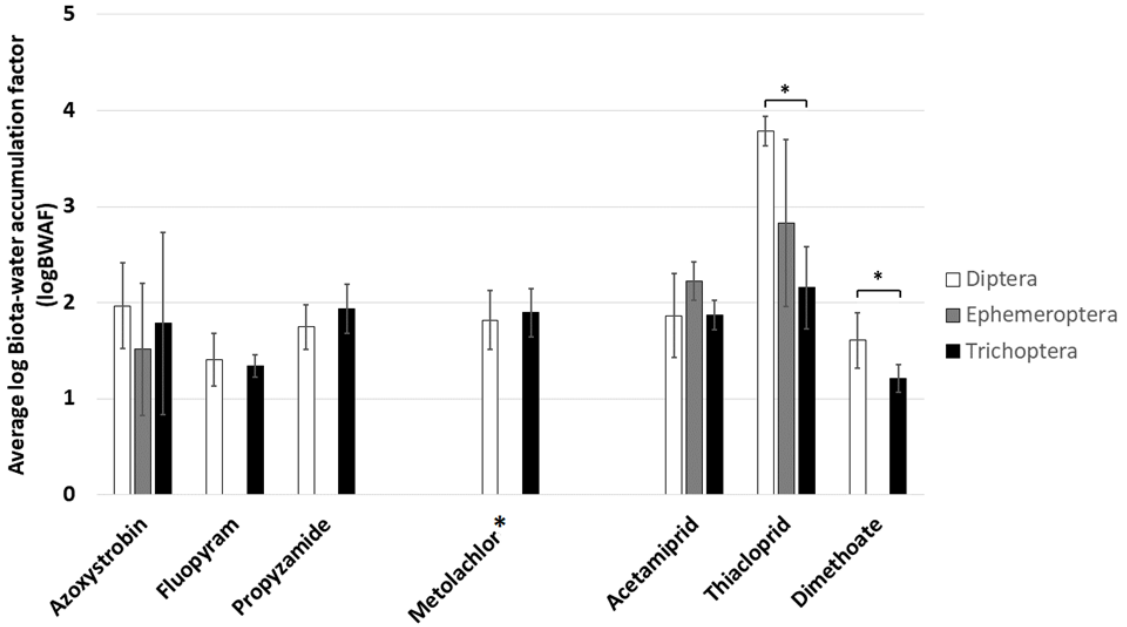

Figure 8. Arithmetic means of biota-water accumulation factors (logBWAFs) for pesticides which were detected in more than 50 % of samples of adult emerging insects from three orders (n = 6 for Diptera, n = 4 for Ephemeroptera and n = 4 for Trichoptera). Error bars indicate the standard deviations. Asterisks indicate a significant difference between orders (Kruskal-Wallis rank sum test with post hoc Dunn's test using Bonferroni correction, $p < 0.05$). *Concentration is reported for the sum of isomers (Source: Appendix III).

Overall, emerging insect samples contained 27 pesticides (Section 4.1, Fig. 3, Appendix III). The majority, 83%, of concentrations were below 1 ng/g, but two neonicotinoids, thiacloprid and imidacloprid, had consistently higher concentrations, which were up to 23.2 and 6.7 ng/g, respectively (Fig. 3). The BWAFs for the emerging insects covered a range of approximately 1.8 – 12300, with 84% of the values lying between 10 to 1000 (Fig. 9). The neonicotinoid, thiacloprid, had the highest BWAFs (up to 12300). The median water concentrations of thiacloprid at the stream sites were 0.0017 and 0.0055 ng/mL, for MB and SB, respectively. These concentrations were therefore a factor of 18 or 60 lower than the lowest treatment level (0.1 ng/mL) of thiacloprid tested in the laboratory study (Section 4.2.2, Appendix II). Adult males and females emerging from this treatment level contained average thiacloprid concentrations (± standard deviation) of 12.2 ± 6.3 and 8.4 ± 2.5 ng/g, respectively (Appendix II). In comparison, thiacloprid concentrations in emerging Diptera from the field sites, MB and SB, were 11.7 ± 3.3 (n = 6) and 23.2 (n = 1), respectively. The results therefore indicate that duration of exposure and not just exposure-concentration potentially affect the mechanism of thiacloprid transfer.

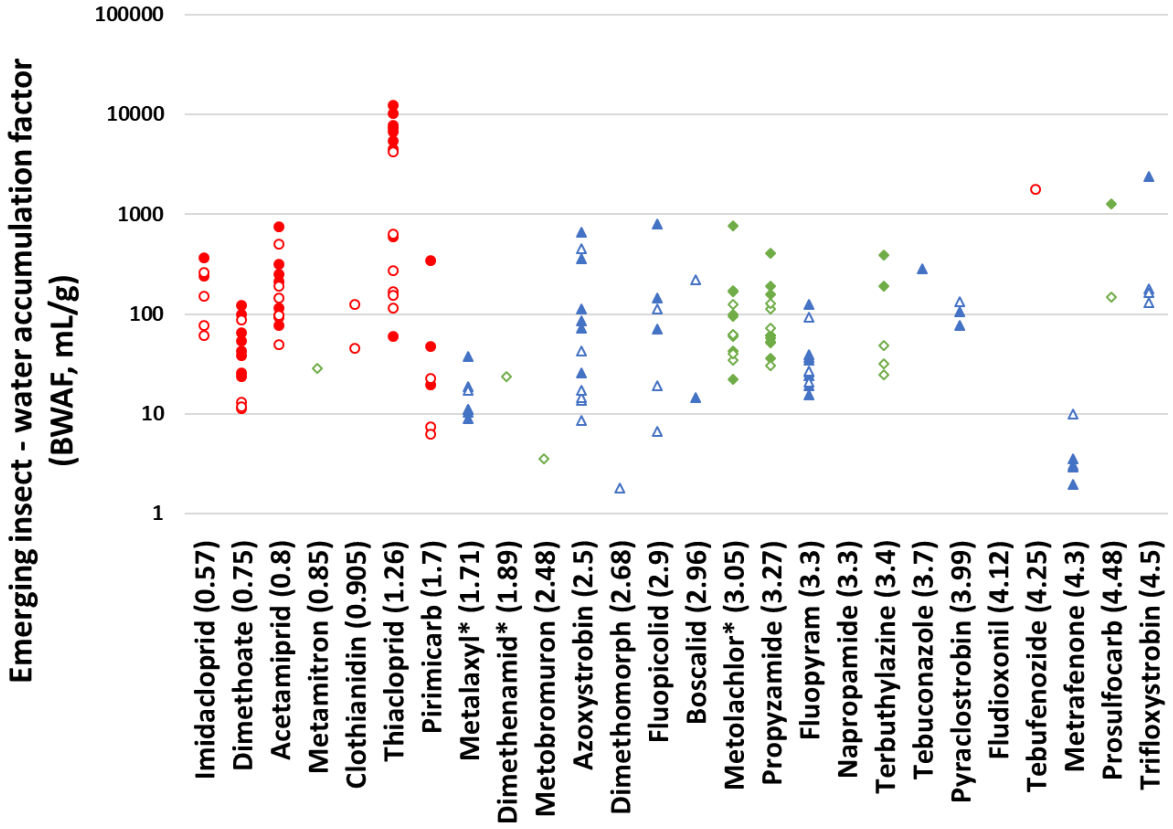

Figure 9. Biota-water accumulation factors (BWAF) for pesticides in emerging aquatic insects. Values shown for fungicides (blue triangles), herbicides (green diamond) and insecticides (red circles) in

samples of emerging insects (including Diptera, Ephemeroptera and Trichoptera) collected from two stream sites affected by agricultural land use, namely the Modenbach (MB, solid shapes) and the Spiegelbach (SB, outlined shapes). Pesticides are arranged from left to right in order of increasing lipophilicity (logKow values are provided in brackets). *BWAFs are reported for the sum of isomers (Source: Appendix III).

Emerging insects, collected from the agricultural stream sites, contained only a fraction of the pesticides present in the aquatic environment (27 out of 49, Appendix III). Similarly, spiders from the same sampling sites contained only 25 pesticides (Appendix III). A principal component analysis of eight parameters associated with physicochemical properties, toxicity and stability of 18 pesticides (Appendix III), which were categorised according to their frequency of detection in abiotic (sediment, aquatic leaf litter and water) and biotic compartments (emerging insects and spiders) as either “transferred” or “not transferred” by emerging insects could not separate the two groups. This result may be due to the fact that the pesticides included all have systemic properties strongly linked to their solubility, which intentionally facilitates their dispersal and movement through plants and insects (Kagabu, 1999). It is therefore unsurprising that differences in the tested properties of the pesticides were not sufficient to differentiate between those pesticides with a high or a low propensity to be transferred by emerging insects.

Three neonicotinoids which were measured in emerging insects, had higher concentrations in the riparian spiders, while the concentrations of fungicides and herbicides generally decreased (see Section 4.3). Thus, implying a mechanism of bioaccumulation specific to neonicotinoid insecticides. This is supported by the results in the second laboratory batch-scale study (Appendix II), where the neonicotinoid, thiacloprid, was selectively transferred due to a slow rate of elimination compared to two other insecticides (Fig. 7). A similar selective bioaccumulation of neonicotinoids in the presence of complex mixtures of pesticides, as in the present study, has been reported in earthworms under laboratory conditions (Chevillot et al., 2017). The neonicotinoids used in Chevillot et al. as well as those from the field study, are so called “first and second generation” neonicotinoids, which share a common structural backbone and stearic conformations that are essential to their systemic behaviour and mode of toxic action (Jeschke & Nauen, 2008; Simon-Delso et al., 2015). Specific binding of neonicotinoids to proteins or other large biomolecules has been put forward by several authors to explain the differences between the predicted and measured toxicokinetics of neonicotinoids in aquatic crustaceans (Lauper et al., 2022; Li et al., 2021). A mechanism involving specific binding is further supported by the enantioselective bioaccumulation rates in earthworms reported for dinotefuran, the only neonicotinoid containing a stereocenter, but not measured in the field study (T. Liu et al., 2018). Furthermore, flupyradifurone, which is a newer generation butenolide insecticide (Nauen et al., 2015) structurally related to the neonicotinoids, was frequently detected at low concentrations in the spiders from the field study. It was, however, not detected in the water or emerging insects (Appendix III). The volume of this insecticide applied was < 1% of the total neonicotinoids applied during the sampling period (Bub et al., 2023), which could potentially have resulted in these concentrations lying below the analytical detection limits. A mechanism of biomagnification similar to the neonicotinoids could explain the results in spiders, it can, however, only be speculated from the current data.

4.3 Dietary exposure of insectivores preying on emerging insects

The estimated average weekly pesticides fluxes mediated by emerging insects were similar between the two stream sites in the field study (10.0 or 11.2 ng/ m^2·week, at SB and MB, respectively). Concentrations of pesticides in Dipterans, which dominated emergence (Section 4.2.3), were composed of 80 – 93% insecticides (Appendix III). This resulted in insecticide fluxes of 7.7 – 9.8 ng/ m^2·week, which is lower than what has been reported in insects emerging from agriculturally impacted wetlands in the USA (0.4 – 26.8 ng/m^2·d) (Kraus et al., 2021). However, in addition to Diptera, Kraus et al. also sampled Odonata, an insect order which was not included in the stream sampling. The concentrations of neonicotinoids measured in wetland insects were in fact the same order of magnitude as those in the stream insects (Section 4.1). The sulfone metabolite of fipronil made a large contribution to the fluxes from wetlands, and was only detected at low concentrations in the insects emerging from the stream sites. The environmental concentrations of the parent compound, fipronil, were not reported in the wetlands, and it was detected at very low infrequent concentrations in the stream water (max concentration of 0.003 ng/mL at SB). The use of fipronil in the European Union was also severely restricted during the sampling period (European Commission, 2019) and its concentrations in the environment are thus presumably lower compared to the sites in the USA. The pesticide fluxes from wetland insects also included pesticides which were not measured in the stream insects, among them a metabolite of the bioaccumulative insecticide, DDT (p-p' DDD), which also has a high propensity to be transferred by emerging insects (Reinhold et al., 1999).

The dominant contribution of Diptera to the pesticide flux (Section 4.2.3) from streams is of great relevance for the exposure of terrestrial insectivores considering that Chironomidae (Diptera) have the longest emergence window among the three emerging insect orders; Diptera, Ephemeroptera and Trichoptera (Raitif et al., 2018). Emergence of this family of Diptera subsides only during the coldest months, and thus implies a potentially continuous flux of pesticides to terrestrial insectivores. Furthermore, the laboratory-scale study results (Section 4.2.1, Appendix I) indicated that pesticide concentrations can persist in the adult insects over the terrestrial life stage (Section 4.2.1, Appendix I), facilitating their transfer as much as 100 m into adjacent terrestrial ecosystems via insect dispersal (Carlson et al., 2016).

The concentrations of pesticides in web-building riparian spiders feeding on emerging aquatic insects was quantified in the field study (Appendix III). Twenty-nine pesticides were detected in spider samples across all ten sampling sites (Fig. 10). Eleven insecticides were measured at relatively high concentrations, with SACs: 2.1 to 94.2 ng/g across all ten sites (Fig. 10 A). Six fungicides and two herbicides were measured at lower concentrations (Fig. 10 B), with sum average concentrations (SACs): < 6.2 and < 1.6 ng/g, respectively (Appendix III). Insecticide SACs, which were up to three orders of magnitude greater than for the fungicides and herbicides at individual sampling sites (Appendix III), were at least a factor of 10 to 20 times lower than what has been reported for sum PFAS and sum PCBs in tetragnathid spiders feeding on emerging insects, but similar to concentrations of pharmaceuticals and endocrine disrupting chemicals (Koch et al., 2021; Previšić et al., 2021; Walters et al., 2010).

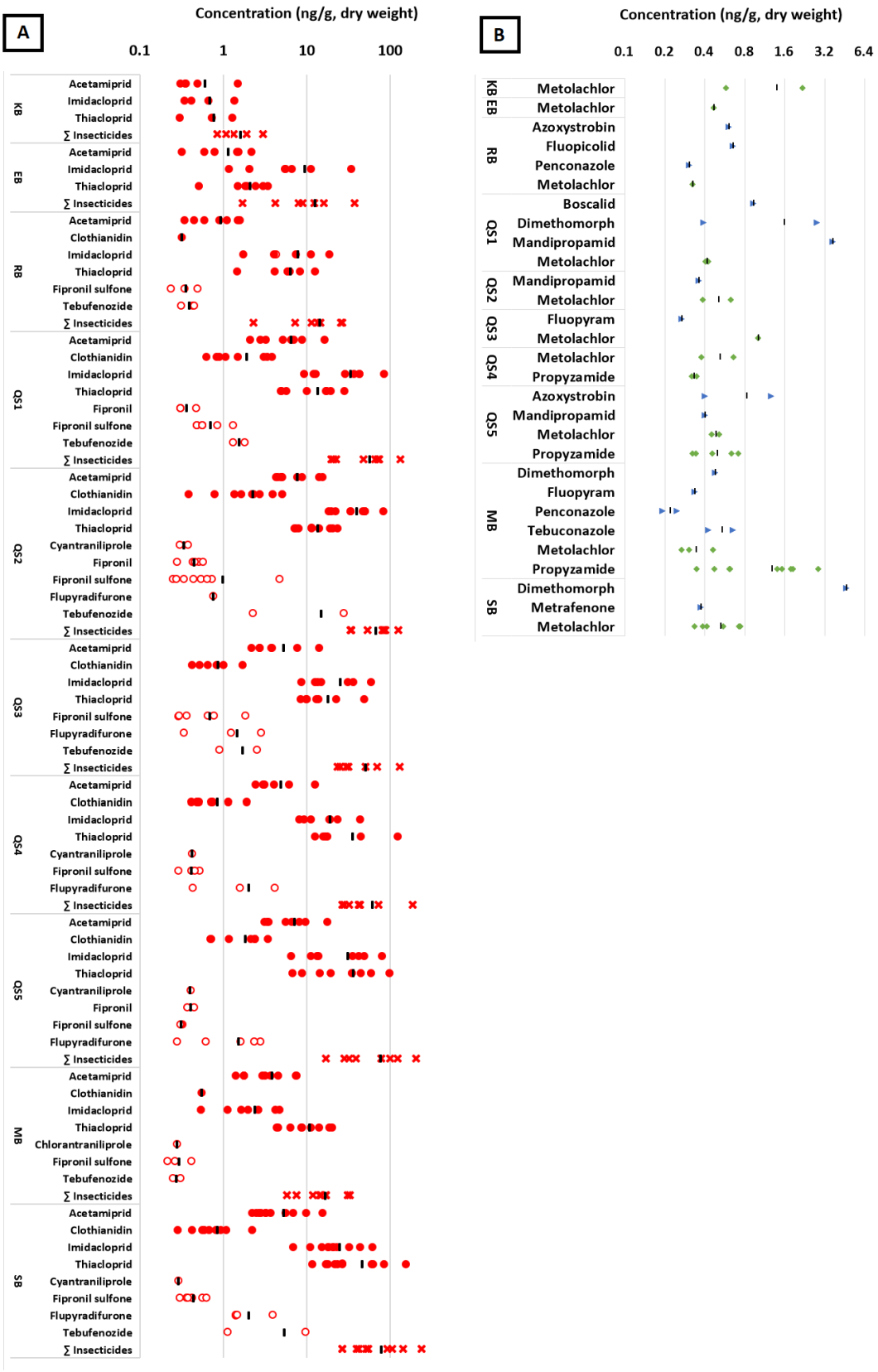

Figure 10. Measured pesticide concentrations in spider samples from ten sampling sites. **(A)** Insecticide concentrations are shown for neonicotinoid insecticides (solid red circles) and other insecticide classes (outlined red circles) are shown for individual samples. **(B)** Concentrations of fungicides (blue triangles) and herbicides (green diamonds) are shown for individual samples. Average concentrations are indicated by black dashes. Sum insecticide concentrations indicated by red crosses (Source: Appendix III).

Four neonicotinoid insecticides, namely acetamiprid, clothianidin, imidacloprid and thiacloprid made up 78 – 100% of insecticide SACs, and were detected in 16 – 100% of spider samples across all ten sites (Appendix III). These four neonicotinoids had the highest site-specific average concentrations (up to 46 ng/g) compared to other insecticide classes (up to 14.9 ng/g, Fig. 10 A). Moreover, the overall median concentrations of acetamiprid, clothianidin, imidacloprid and thiacloprid (3.2, 0.9, 14.4 and 12.6 ng/g, respectively) were all higher than the median concentration of other (non-neonicotinoid) insecticides (0.5 ng/g), fungicides (0.5 ng/g) and herbicides (0.5 ng/g), (Fig. 11).

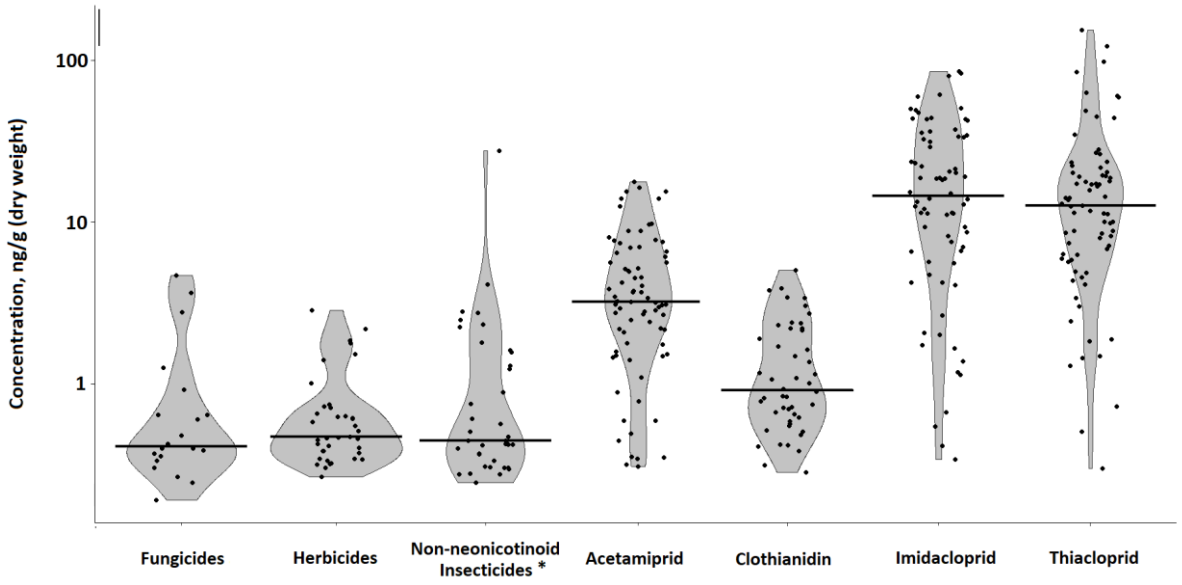

Figure 11. Pesticide concentrations are reported for individual spider-samples (n = 79 samples) collected from ten stream sites. Horizontal lines indicate the median concentrations. *Non-neonicotinoid insecticides in spider samples includes concentrations for fipronil's sulfone metabolite (Source: Appendix III).

Furthermore, compared to the concentrations in emerging aquatic insects, biomagnification was observed for three neonicotinoids and one herbicide in spiders (Fig. 12). Significantly higher concentrations (factor 6 to 15) of the neonicotinoids acetamiprid, imidacloprid and thiacloprid, were observed in female spiders compared to the emerging insect samples collected from SB. Male spiders from this site had concentrations which were a factor of 3 to 5 higher than the emerging insects, although not statistically significant. Acetamiprid concentrations were significantly higher (factor 15 to 32) in both spider sexes at MB, as well as for the herbicide propyzamide in male spiders (by a factor 7).

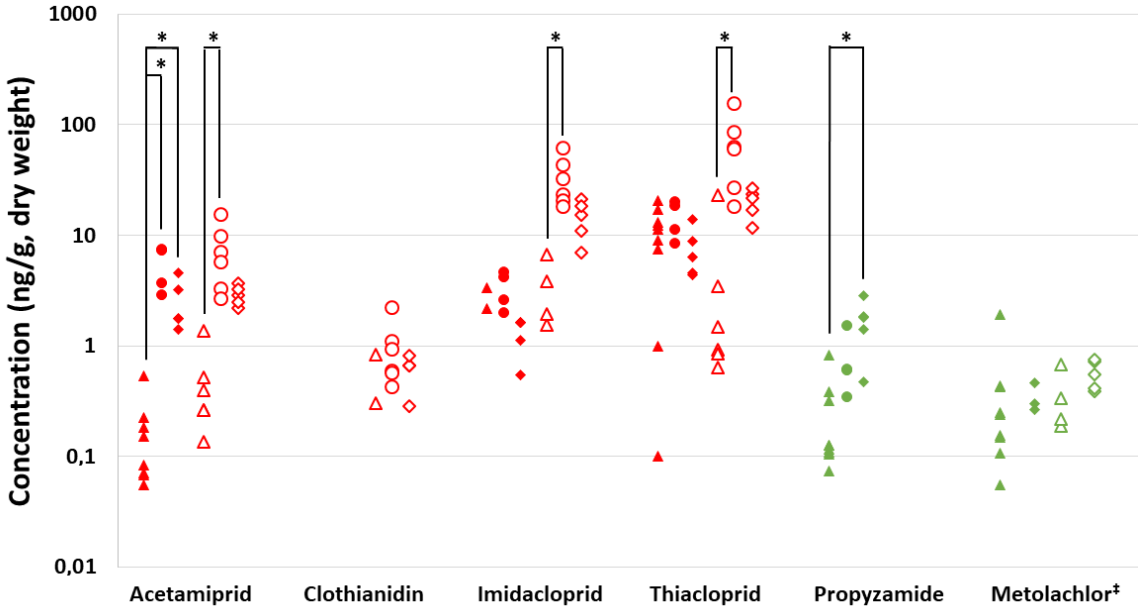

Figure 12. Pesticides concentrations in emerging insects and riparian spiders (adult *Tetragnatha* spp.) Samples collected from two stream sites impacted by agricultural activities (Modenbach, MB – solid black shapes and Spiegelbach, SB – outlined shapes). Pesticide concentrations in emerging aquatic insect samples, comprised of dipterans, ephemeropterans and trichopterans, are indicated by triangles, while concentrations in female and male spiders are indicated by circles and diamonds, respectively. Insecticides concentrations are shown in red and herbicides in green. Asterisks indicate a significant difference in concentrations between groups (Kruskal-Wallis rank sum test with post hoc Dunn's test using Bonferroni correction, $p < 0.05$). ‡Concentrations are reported as the sum of isomers (Source: Appendix III).

Overall, the transfer of predominantly insecticides, among them mixtures of up to four neonicotinoids, by emerging insects has the potential to negatively impact the terrestrial food web. This may be relevant for a wide range of predators, including birds, bats, lizards and spiders, which can obtain a large proportion of their energy requirements through consumption of emerging aquatic insects (Baxter et al., 2005). Negative impacts on terrestrial food webs could arise due to the emerging insects themselves being negatively impacted by retained neonicotinoid insecticides. For example, the neurotoxic mode of action of neonicotinoids has been linked to a range of sublethal effects in non-target insects, such as vision loss, reduced immune response to pathogens and behavioural effects (Pisa et al., 2021; Tasman et al., 2021). Retention of these compounds may therefore have negative impacts on the fitness and longevity of the successfully emerged adults, with potential for further cascading impacts on terrestrial consumers through further reduced food availability. Furthermore, terrestrial insectivores are dietarily exposed to the pesticides in the emerging insects, resulting in the bioaccumulation of several pesticides including neonicotinoids in riparian spiders. Spiders, however, are fairly tolerant towards neonicotinoids in comparison to insects (Song et al., 2009) and could thus potentially create a reservoir for these insecticides in the food web, increasing the likelihood of exposure for other terrestrial insectivores, such as bats and birds (Reinhold et al., 1999; Walters et al., 2010). The impacts of dietary exposure to neonicotinoids on vertebrate predators are challenging to study at the landscape scale due to the confounding effects of multiple stressors. However, laboratory investigations have

revealed sublethal effects on development, behaviour, immune function and reproductive success for a wide variety of terrestrial insectivores, including birds and bats (Gibbons et al., 2015; Pisa et al., 2021; Wu et al., 2020).

5 Conclusion

Emerging aquatic insects transfer current-use pesticides from contaminated aquatic ecosystems to adjacent terrestrial food webs. Thus, adding these contaminants to the growing list of micropollutants which terrestrial insectivores, preying on these insects, may be dietarily exposed to. The current-use pesticides studied included diverse small organic molecules from many chemical classes. This resulted in combined pesticide-specific and organism-specific properties and processes affecting their propensity to be transferred by emerging aquatic insects. A unique propensity to bioaccumulate in emerging insects, and riparian spiders hunting them, was found to be shared by several widely used neonicotinoid insecticides, which is potentially attributed to their conserved molecular structure and stearic shape. This may thus extend to newer classes of insecticides which share these properties, and are intended to replace them. Future studies are therefore needed to further investigate the mechanisms and molecular characteristics involved in the bioaccumulation of these neurotoxic insecticides. The emerging insects and spiders studied in this thesis are widely distributed and also studied across the globe. Moreover, the exposure concentrations used in the mesocosm studies were environmentally relevant based on published data. Furthermore, the concentrations of neonicotinoids measured in water samples from the field study were lower than global values. It can thus be concluded that the results presented in this thesis are relevant for contaminated freshwater ecosystems worldwide. The contamination of emerging insect subsidies with organic pesticides may therefore have widespread but still unknown negative consequences for riparian insectivores. This may be especially relevant for sensitive life stages or species of high conservation value, for example, bird nestlings and threatened bats. Considering the declines in bird and bat populations coinciding with increased pesticide use in recent decades, a better understanding of the potential dietary exposure to organic pesticides via this route is of great relevance.

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7 Declaration

I hereby declare that I independently conducted the work presented in this thesis entitled AQUATIC-TERRESTRIAL TRANSFER OF CURRENT-USE PESTICIDES BY EMERGING AQUATIC INSECTS AND POTENTIAL FOR DIETARY EXPOSURE OF TERRESTRIAL INSECTIVORES. All used assistances are mentioned and involved contributors are either co-authors of or are acknowledged in the respective publication.

This thesis has never been submitted elsewhere for an examination, as a thesis or for evaluation in a similar context to any department of this university or any scientific institution. I am aware that a violation of the aforementioned conditions can have legal consequences.

Landau in der Pfalz, 27.02.2023

Place, date Signature

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9 Curriculum Vitae

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2016 – 2018	Master of Science in Chemistry, Department of Chemistry, University of Pretoria, Pretoria, South Africa
	Dissertation Title: A new sampling method for human skin volatile analysis by comprehensive gas chromatography and mass spectrometry.
2015 – 2016	Bachelor of Science (Honours) in Chemistry, Department of Chemistry, University of Pretoria, Pretoria, South Africa
2012 – 2015	Bachelor of Science in Chemistry, University of Pretoria, Pretoria, South Africa

10 Appendices

Appendix I

Roodt, A.P., Röder, N., Pietz, S., Kolbenschlag, S., Manfrin, A., Schwenk, K., Bundschuh, M., Schulz, R. (2022) Emerging midges transport pesticides from aquatic to terrestrial ecosystems: importance of compound- and organism-specific parameters. *Environmental Science & Technology* 56, 5478-5488.

Appendix II

Roodt, A.P., Schaufelberger, S., Schulz, R. (2023) Aquatic-terrestrial insecticide fluxes: midges as neonicotinoid vectors. *Environ. Toxicol. Chem*. 42, 60–70.

Appendix III

Roodt, A.P., Huszarik, M., Entling, M.H., Schulz, R. (2023) Aquatic-terrestrial transfer of neonicotinoid insecticides in riparian food webs. *J. Hazard. Mater.* 455, 131635.

Appendix I

Roodt, A.P., Röder, N., Pietz, S., Kolbenschlag, S., Manfrin, A., Schwenk, K., Bundschuh, M., Schulz, R. (2022) Emerging midges transport pesticides from aquatic to terrestrial ecosystems: importance of compound- and organism-specific parameters. *Environmental Science & Technology* 56, 5478-5488.

Supplementary Material – Emerging midges transport pesticides from aquatic to terrestrial ecosystems: importance of compound- and organism-specific parameters.

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Article

Emerging Midges Transport Pesticides from Aquatic to Terrestrial Ecosystems: Importance of Compound- and Organism-Specific Parameters

Alexis P. Roodt,* Nina Röder, Sebastian Pietz, Sara Kolbenschlag, Alessandro Manfrin, Klaus Schwenk, Mirco Bundschuh, and Ralf Schulz

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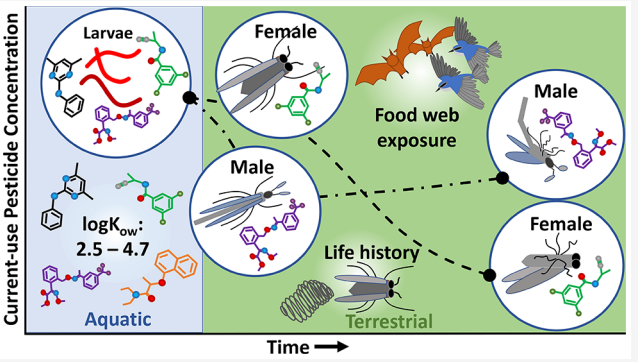
ABSTRACT: Emerging aquatic insects have the potential to retain aquatic contaminants after metamorphosis, potentially transporting them into adjacent terrestrial food webs. It is unknown whether this transfer is also relevant for current-use pesticides. We exposed larvae of the nonbiting midge, *Chironomus riparius*, to a sublethal pulse of a mixture of nine moderately polar fungicides and herbicides (logK_{ow} 2.5−4.7) at three field relevant treatment levels (1.2−2.5, 17.5−35.0, or 50.0−100.0 μg/L). We then assessed the pesticide bioaccumulation and bioamplification over the full aquatic−terrestrial life cycle of both sexes including the egg laying of adult females. By applying sensitive LC−MS/MS analysis to small sample volumes (~5 mg, dry weight), we detected all pesticides in larvae from all treatment levels (2.8−1019 ng/g), five of the pesticides in the adults from the lowest treatment level and eight in the higher treatment levels (1.5−3615 ng/g). Retention of the pesticides through metamorphosis was not predictable based solely on pesticide lipophilicity. Sex-specific differences in adult insect pesticide concentrations were significant for five of the pesticides, with greater concentrations in females for four of them. Over the duration of the adults' lifespan, pesticide concentrations generally decreased in females while persisting in males. Our results suggest that a low to moderate daily dietary exposure to these pesticides may be possible for tree swallow nestlings and insectivorous bats.



KEYWORDS: *aquatic−terrestrial linkage, current-use pesticides, food web, organic pesticides, aquatic insects*

INTRODUCTION

During their development in freshwater ecosystems impacted by agriculture, industry, or waste water, the aquatic life stages of emerging insects are regularly exposed to a broad range of anthropogenic contaminants.[1−7] Contaminants with low toxicities can potentially bioaccumulate in the developing aquatic life stages of emerging insects with minimal effect on emergence success and, when retained by the emerging adult insects after metamorphosis, can be transported into adjacent terrestrial ecosystems.[8] A wide range of contaminants have been observed to be retained by the adult aquatic insects after metamorphosis, for example, some pesticides, metals, metal-based nanoparticles, pharmaceuticals, polychlorinated biphenyls (PCBs), per- and polyfluorinated alkyl substances, and polycyclic aromatic hydrocarbons (PAHs).[9−17] After successful emergence, these insects serve as important nutritional sources for spiders, lizards, birds, and bats in adjacent terrestrial food webs.[18] Retention of contaminants by emerging insects therefore has the potential to increase the dietary exposure of terrestrial insectivores, including species of high conservation value.[19] Currently, there is insufficient data on the extent to which many current-use pesticides are transported from the aquatic environment into terrestrial food webs via emerging insects.[17,20] Furthermore, the ubiquitous presence of current-use fungicides and herbicides in aquatic environments, which do not necessarily have a high toxicity to developing insect larvae and thus will likely not reduce emergence success, potentially has great relevance in this context.[4,21]

Bioaccumulation of anthropogenic contaminants by aquatic insects refers to the process of assimilation of these substances from the environment into the organism, both passively and through dietary exposure.[22] Compound-specific parameters of contaminants, such as increasing lipophilicity and decreasing

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molecular mass, can correlate positively with the potential for bioaccumulation in aquatic organisms and are therefore often used as key predictors in this regard.[23−26] The propensity for assimilation of a contaminant is offset by its elimination from the organism, for example, by metabolism of the contaminants during the aquatic life stage.[27] However, in the case of emerging aquatic insects, the full life cycle has the potential to alter the concentrations of bioaccumulated contaminants. Metabolic and life cycle processes, which include metamorphosis of insect larvae and continue through the adult life stage, involve the interplay between contaminant enrichment, through body weight loss, and contaminant elimination, i.e., bioamplification.[28] The relationship between contaminant lipophilicity and retention over metamorphosis has been investigated for two main groups of organic contaminants, namely, PAHs and PCBs. These studies reveal an inverse relationship for contaminants with log octanol/water partition coefficients ($\log K_{ow}$) between 3 and 5. Currently, there is still a lack of data for current-use pesticides in this regard. The changes in contaminant concentrations over metamorphosis can have profound effects on the estimation of the dietary exposure risk of terrestrial insectivores; for example, enrichment of larval concentrations by up to threefold has been reported for some organic contaminants in emergent adults.[17]

Additionally, sexual dimorphism in insect development and life cycles has the potential to influence the mechanism of bioamplification through differences in body weight loss and egg production, although the effects of these aspects on contaminant transfer by emerging insects is not often studied.[17] Sex-specific differences in the contaminant concentrations have however been observed for PCBs and zinc in adults of several mayfly species, as well as for halogenated organic pollutant (HOP) concentrations in terrestrial species of Lepidoptera after exposure as larvae and subsequent metamorphosis.[10,29,30] Furthermore, this aspect of pesticide transfer into terrestrial food webs can potentially be of great relevance due to the sexual dimorphism of adult body weight and differences in the nutritional value.[31,32]

Currently, insufficient data on the relationship between contaminant-related parameters (such as lipophilicity) and the importance of life cycle characteristics (e.g., metamorphosis or egg production) on the aquatic to terrestrial transport of current-use pesticides by emerging insects are available. We used laboratory microcosms to expose larvae of the holometabolous midge, *Chironomus riparius* (Insecta: Diptera) to a single pulse exposure of a mixture of nine current-use fungicides and herbicides at one of three sublethal field relevant concentration levels, with concentrations ranging between 1.2 and 100 μg/L for individual pesticides. Mixtures of pesticides in agricultural streams, containing many of the pesticides used, can reach 7−83.4 μg/L during runoff events, with some reported concentrations exceeding levels set for environmental quality standards.[33,34] In addition to the environmental prevalence, the selected pesticides are all small molecules (<500 Da), with a range of log octanol/water partition coefficients ($\log K_{ow}$) between 2.5 and 4.7 and few proton donor and acceptor groups. Conforming with these criteria implies that the compounds can partition across biological membranes and are potentially easily absorbed by organisms.[35]

We hypothesized that the bioaccumulation of the selected current-use pesticides in the aquatic larvae and their retention through metamorphosis would correlate with pesticide lipophilicity. Furthermore, we hypothesize that larger reductions in body weight between the larvae and adult in male insects compared to females would result in a bias toward higher concentrations directly after metamorphosis in the males. Finally, we hypothesize an increase in pesticide concentrations over the course of the adult life stage due to consumption of energy reserves and body weight loss and that this increase would be offset by oviposition in female insects. In addition, we contextualize the potential implications of our finding for terrestrial insectivores, which feed on emerging midges, by calculating a preliminary estimate of their daily dietary exposure to the pesticides.

MATERIALS AND METHODS

Chemicals and Reagents. Seven fungicides, namely, azoxystrobin (AZO), boscalid (BOS), cyflufenamid (CYF), fluopyram (FLU), tebuconazole (TEB), pyrimethanil (PYR), and trifloxystrobin (TRI), and two herbicides, napropamide (NAP) and propyzamide (PRO), were used in the current study. Pesticide formulation products, in the form of suspension concentrates, oil in water emulsions, or water dispersible granules, were used except for napropamide, for which the formulation product was not available and the pure substance was used instead. Formulation products, which contain formulants such as surfactants that are designed to improve the solubility and longevity of the active ingredients, are known to affect the distribution and persistence of pesticides in the environment.[36] They were therefore used, as opposed to pure pesticide active ingredients, to provide an experimental simulation closer to the situation in the field. The products and suppliers of all materials, chemicals, and reagents are listed in the Supporting Information (Table S1). All of the formulation products used in the current study are approved for use in Germany.[37] Examples of concentrations of the selected pesticide active ingredients found in environmental water samples, as well as the molecular masses, $\log K_{ow}$ values and aqueous solubilities, are provided in the Supporting Information (Table S2).

Experimental Microcosms. Three types of enclosures were used as microcosms for different life stages of the organisms during the experiment. Midge larvae were raised in sediment-water microcosms. Glass vessels with a diameter of 14 cm and volume of 0.9 L were used for the microcosms in which larvae were raised and collected before pupation and emergence. Separate glass vessels, approximately 32 × 22 cm, with a total volume of 3.75 L were covered with a 0.6 mm polyester mesh tent (38 × 23 × 24 cm) and used to raise larvae until emergence. The former and the latter vessels are referred to as larval and emergence microcosms in the remaining text, respectively. Separate microcosms were used for the collection of larvae and adults because the extraction of the larvae from the sediment is a destructive process, with the potential to disrupt the pesticide exposure of the larvae and emergence dynamics of the adult insects if a single microcosm was used. A portion of the adult insects collected in the emergence microcosms was transferred to separate cages (55 × 27.5 × 55 cm, 0.6 mm polyester mesh) where mating could take place. Each mating cage was equipped with a glass crystallization dish containing the medium for egg laying, which was exchanged daily.

Larval and emergence microcosms contained sediment with a depth of approximately 2 cm, which was prepared in accordance with the OECD 219 sediment-water chironomid

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Environ. Sci. Technol. XXXX, XXX, XXX−XXX

toxicity test.[38] The SAM-5S medium was used as the overlying aqueous phase with a depth of approximately 4 cm in both microcosm types.[39] A 700 μg/L pesticide stock solution was prepared from all the formulation products and napropamide in the SAM-5S medium. The pesticide stock solution was used to spike the medium above the sediment in the treated microcosms. Four replicate microcosms were prepared for each treatment level with nominal concentrations of 0, 2.5, 35, and 100 μg/L for each of the nine pesticides in the mixture. The actual pesticide concentrations of the stock solution were quantified by liquid chromatography coupled with tandem mass spectrometry (LC−MS/MS). The results of which indicated that the concentrations of individual active ingredients in the 2.5, 35, and 100 μg/L treatment levels were 1.6 ± 0.42, 23.19 ± 5.84, and 66.25 ± 16.70 μg/L, respectively, for the pesticides applied as formulation products (Table S3). Due to the differences between concentrations of each individual pesticide in the same treatment level, the treatment levels will be referred to as low, medium, and high as opposed to the nominal concentrations in the remaining text. After the addition of pesticides, the microcosms were allowed to equilibrate for 24 h before the test organisms were introduced.

Larvae were raised from eggs collected from an in-house laboratory culture. Freshly hatched larvae (<3 days old) were counted, and 50 or 400 individuals were separated into larval and emergence microcosms, respectively, which resulted in a ratio of approximately 5−6 mL of medium and 2−3 cm^2 sediment surface area per larva. Larvae were fed every second day with pulverized Tetramin fish food at a concentration of 0.5 mg/larva. Once the adult insects had started to emerge, the volume of food added to each vessel was reduced based on the total number of adults collected. Approximately, 65% of the daily emergence of each sex from the low and medium treatment level microcosms was transferred to replicate-specific mating cages.

The microcosms were maintained at 20 °C with 70% relative humidity and a 16:8-hour day/night cycle in a climate-controlled chamber throughout the experiment. Replicate microcosms were arranged in a pattern to randomize any potential effects of temperature or light gradients. Air pumps provided aeration of the medium, and a fresh medium was added to the vessels when needed to replace water lost due to evaporation. Water temperature, dissolved oxygen, conductivity, and pH were measured weekly in a subsample of the replicates from each treatment level and were consistent throughout the four weeks of the experiment's duration (Table S4).

Sample Collection. Aqueous medium samples (2 mL) for pesticide analysis were collected on days d0, d8, and d14 from the larval microcosms and d0, d14, and d28 from emergence microcosms. Samples were stored in polypropylene tubes at −20 °C. Larvae were collected after 14 days of development. Individuals that had begun to pupate were excluded (total of four individuals out of all replicates). Larvae were frozen in liquid nitrogen before being rinsed with HPLC-grade water. Adults were collected daily from each emergence microcosm, separated by sex, and counted. The adults (approximately 35%) that were not transferred to the mating cages were frozen in liquid nitrogen. Dead adults were collected daily from the floors of the mating cages, separated by sex, counted, and frozen at −80 °C. All insect samples were stored at −80 °C until being freeze-dried prior to pesticide extraction. Adult midges that were collected within 24 h of their emergence and those collected after having died in the mating cages are referred to as live and dead adults, respectively, in the remaining text. Individual insects from each replicate (n = 4) were pooled in order to achieve the required biomass for pesticide analysis (approximately 5 mg). For larvae, all the individuals recovered from the respective microcosm were pooled. Live and dead adult insect samples were prepared for each replicate by pooling individuals from different time points over the entire emergence and terrestrial life stage periods, respectively. Pooled samples of larvae (n = 45 ± 5 individuals per sample), adult males (n = 8 ± 2) and females (n = 4 ± 1), dead collected males (n = 15 ± 5), and dead collected females (n = 8 ± 3) were homogenized using a bead mill (Retsch MM 301, Haan, Germany) and steel beads. A subsample of 4.6 ± 1.0 mg dry weight was then weighed out from each sample and used for pesticide analysis.

Life History Parameters of Adult Midges. The sex-specific duration of the terrestrial life stage of the adult midges was determined by calculating the number of days between the date with the greatest number of emerged individuals and the date with the greatest number of dead individuals in each replicate (Figure S1). Further details on the average total emergence, average sex-specific emergence, adult dry weights, adult lifespan, sex ratio, and number of egg masses collected from each treatment level are provided in the Supporting Information (Table S5). The pesticide concentrations used in this study are considered sublethal due to the lack of a significant treatment-dependent effect observed for total emergence or estimated adult lifespan relative to the control (Kruskal-Wallis H test, $p > 0.05$).

Analysis of Pesticide Concentrations in Insects and the Aqueous Medium. Extraction of pesticides from insect samples was performed by ultrasound-assisted solid−liquid extraction. Five subsequent extractions using 1 mL aliquots of solvent were performed. Three of the extractions were performed with acetonitrile and two with methanol. The samples were placed in an ultrasonic bath at 25 °C for 5 min during each extraction. After centrifugation, the solvent extracts were pooled and then evaporated to dryness under a stream of nitrogen gas at 20 °C. The extracts were redissolved in 0.5 mL of a mixture of water and methanol (70:30) containing 0.1% formic acid. The aqueous medium samples were diluted with methanol containing formic acid to achieve a final water to methanol ratio of 70:30 with 0.1% formic acid. All samples were transferred to glass HPLC-vials after being filtered using a 0.2 μm PTFE membrane. The samples were stored at −20 °C before and 16 °C during analysis. All measurements were performed by LC−MS/MS using matrix-matched calibration standards (i.e., aqueous medium, larvae, adult males, and adult females). The interpretation of measured concentrations in dead adults should, however, be evaluated with caution because the quantitative LC−MS/MS analysis did not include separate matrix-matched standards for the dead collected adults. Sex-specific calibration standards were prepared from live collected adult individuals. Further details on the matrix effects are provided in the Supporting Information (Figure S2). Briefly, the observed matrix effects resulting in reduced measurement sensitivity correlate with the average individual body masses of the different insect matrices (i.e., larvae > females > males). A reduced matrix effect, arising due to lower body masses, would thus potentially cause analyte signals to be higher in dead individuals compared to their live

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Environ. Sci. Technol. XXXX, XXX, XXX−XXX

counterparts. We therefore consider decreases in dead adult sample concentrations relative to live adults as more reliable than increases or equality of concentrations. The instrumental analysis parameters are listed in the Supporting Information (Tables S6 and S7). The exact dry weight of each sample was used for the normalization of pesticide concentration measurements based on sample dry weight.

Analytical Method Validation and Quality Assurance. Method validation for the pesticide analyses was performed in accordance with the International Conference on Harmonisation, ICH Harmonised Tripartite Guideline.[40] Details of the method validation are provided in the Supporting Information. Briefly, limits of quantification (LOQs) were determined for each pesticide based on the quantifier-MRM transition signal-to-noise ratio (S/N). Pesticide LOQs were validated for each matrix separately, and the values varied between 0.25 and 5 ng/g, based on sample dry weight (Table S8). Replicate samples (n = 5) fortified at concentrations close to the LOQ were analyzed to determine pesticide recoveries and method precision (Table S9). LOQs were similar between adults (of both sexes) and larvae. Recoveries were within an acceptable range of 60−125% for all the pesticides with the exception of pyrimethanil in larvae and tebuconazole in adult males. Measured signals had acceptable precision, with relative standard deviations (RSDs) below 15% for all pesticides except pyrimethanil in larvae, which had an RSD of 20%. No correction factors were applied to any measured concentrations of pesticides based on the recoveries in the validation, including those with recoveries below 70%, because the measured concentrations of those pesticides in actual samples were much larger than the fortified concentrations in the recovery experiments.

Data Analysis. Pesticide-specific concentration factors (CFs) were calculated for larvae and live adult samples using the relevant dry weight normalized pesticide concentration (P) and time-weighted average pesticide exposure concentration (TWAC, for details see the Supporting Information) with eq 1.

$$\mathrm{CF}_{ijk} = \frac{P_{ijk}}{\mathrm{TWAC}_{ij}} \tag{1}$$

where i represents the type of insect sample (larvae, adult male, or adult female), j represents the treatment level and k represents the replicate. Bioconcentration factors (BCFs) and bioaccumulation factors (BAFs) are used to describe the accumulation of a contaminant in an aquatic organism from its environment via absorption through respiratory and dermal surfaces or through all routes of exposure, respectively.[22] Both factors can be calculated as the ratio of the concentrations of a contaminant in the organism to the concentration in the external environment under steady-state conditions. The term CF was therefore used instead of BAF because a steady-state exposure concentration was not maintained throughout the course of the experiment. Between 4 and 11 days passed between the date of larvae sampling and the date of peak adult emergence, for the pesticide-treated replicates (Figure S1). Comparison of larval and adult CFs was therefore performed in order to mitigate the effect of pesticide degradation on the exposure over the different durations taken to reach each life stage. Not conforming to the steady-state criteria may result in the calculated CFs underestimating the accumulation of the pesticides compared to the respective BCFs or BAFs. Pesticide relative concentration factors (RCFs) were calculated for each pesticide to evaluate the effect of metamorphosis on pesticide concentrations in the organisms using eq 2.

$$\mathrm{RCF} = -\frac{\mathrm{CF}_{(\mathrm{larvae})}}{\mathrm{CF}_{(\mathrm{average\ adults})}} \tag{2}$$

The average of the adult male and female CFs from each replicate was used in this calculation.

The proportion of each pesticide not adsorbed to sediment or the microcosm surfaces at the start of the experiment was determined as the percentage of the initial spiked concentration at d0 after the 24-hour equilibration period. The rates of pesticide degradation in the microcosms were evaluated by calculating the percentage change in pesticide concentrations in the medium over 14 days with eq 3.

$$\%\mathrm{Pesticide\ Concentration\ Change}_{xyz} = \frac{(P_{\mathrm{d14},xyz} - P_{\mathrm{d0},xyz})}{P_{\mathrm{d0},xyz}} \times 100 \tag{3}$$

where P denotes the concentration of the pesticide measured in the medium, x represents the microcosm type (larval or emergence), y represents the treatment level, and z represents the replicate. The time points of sample collection are denoted by d0 and d14.

Statistical Analysis. Nonparametric analyses were used because of the limited number of replicates in each treatment level. The Kruskal-Wallis H test was used to test for differences based on the treatment level for total emergence, larval CFs, sex ratio of emergent adults, sex-specific estimated adult lifespan, and sex-specific pesticide concentrations in adult midges. This was followed by a post hoc Dunn's test with Bonferroni correction when treatment level effects were detected. A paired Wilcoxon signed-rank test was used to test for sex-specific differences in concentrations of each pesticide between live and dead adults from the same treatment level. The Mann−Whitney U test was used to test for differences in pesticide degradation rates between microcosm types (larval or emergence), in CFs between larvae and adults, and treatment level-specific differences in sex-specific adult pesticide concentrations. Spearman correlation analysis was performed to test for correlation between pesticide lipophilicity and larval CF or organism RCFs. The significance level, α, was set at 0.05 for all tests. Statistical analyses were performed in R Version 4.0.3.[41]

Potential Dietary Exposure of Terrestrial Insectivores. The pesticide concentrations measured in the live adult midges from the lowest treatment level, the sex-specific adult dry weights, and the sex ratio of emergent adults were used to estimate the total annual pesticide transport out of agricultural streams (ng/m^2·year), as well as the daily dietary intake of pesticides (μg/kg·d) by tree swallow nestlings and insectivorous bats. The former estimation was calculated based on published data for the emergent mass of Chironomidae from agricultural streams. The latter two estimations were based on published food bolus content, mass and feeding rates of tree swallows (*Tachycineta bicolor*), and the feeding rates of widespread insectivorous bat species, *Myotis daubentonii* and *Myotis lucifugus*. A full description of the calculations is provided in the Supporting Information (Tables S10 and S11).

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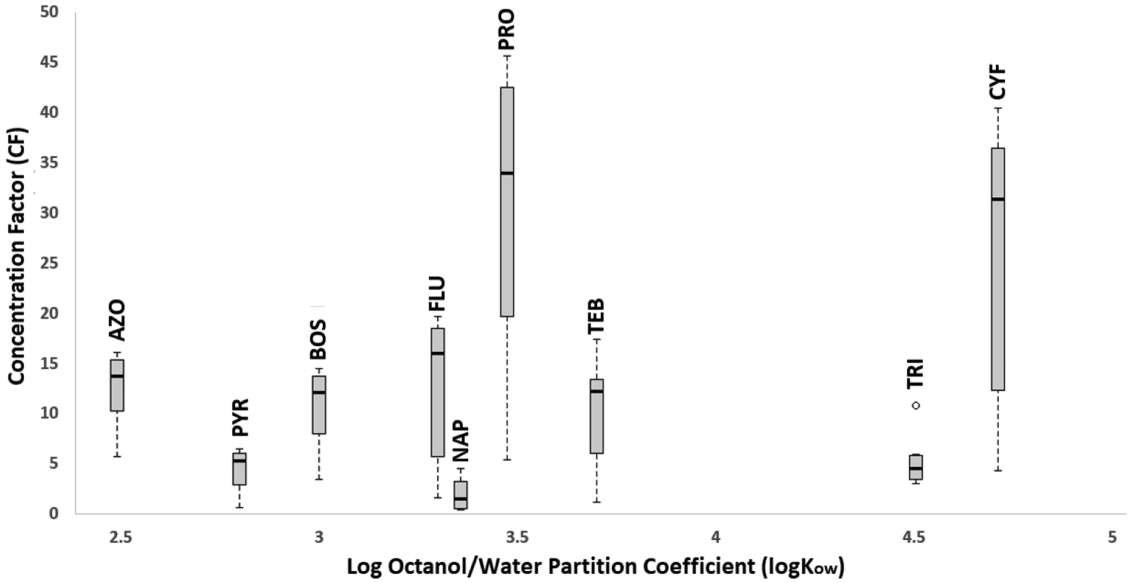

Figure 1. Box plots of pooled (medium and high treatment levels, $n = 8$) concentration factors (CFs) of nine pesticides with increasing logK_{ow} in midge larvae. CFs, which are larger than 1.5 times the interquartile ranges, are depicted as open circles. AZO = azoxystrobin, BOS = boscalid, FLU = fluopyram, NAP = napropamide, PRO = propyzamide, TEB = tebuconazole, TRI = trifloxystrobin, and CYF = cyflufenamid.

RESULTS AND DISCUSSION

Pesticides in Larvae. Accumulation of all nine pesticides was observed in larvae from all treated microcosms but not the controls, regardless of the treatment level. A significant treatment level effect on the calculated CFs was observed for three of the pesticides (Kruskal-Wallis H test, $p < 0.05$, Figure S3). Differences in the CFs between treatments likely resulted from differences in the rate of pesticide degradation between treatment levels (Figure S4). The rates of the pesticide concentration decrease were the slowest in the low treatment level compared to the medium and high treatment levels but were only significant when compared with the low and high treatment levels (Dunn's test, $p < 0.05$). This observation can be the result of processes such as oxidative or photodegradation, which have concentration-dependent reaction rates.[42] No significant differences were detected in the CFs or pesticide degradation rates between the medium and high treatment levels for any of the pesticides. The CFs from these two treatment levels were thus pooled before correlation analysis (Figure 1).

No relationship between the pesticide CFs and their respective logK_{ow} values was observed (Spearman's rank correlation: $\rho = 0.1$, $p > 0.05$). This is in contrast to a strong positive linear relationship ($R^2 = 0.98$), which has been reported for BCFs in midge larvae of a series of increasingly halogenated chlorobenzenes, with the same range of logK_{ow} values as were used in the current study.[43] Furthermore, a review of published *Chironomus* spp. BCFs for 14 structurally unrelated pesticides with logK_{ow} values between 2.4 and 8.1 showed a weak, yet still positive, linear correlation ($R^2 = 0.5$).[27] The lack of a strong positive linear relationship between the pesticide CF and logK_{ow} in the current study could result from a decreased bioavailability of the sediment bound fraction of each of the pesticides (Figure S5). However, the larval CFs only exhibited a very weak positive correlation with the percentage of the total pesticide concentration in the medium after equilibration (Spearman's rank correlation: $\rho = 0.1$, $p > 0.05$). The presence of surfactants, such as those used as adjuvants in the pesticide formulation products, can reduce the uptake of organic chemicals such as pesticides or pharmaceuticals in aquatic organisms.[44,45] However, the formation of surfactant micelles necessary for this is concentration-dependent. Therefore, considering that the increasing pesticide treatment levels also contain increasing concentrations of adjuvants, this would potentially result in slower pesticide degradation and lower larval concentrations with the increasing treatment level, neither of which was observed in our results (Figure S4 and Table S12).

The median pesticide CFs from the pooled treatment levels were between 1 and 35 (Figure 1). Propyzamide and cyflufenamid exhibited the greatest accumulation, both with a median CF larger than 30, double that of the medians of the next most accumulated pesticides. Large variation in the CFs of several pesticides may be attributed to differing rates of pesticide accumulation and body growth by different larval instars, and the distributions thereof, in each sample of pooled larvae.[46,47] Limited information on the accumulation potential of the pesticides used in the current study in aquatic invertebrates is available. Reported BAFs of azoxystrobin (BAF ≈ 31) and tebuconazole (BAF ≈ 32) are larger in the aquatic crustacean, *Gammarus pulex*, but have the same order of magnitude, compared to the current study.[48] Some of these differences may be the result of differing feeding strategies, contaminant metabolism, or respiration rates between the organisms.[27] Much larger BAFs have been reported for atrazine (logK_{ow} = 2.7; BAF = 1900) and trifluralin (logK_{ow} = 5.27; BAF = 210) in *C. riparius* larvae under chronic exposure conditions in the presence of sediment.[49] The calculated CFs in this study could underestimate the actual BAFs in the larvae because the exposure concentration decreases over the duration of the larval development. Nevertheless, the differences in the propensities of pesticides with very similar lipophilicities (differences in logK_{ow} < 0.5) to be accumulated in midge larvae, as well as the decreasing tendency for a strong linear correlation between contaminant lipophilicity and accumulation potential with the increasing diversity of contaminant molecular structure, as observed in the present

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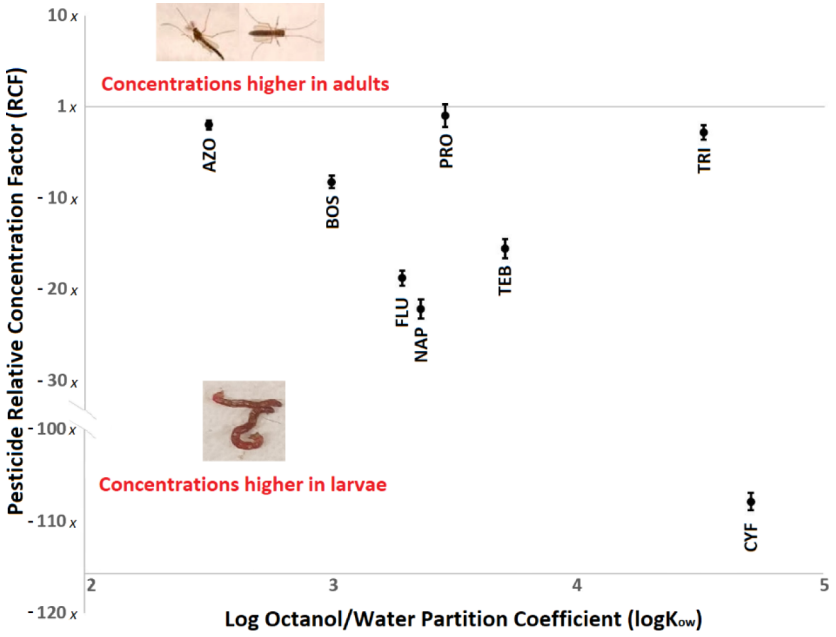

Figure 2. Average relative pesticide concentration factors (RCFs) for pooled medium and high treatment levels (n = 8). Values below the unity line indicate larger average CFs for the larvae compared to the adults. Error bars represent the RSDs. The y-axis is broken between 30 and 100× in the larval direction. AZO = azoxystrobin, BOS = boscalid, FLU = fluopyram, NAP = napropamide, PRO = propyzamide, TEB = tebuconazole, TRI = trifloxystrobin, and CYF = cyflufenamid.

study, suggest the important role of pesticide metabolism in determining the pesticide-specific potential for accumulation of the investigated pesticides in the larvae.[27] Compound-specific metabolic rate constants for the pesticides used in the present study are not available for aquatic insect larvae. Estimations of these values can be based on model organisms for which more data are available (e.g., fish).[26] Compound-specific metabolic rates can, however, vary widely between organisms, and a more detailed investigation of the metabolism of these pesticides in aquatic insect larvae is therefore necessary.

Retention of Pesticides after Emergence. All pesticides included in this study, with exception of pyrimethanil, were measured above their respective LOQs in the live adults (<24 h old) from the medium and high treatment levels. Five of the nine pesticides, namely, azoxystrobin, boscalid, propyzamide, tebuconazole, and trifloxystrobin, were even measured in the live adults from the low treatment level. There were no significant differences between the larval microcosms and emergence microcosms in the rates of pesticide degradation in the medium over the first 14 days for seven of the nine pesticides in the pooled medium and high treatment levels (Mann−Whitney U test, $p > 0.05$). Degradation of napropamide and propyzamide was faster in the emergence microcosms compared to the larval microcosms. The differences in final concentrations were 15 and 10% for napropamide and propyzamide, respectively. This may have resulted in a bias toward the larvae when calculating the RCFs for these two substances.

Metamorphosis was found to significantly lower concentrations of seven of the eight pesticides measured in the adults (average of male and female CFs) compared to the larvae, with reductions between approximately 20 and 100% (Mann−Whitney U test, $p < 0.05$, pooled medium and high treatment levels). Propyzamide, which had approximately equal average CFs for larvae and adults, was the only exception (Figure 2). The majority of pesticides had CFs in the adults, which were less than 20% of their respective CFs in the larvae. The two strobilurins, azoxystrobin and trifloxystrobin, had a very similar propensity to be retained by the adults despite two orders of magnitude difference in lipophilicity between them, with average CFs, which were approximately 40 and 30% of the value in the larvae, respectively. The greatest difference between developmental stages was observed for cyflufenamid, which had one of the largest average CFs in the larvae but less than 1% of this CF in the adults. The opposite trend has been reported for the concentrations of 20 pharmaceuticals and endocrine-disrupting chemicals between larvae and adults of holometabolous Trichoptera, where either equality or an increase (i.e., bioamplification) in concentration with the sequential development stage for 19 of the substances has been reported.[13] The contaminants measured by these authors occupy the same chemical space, with regard to lipophilicities and molecular masses, as the pesticides used in the current study. These differences may be the result of differing rates of contaminant metabolism, which are known to vary in holometabolic insect larvae depending on their order.[50,51] Such differences are likely to extend to the later life stages, such as the pupal stage. Additionally, differences in the life histories, such as larval development duration or the percentage body mass loss during pupation, will likely play an important part in determining the organism-specific bioamplification of contaminant concentrations between life stages.

Overall, we observed a weak negative trend (Spearman's rank correlation: $\rho = 0.33$, $p > 0.05$) between the RCFs and the lipophilicities of the tested pesticides. This result is consistent with the strong negative correlation ($R^2 = 0.96$), which has been reported for contaminants with logK_{ow} values ranging from 3 to 5.[17] The stronger correlation reported by these authors is predominantly based on the retention of PAHs, which is a more structurally uniform class of chemicals in comparison to the pesticides used in the current study. These authors hypothesized that metabolism played an important

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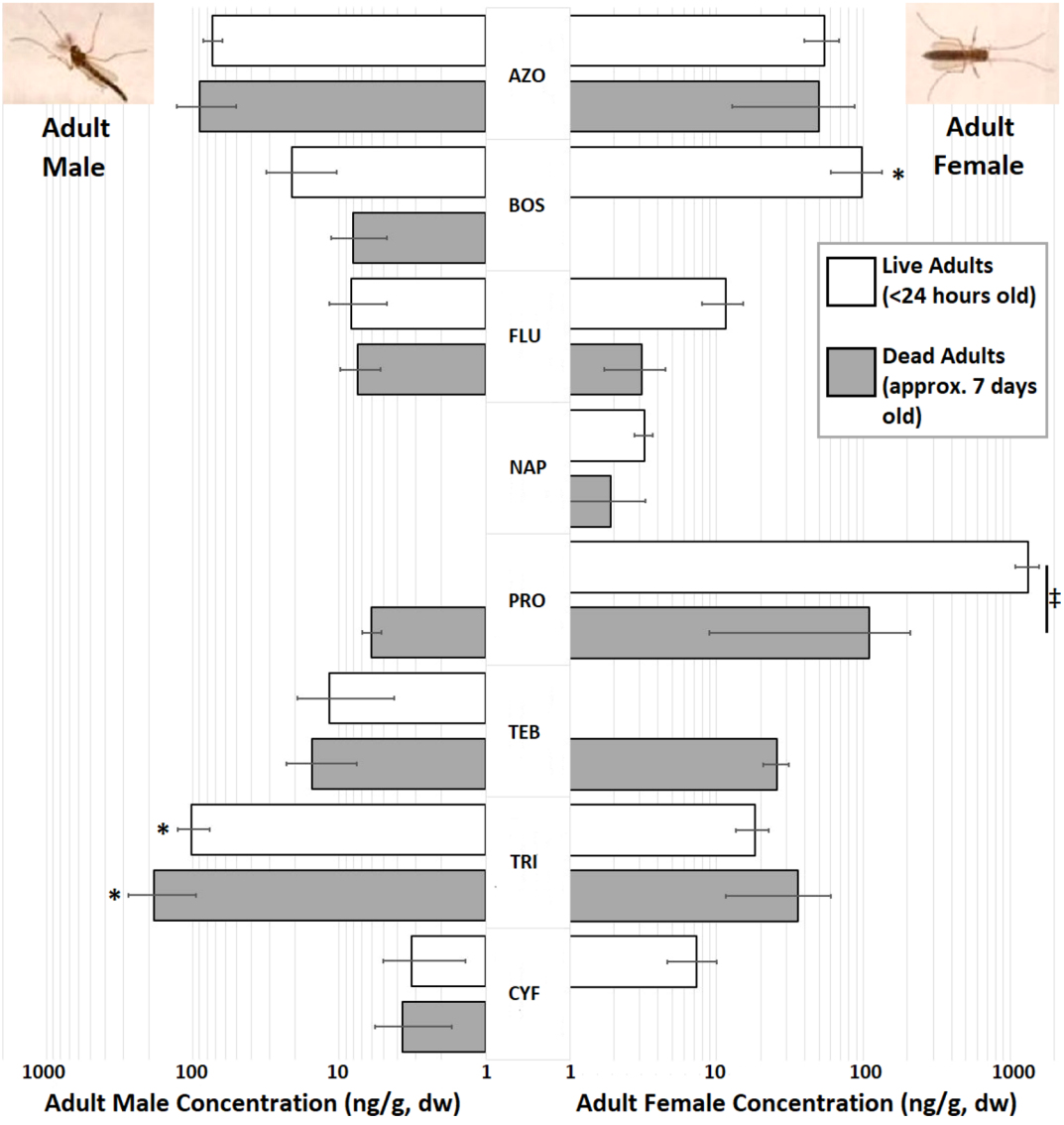

Figure 3. Average (n = 4) sex-specific pesticide concentrations in live (white bars) and dead (gray bars) adult male and female midges that emerged after exposure as larvae to the medium pesticide treatment level. Error bars indicate the standard deviation. Asterisks indicate a sex-specific significant difference between the adults from the same life stage (Mann−Whitney U test, $p < 0.05$). The symbol ‡ indicates significant differences between life stages of the same sex (paired Wilcoxon signed-rank test, $p < 0.05$). The figure uses a base-10 log scale for the x-axis. Pesticides are arranged from top to bottom in order of increasing lipophilicity. AZO = azoxystrobin, BOS = boscalid, FLU = fluopyram, NAP = napropamide, PRO = propyzamide, TEB = tebuconazole, TRI = trifloxystrobin, and CYF = cyflufenamid.

role, affecting the relationship between the persistence of these contaminants and their lipophilicity. This would provide a potential explanation for the difference in the strengths of the correlation between persistence and logK_{ow} for the two groups of chemicals as well (i.e., uniformity of the contaminant molecular structure improves the correlation by affecting the rate of metabolism). Deviations from the linear correlation between the pesticide RCFs and logK_{ow}s may occur as a result of differing expression profiles of detoxification enzymes, which are known to be distinct between the larval, pupal, and adult life stages of dipterans.[52] For example, relatively slower compound-specific metabolism could explain the similar propensity for retention of the two strobilurins, azoxystrobin and trifloxystrobin, which deviate from a linear trend with lipophilicity in our results but have similar molecular structures and contain chemical moieties, which are unique in the group of pesticides tested. In addition to metabolism, the excretion of contaminants as part of the exuviae or meconium during pupation has the potential to affect the pesticide concentrations in the adults, as has been observed for heavy metals and several organic contaminants in holometabolous insects from various orders.[17,30] The metabolism of pesticides in aquatic insect larvae has received some attention for its relevance to bioconcentration, bioaccumulation, and toxicity to these organisms.[27] The investigation of contaminant metabolism during the metamorphosis of aquatic insects has however received far less attention. Despite this, measurements of HOP concentrations (including organochlorine pesticides, PCBs, and flame retardants) in the developing and adult stages of aquatic and terrestrial insects from a range of taxa have provided evidence for common metabolic pathways of detoxification during metamorphosis regardless of whether species are hemimetabolous, holometabolous, or paurometabolous.[53] More detailed investigation of the effects of

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Environ. Sci. Technol. XXXX, XXX, XXX−XXX

metabolism on contaminant concentrations during metamorphosis of aquatic insects from different orders is required to further investigate the potential for a diverse range of pesticides to be transported between ecosystems via this vector.

Sex-Specific Effects on Pesticide Retention. Greater concentrations of boscalid, napropamide, cyflufenamid, and propyzamide were retained by live collected (<24 h old) females than the respective males after emergence (Table S12). The differences between sexes were consistent for all treatment levels in which pesticide concentrations were measured above their respective LOQs and were significant in the high treatment level when measured in both sexes (Mann−Whitney U test, $p < 0.05$). The sex-specific difference was between a factor of 2 and 6 for the first three pesticides and the most pronounced for propyzamide, for which the females contained approximately 270 times greater concentrations than the males (high exposure treatment, Table S12). The bioamplification of propyzamide in females thus offsets the very low retention in males, resulting in the equality of average RCFs in both sexes (Figure 2). Azoxystrobin and trifloxystrobin concentrations were consistently greater in males than in females, regardless of the treatment level. This was, however, only significant for trifloxystrobin, which had approximately five times greater concentrations. Comparison of the sex-specific differences in the concentrations of tebuconazole was inconclusive due to inconsistency between treatments. Measurable concentrations of tebuconazole were only observed in males from the low and medium treatment levels, but higher concentrations were observed in the females compared to the males in the high treatment level. The effect of emergent aquatic insect sex on contaminant retention through metamorphosis has not been well documented for a wide range of contaminants. Bioamplification of contaminant concentrations, which results from weight loss during pupation, could be greater in male insects when compared to females. This hypothesis is based on the retention of PCBs, for which biota-sediment accumulation factors are greater in male *Chironomus* spp.[54] Similarly, males of a terrestrial lepidopteran had greater concentrations of HOPs after dietary exposure as larvae.[30] Our results, however, reveal a more complex pattern for the pesticides used in our study, indicating that sexual dimorphism during larval development and pupation (i.e., reduction in adult weight relative to the final larvae stage) cannot fully explain the sex-specific differences in pesticide concentrations of emergent adults.[31] Additional factors could therefore be considered; for example, a longer duration of larval development for females may result in increased exposure and correspondingly higher uptake of certain pesticides.[55] Finally, sex-specific differences in pesticide metabolism may further modify the concentrations of accumulated pesticides.[56]

Pesticide Concentrations in Newly Emerged versus Postmating Adults. After completion of the terrestrial life stage, the concentrations of propyzamide in female midges had decreased by a factor of 8−12 (Figure 3 and Table S12, Paired Wilcoxon signed-rank test, $p < 0.05$). Similarly, reduction of boscalid and cyflufenamid during the terrestrial life stage resulted in no detectable concentrations of these pesticides in dead females at any treatment level. A trend toward a decreased concentration of fluopyram was observed in the medium treatment level, but this was not confirmed by the results of the lowest treatment level, where the concentration did not change with the life stage (Table S12). Pesticide concentrations in males were generally not reduced during the terrestrial life stage, with the exception of a trend toward lower boscalid concentrations. Furthermore, the concentrations of azoxystrobin and napropamide were similar between life stages for both sexes.

Increases in the concentrations of propyzamide and tebuconazole in males and females, respectively, as well as a trend toward an approximate twofold increase in the trifloxystrobin concentration in both sexes can be explained by bioamplification arising due to body mass loss coupled with limited pesticide loss over the course of the terrestrial life stage. Adult midges that were transferred to mating cages after emergence lived for approximately 6−7 days (Table S5) during which time the average dry weights of males and females decreased by approximately 28 and 55%, respectively. The general trend toward decreases in pesticide concentrations in female insects suggests the potential for sex- and compound-specific loss through depuration, metabolism, or oviposition over the course of the terrestrial lifespan. Across all treatments, 0.9 ± 0.3 egg masses per female ($n = 876$) were collected, implying that the majority of females had successfully oviposited during the terrestrial life stage. Pesticide loss through oviposition would potentially result in the trans-generational transport of the pesticides in midges. Similar maternal transfers of organic contaminants via egg masses are known from other organisms, such as fish, frogs, and terrestrial moths.[30,57−59] Furthermore, this route of contaminant loss is supported considering the general trend toward retention or bioamplification of the same pesticides in males.

More generally, the results from both sexes imply that the potential dietary exposure of terrestrial predators is persistent for many of the pesticides over the duration of the terrestrial lifespan of the adult midges serving as prey. This may result in a greater risk of dietary exposure for the terrestrial food web considering the prophylactic application of especially fungicides, the broad window of aquatic insect emergence (especially for multivoltine species, such as midges), and the potentially wide dispersal of emergent insects from aquatic sources.[4,60,61] Additionally, the observed female contributions to total emergent dry weight were approximately double that of the males in the current study (Table S5), which may therefore increase the exposure risk of terrestrial predators to specific pesticides (i.e., propyzamide). Overall, our results further highlight the potential relevance of including sex-specific analyses when evaluating the compound-specific exposure risk of terrestrial food webs via insect-mediated contaminant transfer.

Potential Effects on Terrestrial Insectivores. Based on the concentrations of pesticides in the live adults from the lowest treatment level and published emergent biomass of midges from agricultural streams, we estimated the annual mass flux of the pesticides used in the current study, from aquatic to terrestrial ecosystems, to be 10.4−94.0 ng/m^2·year (Table S10).[61,62] The results from the low treatment level were used for this estimation because the chronic pesticide exposure in freshwater environments generally takes place at low concentrations (Table S2). The estimated value is however considerably lower than what has been observed in field studies, where estimates of daily fluxes of insecticides from wetlands are between 0.4 and 26.8 ng/m^2·d, when also including other aquatic insect orders.[9] The specific contributions of midges in this context are, however, of relevance considering that Chironomidae are the most abundant and have the longest emergence window among the common

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Environ. Sci. Technol. XXXX, XXX, XXX−XXX

emerging insect orders, thus making them an important nutrient source in terrestrial food webs.[61,63] Moreover, aquatic insect communities in polluted aquatic environments often show a shift toward dipterans dominating the remaining insect fauna due to their tolerance of a wide range of anthropogenic disturbances, for example, in aquatic environments disturbed by agriculture.[64] More generally, our results show that midge-mediated transport of current-use organic pesticides from aquatic to terrestrial ecosystems takes place at environmentally relevant pesticide concentrations. Current-use organic pesticides therefore add to the growing list of anthropogenic substances contained in emergent aquatic insect subsidies.

In order to provide a preliminary estimate of the dietary exposure of potentially impacted terrestrial insectivores, we calculated daily dietary pesticide intake based on published feeding rates for two common predators of midges, namely, tree swallow nestlings (*Tachycineta bicolor*) and two species of bats, *Myotis daubentonii* and *Myotis lucifugus* (Table S11).[65−67] The tree swallow nestling daily dietary pesticide intake was estimated to be between 1.3 ± 0.3 and 24.0 ± 5.0 μg/kg·d. Similarly, the daily dietary intake of pesticides by bats was calculated to be 4.9 ± 1.0−20.3 ± 4.3 and 9.7 ± 2.0−18.2 ± 3.8 μg/kg·d for male and female bats, respectively. The estimated dietary exposure rates for pesticides are the same order of magnitude as what has been estimated for pharmaceuticals and endocrine-disrupting compounds through consumption of emerging aquatic insects by birds and bats.[13] The actual dietary exposure risk is dependent on more factors, for example, the bioavailability of the pesticides from consumed insects. Nevertheless, current-use pesticides add to the diverse range of organic contaminants already known to be in the diets of tree swallows and insectivorous bats, some of which can already be present at high concentrations.[54,68,69] Research on the potential sublethal effects of dietary exposure to contaminant mixtures containing the pesticides used in the current study in nontarget organisms is still in its infancy. However, endocrine-disrupting effects in mammals and amphibians are known for tebuconazole and trifloxystrobin, respectively.[69,70] The contamination of insect subsidies with organic pesticides may therefore have still unknown negative consequences for sensitive life stages or species of insectivores, among which are bird nestlings and threatened bats. Considering the declines in bird and bat populations coinciding with increased pesticide use in recent decades, a better understanding of the potential dietary exposure to organic pesticides is of great relevance.[71,72]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.1c08079.

Chemical and reagent suppliers, physicochemical properties and examples of measured field concentrations of the selected pesticides, emergence dynamics summary, instrumental analysis parameters, validation and quality control, pesticide TWAC calculation, potential effects on terrestrial predators' calculations, pesticide concentrations in the test medium and pesticide concentrations in larvae and adults from each treatment level (PDF)

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Author Contributions

All authors designed the study. A.P.R., N.R., S.P., and S.K. carried out the experimental work. A.P.R. performed the pesticide measurements and data analyses. A.P.R. wrote the manuscript. K.S., M.B., and R.S. supervised the study. All authors revised and contributed to the final manuscript.

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Notes

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Emerging midges transport pesticides from aquatic to terrestrial ecosystems: importance of compound- and organism-specific parameters

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21 pages, 12 Tables, 5 figures

Table S1. Pesticide formulation products used in microcosm experiments.

Pesticide	Formulation Product Name	Formulation Product Type	Supplier
Azoxystrobin	Ortiva®	Suspension concentrate	Syngenta (Maintal, Germany)
Boscalid	Cantus®	Water dispersible granule	BASF Agrar (Ludwigshafen, Germany)
Cyflufenamid	Vegas®	Oil in water emulsion	Certis Europe B.V. (Hamburg, Germany)
Fluopyram / Tebuconazole	Luna Experience®	Suspension concentrate	Bayer Crop Science (Langenfeld, Germany)
Propyzamide	Kerb Flo®	Suspension concentrate	Dow AgroSciences (Munich, Germany)
Pyrimethanil	Pyrus®	Suspension concentrate	Arysta LifeScience (Düsseldorf, Germany)
Trifloxystrobin	Flint®	Water dispersible granule	Bayer Crop Science (Langenfeld, Germany)

Chemical and reagent suppliers. Pure active ingredients for all the pesticides were obtained from LGC Standards (Wesel, Germany). Solvents (LC-MS Grade) were purchased from Honeywell (Seelze, Germany). All other chemical reagents (>99% purity) and clay were purchased from Carl Roth (Karlsruhe, Germany). Aquarium sand and peat were obtained from Schicker mineral (Bad Berneck, Germany) and Floragard (Oldenburg, Germany) respectively.

Table S2. Physicochemical properties and examples of measured field concentrations of the pesticides used in the current study.

Pesticide name and abbreviation	Molecular mass (Da)	Log K_{ow}*	Aqueous solubility at 20°C (mg/L)*	Reported Environmental Concentrations (µg/L)	Sampling Site Description	Reference
Azoxystrobin (AZO)	403	2.5	6.7	29.7	Stream water during runoff event	1
				1.8	Stream water during rainfall event	2
				3	Edge-of-field runoff	2
				0.51	Stream water	3
Pyrimethanil (PYR)	199	2.8	110	1.3	River water	4
				4.4	Stream water during rainfall event	2
				5.9	Edge-of-field runoff	2
Boscalid (BOS)	343	3	4.6	0.72	Stream water	3
				1.3	River water	4
				24	Stream water during rainfall event	2
				17	Edge-of-field runoff	2
Fluopyram (FLU)	397	3.3	16	No reference found	-	-
Napropamide (NAP)	271	3.4	74	0.0055	WWTP effluent	5
				0.02	WWTP effluent	6

Table S2 Continued.

Propyzamide (PRO)	256	3.5	9	0.43	Stream water	3
				4.9	River water	4
Tebuconazole (TEB)	308	3.7	36	0.5	WWTP effluent	7
				0.24	Stream water	3
				11.49	Vegetated ditches and ponds	8
				9.1	Stream water during runoff event	1
				81	Edge-of-field runoff	9
Trifloxystrobin (TRI)	408	4.5	0.61	0.05	Vegetated ditches and ponds	8
				3.8	Edge-of-field runoff	2
Cyflufenamid (CYF)	412	4.7	0.52	0.05	Stream water	10

*Source: Pesticide properties database (PPDB), Agriculture & Environment Research Unit (AERU) at the University of Hertfordshire. (available at: https://sitem.herts.ac.uk/aeru/ppdb/). WWTP – Waste water treatment plant.

Table S3. Pesticide stock solution and treatment level concentrations.

Formulation product	Active substance, label concentration in parentheses	Stock Solution Measured Concentration (µg/L)*	Low treatment concentration (µg/L)	Medium treatment concentration (µg/L)	High treatment concentration (µg/L)
Ortiva®	Azoxystrobin (250 g/L)	400	1.43	20.00	57.14
Pyrus®	Pyrimethanil (400 g/L)	510	1.82	25.50	72.86
Cantus®	Boscalid (500 g/kg)	702	2.51	35.10	100.29
Luna Experience®	Fluopyram (200 g/L)	386	1.38	19.30	55.14
-	Napropamide‡	700	2.5	35	100
Luna Experience®	Tebuconazole (200 g/L)	366	1.31	18.30	52.29
Flint®	Trifloxystrobin (500g/kg)	468	1.67	23.40	66.86
Vegas®	Cyflufenamid (51.3 g/L)	350	1.25	17.50	50.00
Kerb Flo®	Propyzamide (400 g/L)	528	1.89	26.40	75.43

*A solution, containing all nine pesticides, with a nominal concentration of 700 µg/L was prepared in SAM-5S medium. ‡Napropamide was used as a pure substance.

Table S4. Summary of weekly temperature, conductivity, dissolved oxygen and pH measurements of larval and emergence microcosms.

Parameter:	Total number of measurements:	Average ± standard deviation of measured values:
Temperature (°C)	40	21 ± 0.65
Specific conductivity (µS/cm)	37	884 ± 154
Dissolved oxygen (mg/L)	29	5.9 ± 1.2
pH	44	7.43 ± 0.33

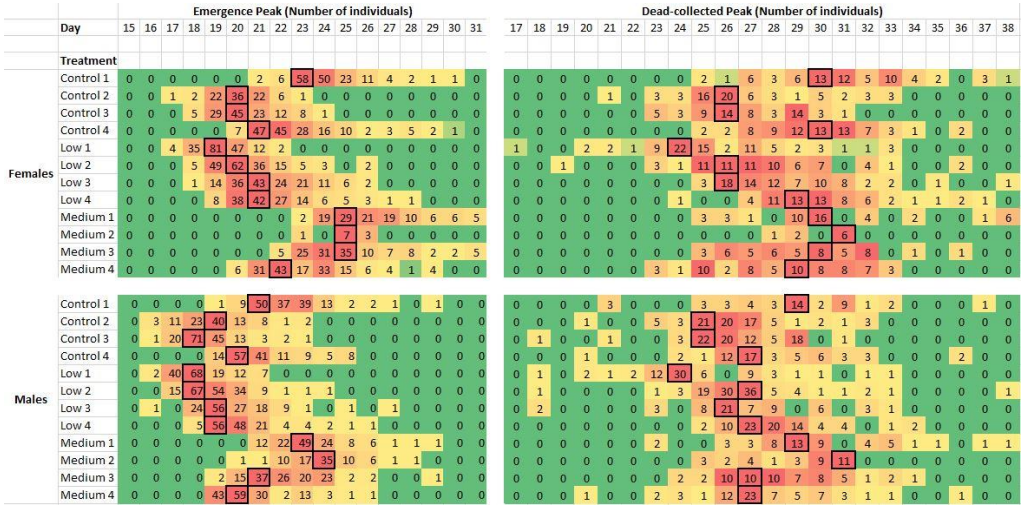

Figure S1. Heat map indicating the number of emerged adult insects and the number of dead collected insects each day for each sex and in each treatment replicate. Black boxes indicate the days used in the calculation of the sex-specific adult lifespans.

Table S5. Average (± standard deviation) of the total emergence, sex-specific emergence, sex-specific dry weight, sex-specific lifespan, sex ratio as percentage females and number of egg-masses collected per female in the mating cages.

Treatment	Total Emergence (individuals)	Sex Ratio (Percentage Females, %)*	Egg-masses per female	Males			Females		
				Total Emergence (individuals)	Dry weight (mg, dw)	Life-span (Days)‡	Total Emergence (individuals)	Dry weight (mg, dw)	Life-span (Days)‡
Control	273.5 ± 57.1	49	0.8 ± 0.4	139.3 ± 26.0	34.8 ± 6.5	7.0 ± 0.8	134.3 ± 34.9	67.2 ± 17.5	7.0 ± 1.4
Low	317.8 ± 32.9	52	1.0 ± 0.3	152.5 ± 20.1	38.1 ± 5.0	7.5 ± 1.3	165.3 ± 16.8	83.0 ± 8.4	6.0 ± 1.4
Medium	226.0 ± 93.7	46	0.9 ± 0.3	121.5 ± 29.1	30.4 ± 7.3	6.5 ± 0.6	104.5 ± 64.9	53.0 ± 33	6.3 ± 0.9
High	211.7 ± 91.0	53	-	99.7 ± 55.0	24.9 ± 13.8	-	112.0 ± 37.0	56 ± 18.5	-

‡Determined by the replicate-specific differences in dates of peak emergence and dead collected individuals.
*Calculation based on average emergence. Treatment dependent effects on total emergence and average adult lifespan were not significant (Kruskal-Wallis H test, $p > 0.05$).

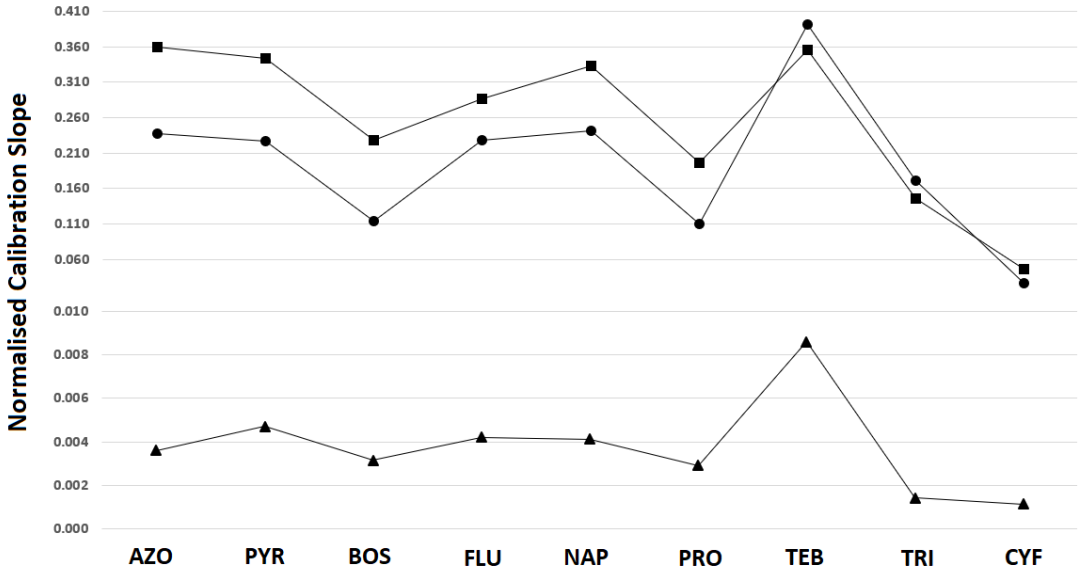

Figure S2. LC-MS/MS instrument response sensitivities of the selected pesticides prepared in insect matrices. Calibration gradients prepared in insect matrices were normalised to the respective gradient in pure solvent (H_2O/MeOH, 70:30). The matrix matched calibration curves were prepared in midge larva (triangles), adult male (squares) and adult female (circles) matrices. Pesticides are arranged from left to right in order of increasing lipophilicity. The y-axis is broken between 0.010 and 0.060.

Matrix effects in pesticide measurements. Consideration of matrix effects, such as ion suppression, is an important aspect for accurate quantitative comparisons of measurements made for analytes in different matrices when applying electrospray ionisation coupled with mass spectrometry (ESI-MS/MS).[11] The pesticide specific gradients of the linear calibration curves prepared in solvent (H_2O/MeOH, 70:30), larvae, adult males and adult females were compared in order to determine the extent of analyte-specific matrix effects in each sample matrix (Figure S4). The larvae matrix had the strongest effect, reducing the signals for all the analytes. Adult female matrix had the next strongest effect, which reduced the responses for six of the nine pesticides when compared to the results in adult male matrix.

Table S6. Instrument parameters for UHPLC-MS/MS pesticide analysis

Liquid chromatography (UHPLC*) parameters:	
Instrument:	Agilent 1260 Infinity II HPLC System
Column:	ZORBAX Eclipse Plus C18 (2.1 ID x 50 mm, 1.8 micron)
Eluent A:	H_2O/MeOH (98:2), 0.1% Formic acid, 4 mM Ammonium formate
Eluent B:	H_2O/MeOH (2:98), 0.1% Formic acid, 4 mM Ammonium formate
Injection volume for insect samples:	90 µL
Injection volume for water samples:	5 µL
Flow rate:	0.35 mL/min
Column temperature:	50°C
Mass Spectrometry (MS/MS**) parameters:	
Instrument:	Agilent 6495 Triple Quadrupole Mass Spectrometer
Capillary voltage:	3000 V
Nozzle voltage:	0 V
Gas flow:	11 L/min
Gas temperature:	250 °C
Sheath gas flow:	12 L/min
Sheath gas temperature:	350 °C
Nebulizer pressure:	38 psi

*Ultrahigh Performance Liquid Chromatography, **Tandem Mass Spectrometry

Table S7. Pesticide retention times and MRM transitions

Pesticide	Retention Time (Min)	Quantifier MRM*	Qualifier MRMs*
Azoxystrobin	2.6	404.1 -> 372.1 (8)	404.1 -> 344.1 (24) 404.1 -> 329.1 (32)
Boscalid	3.0	343.0 -> 307.1 (16)	343.0 -> 272.1 (32) 343.0 -> 271.2 (32)
Cyflufenamid	6.5	413.1 -> 295.1 (10)	413.1 -> 359.1 (10) 413.1 -> 223.0 (20)
Fluopyram	4.0	397.0 -> 145.0 (61)	397.0 -> 207.9 (37) 397.0 -> 172.9 (41)
Napropamide	4.3	272.2 -> 129.0 (12)	272.2 -> 171.1 (16) 272.2 -> 58.1 (26)
Propyzamide	3.3	256.0 -> 190.0 (10)	256.0 -> 173.0 (20) 256.0 -> 145.0 (36)
Pyrimethanil	2.6	200.1 -> 82.0 (25)	200.1 -> 106.9 (20) 200.1 -> 77.1 (40)
Tebuconazole	5.8	308.1 -> 70.0 (40)	308.1 -> 124.9 (47) 308.1 -> 57.1 (28)
Trifloxystrobin	6.9	409.1 -> 186.0 (12)	409.1 -> 206.1 (8) 409.1 -> 145.0 (52) 409.1 -> 116.0 (24)

*MRM – Multiple-Reaction-Monitoring transition with collision energy in parentheses. All analytes were measured in positive ionization mode.

Determination of pesticide quantification limits. The calibration curves of the pesticides in matrix matched standards were found to be linear over the measured ranges, 0.125 – 1000 ng/g in insects and 0.05 – 30 µg/L in medium samples. At least two MRMs were used for each pesticide, with qualifier MRMs having a ratio of 80-120% relative to the quantifier. Limits of quantification (LOQs) were determined for each pesticide based on an analyte signal-to-noise ratio (S/N) greater than 10 (Table S8). In addition, five replicate

samples of each insect matrix, from uncontaminated cultures, were fortified at a concentration near to the LOQ, dried and extracted. The analysis of these fortified samples provided information on the method recoveries and the relative standard deviations of the pesticide signals (Table S8).

Table S8. Pesticide limits of quantification (LOQs) in insect matrices and medium.

Pesticide	LOQ in larvae (ng/g, dw)	LOQ in adult males (ng/g, dw)	LOQ in adult females (ng/g, dw)	LOQ in medium samples (µg/L)
Azoxystrobin	0.25	0.5	0.25	0.1
Pyrimethanil	2.5	5	5	0.1
Boscalid	5	5	5	0.15
Fluopyram	0.5	0.5	1	0.1
Napropamide	0.25	0.5	0.25	0.1
Tebuconazole	0.5	0.5	1	0.1
Propyzamide	0.5	1	2.5	0.1
Trifloxystrobin	0.5	0.5	0.25	0.05
Cyflufenamid	2.5	0.5	5	0.1

Table S9. Pesticide fortification concentrations, average recoveries and percentage signal relative standard deviations (RSDs) in insect matrices.

Pesticide	Larvae			Adult Males			Adult Females		
	Fortified Concentration, (ng/g, dw)	Recovery, n = 5 (%)	RSD (%)	Fortified Concentration, (ng/g, dw)	Recovery, n = 5 (%)	RSD (%)	Fortified Concentration, (ng/g, dw)	Recovery, n = 5 (%)	RSD (%)
Azoxystrobin	0.25	98.1	6.7	0.5	110.3	1.9	1.0	118.8	2.4
Pyrimethanil	1.0	16.1	20.0	5.0	69.9	7.2	5.0	115.6	3.2
Boscalid	1.0	60.5	11.6	5.0	105.2	9.9	5.0	107.9	3.7
Fluopyram	0.5	84.8	6.4	0.25	80.9	7.6	1.0	117	1.5
Napropamide	0.25	115.3	0.8	0.5	118.7	6.7	0.5	111.7	3.9
Tebuconazole	0.25	120.7	16.0	5.0	57.9	9.8	1.0	98.4	13.7
Propyzamide	0.25	108.4	9.2	1.0	116.6	5.8	1.0	122.6	6.7
Trifloxystrobin	0.5	110.7	9.0	0.5	98.6	6.3	1.0	121.4	6.9
Cyflufenamid	0.5	96	7.5	0.5	88.4	4.42	5	95.2	10.8

Pesticide exposure time weighted average concentrations (TWACs). Treatment level specific TWACs were calculated for larvae and emergence microcosms separately based on measurements of the temporal decline of pesticide concentrations in the overlying medium. Medium concentrations were drawn on days d0, d8 and d14 or on days d0, d14 and d20 for larvae and emergence microcosms respectively. The pesticide concentrations in the treatment replicate (n = 4) were used to create a linear regression describing the pesticide concentration decline over time in each treatment level. The coefficient of determination (R^2) was greater than 0.9 for all treatment levels in both microcosm types. It is assumed that an equilibrium in the distribution of pesticides between different compartments (sediment, aqueous phase, dissolved organic matter, etc.) was reached during the 24-hour equilibration time before the start of the experiment. Sediment containing organic matter is known to act as a reservoir of contaminants in aquatic systems. Degradation of contaminants in the overlying aqueous phase by photodegradation or oxidation therefore results in a continuous re-establishment of this equilibrium partitioning of the pesticides between the sediment and the overlying aqueous phase throughout the experiment. The equation of the linear regression was thus used in combination with the measured pesticide concentrations in the stock solution (Table S3) to estimate the total concentration of

pesticides distributed throughout the system at the final timepoint. The TWAC was calculated as the average of the concentrations at the first and last time points for each microcosm type.

Potential effects on terrestrial predators - emergence mediated pesticide transfer. The total annual dry weight of Chironomidae emerging from agricultural streams in western France has been observed to range between 200 and 2200 mg/m^2.[12] Similarly, the annual dry weight of emergence of Chironomidae from an agricultural stream in northern Germany was recorded to be between 559 and 1272 mg/m^2.[13] Based on the extremes of these reference values for annual emergence, in combination with the ratio of sex-specific dry weight contributions observed in this study (Table S5), the annual insect mediated transfer of the pesticides based on the lowest, and most environmentally relevant, treatment level was estimated (Table S10).

Table S10. Estimation of the average (± standard deviation) mass flux of annual chironomidae mediated pesticide transport from agricultural streams.

Pesticide	Insect mediated pesticide transport ng/m^2·year		
	Males	Females	Average of both sexes
Azoxystrobin	0.31 ± 0.10 – 3.4 ± 1.12	0.4 ± 0.16 – 4.8 ± 1.70	0.36 – 4.1
Pyrimethanil	-	-	-
Boscalid	-	1.2 ± 0.42 – 13.2 ± 4.58	0.6 – 7.6
Fluopyram	0.13 ± 0.06 – 1.4 ± 0.70	0.2 ± 0.06 – 2.2 ± 0.58	0.7 – 1.8
Napropamide	-	-	-
Propyzamide	-	13.3 ± 3.48 – 146.2 ± 38.30	7.7 – 73.1
Tebuconazole	0.22 ± 0.05 – 2.36 ± 0.42	-	0.1 – 2.2
Trifloxystrobin	0.65 ± 0.21 – 7.13 ± 2.29	0.4 ± 0.12 – 3.2 ± 1.2	0.9 – 5.2
Cyflufenamid	-	-	-
Total Average:	1.3 – 21.5	15.5 – 169.6	10.4 – 94.0

Estimate of daily dietary exposure of Tree swallow (*Tachycineta bicolor*) nestlings to pesticides. During the summer breeding season, we assume that adult tree swallows are able to forage for 14 hours per day. Field

observations of tree swallows with access to both aquatic and terrestrial prey, and having an average clutch size (four nestlings), found that male and female birds made approximately 56 foraging trips per nestling per day.[14] Similarly, adults from a lakeside colony made approximately 32 foraging trips per nestling per day.[15] Fledglings in the latter study had an approximate body weight of 24 g at 16 days of age. Collection of 71 insect boluses containing midges from riverside colonies found 2187 individuals in total.[16] Assuming that male and female insects were caught in equal numbers, we estimated (based on average male and female dry masses of 0.25 and 0.5 mg respectively) the average dry weight of midges per bolus to be 11.5 mg. This value is consistent with the reported range of midge dry weights, 11.6 ± 1.7 – 122 ± 1.1 mg, in samples collected from lakeside colonies.[17] Daily exposure was therefore calculated for a 24 g nestling based on the extremes of the feeding frequencies (32 – 56 trips per nestling per day) and midge content of the delivered boluses (11.5 – 122 mg total dry weight) from the literature, in combination with the pesticide concentrations in the newly emerged adults from the low exposure treatment level in our study (Table S11).

Table S11. Estimated daily dietary pesticide exposures for tree swallow nestlings.

Pesticide	Pesticide exposure (ng) at 368 mg (dw) midges consumed per day	Pesticide exposure (ng) at 6832 mg (dw) midges consumed per day
Azoxystrobin	1.4 ± 0.3	25.8 ± 6.4
Pyrimethanil	-	-
Boscalid	2.2 ± 0.8	41.5 ± 14.4
Fluopyram	0.6 ± 0.2	11.0 ± 2.9
Napropamide	-	-
Propyzamide	24.7 ± 6.5	458.9 ± 120.2
Tebuconazole	-	-
Trifloxystrobin	1.8 ± 0.4	32.5 ± 8.2
Cyflufenamid	-	-
Total:	31.0 ± 6.5	577.1 ± 121.5

Estimate of daily dietary pesticide exposure of insectivorous bats. Female and male bats feeding rates are known to vary depending on energy requirements, for example during pregnancy. Estimates of feeding rate are also dependent on the assumed success rate of quantified feeding attempts. We took the extremes of the published values for daily feeding rates, namely 0.46 – 0.86 g and 0.23 – 0.96 g insect material (wet weight) per gram body weight for male and female bats respectively.[18,19] The conversion of insect wet weight to dry weight was based on the results of the current study, where dry weights of adult insects were an average of 25% of the respective wet weights.

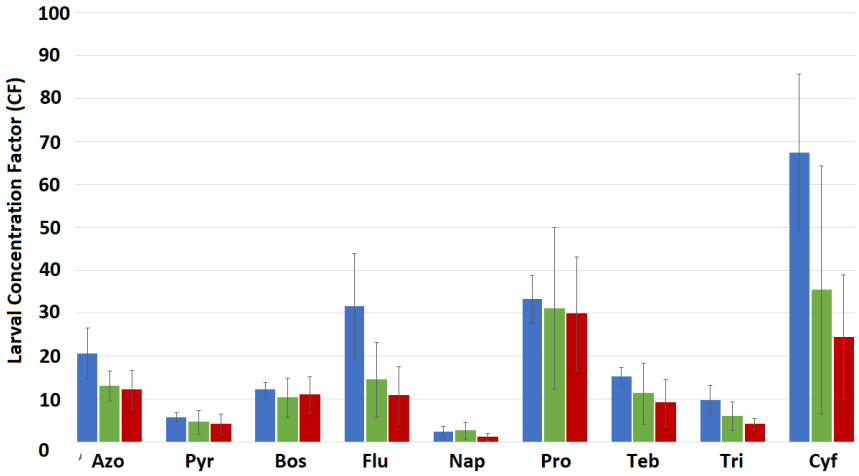

Figure S3. Average (n = 4) pesticide specific larval Concentration Factors (CFs), in order of increasing treatment level (low – blue, medium – green and high – red) for each of nine pesticides. Error bars represent the standard deviations. Pesticides are arranged from left to right in order of increasing $\log K_{ow}$.

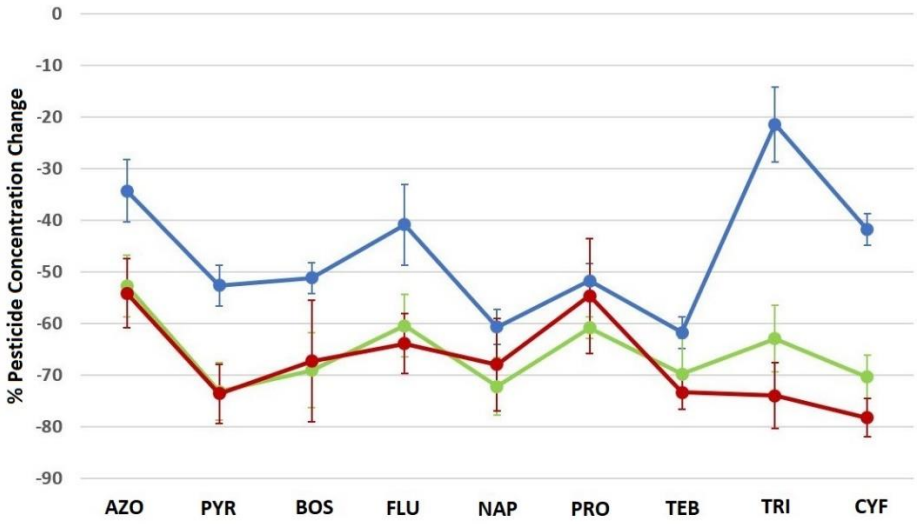

Figure S4. Treatment level-specific percentage changes in average (n = 4) pesticide concentrations in medium from larval microcosms after 14 days. Treatments levels are indicated with different colours, i.e. low (blue), medium (green) and high (red). Percentage change is calculated using the difference between the concentrations on day 14 and day 0. Pesticides are arranged from left to right in order of increasing $\log K_{ow}$. Error bars indicate the standard deviation.

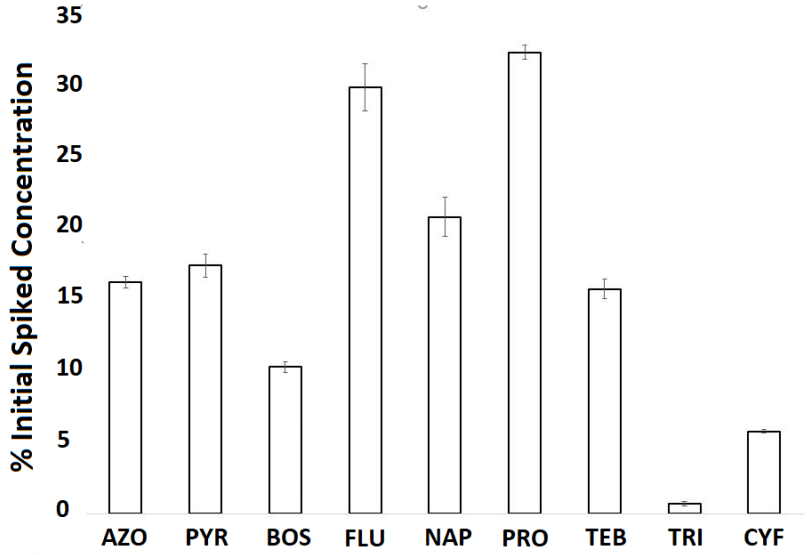

Figure S5. Percentage of average (n = 8, medium and high treatments) total pesticide concentration measured in the medium of larval cultures after 24 hours of equilibration with sediment. Error bars indicate the standard deviation. Pesticides are arranged from left to right in order of increasing $\log K_{ow}$.

Table S12. Average (± standard deviation) measured pesticide concentration in larvae, live and dead collected adults and pesticide time weighted average exposure concentrations (TWACs).

Pesticide	Larvae low treatment level		Emergent adults from low treatment level				
	TWAC (µg/L)	Larvae pesticide concentration (ng/g, dw) (n =4)	TWAC (µg/L)	Adult insect pesticide concentrations (ng/g, dw)			
				Live males (n = 4)	Dead males (n = 4)	Live females (n = 4)	Dead females (n = 4)
Azoxystrobin	0.61	12.7 ± 3.7	0.61	4.6 ± 1.6	6.6 ± 3.1	3.4 ± 1.2	3.1 ± 2.2
Pyrimethanil	0.83	4.8 ± 0.9	0.77	ND	ND	ND	ND
Boscalid	2.26	27.8 ± 3.6	1.17	<LOQ	<LOQ	9.1 ± 3.2	ND
Fluopyram	0.53	16.8 ± 6.5	0.55	1.9 ± 1.0	5.6 ± 1.5	1.5 ± 0.4	2.4 ± 0.8
Napropamide	1.14	2.8 ± 1.4	1.13	<LOQ	<LOQ	<LOQ	<LOQ
Propyzamide	0.78	26.6 ± 4.4	0.77	<LOQ	<LOQ	100.7 ± 26.4	13.1 ± 3.5
Tebuconazole	0.61	9.3 ± 1.3	0.56	3.3 ± 0.6	10.1 ± 5.5	ND	ND
Trifloxystrobin	1.78	17.5 ± 6.0	1.81	9.8 ± 3.2	27.8 ± 12.0	2.2 ± 0.9	3.0 ± 1.7
Cyflufenamid	0.58	38.9 ± 10.6	0.63	<LOQ	5.5 ± 2.6	<LOQ	ND

Table S12 continued.

Pesticide	Larvae medium treatment level		Emergent adults from medium exposure level				
	TWAC (µg/L)	Larvae pesticide concentration (ng/g, dw) (n =4)	TWAC (µg/L)	Adult insect pesticide concentrations (ng/g, dw)			
				Live males (n = 4)	Dead males (n = 4)	Live females (n = 4)	Dead females (n = 4)
Azoxystrobin	9.24	120.6 ± 32.3	9.05	73.9 ± 11.1	89.9 ± 39.2	54.1 ± 14.70	50.0 ± 37.2
Pyrimethanil	12.24	56.7 ± 33.5	12.40	ND	ND	ND	ND
Boscalid	32.20	332.2 ± 149.0	16.32	21.1 ± 10.7	8.0 ± 3.3	97.3 ± 36.8	ND
Fluopyram	8.52	124.1 ± 74.2	8.03	8.2 ± 3.5	7.53 ± 2.3	11.5 ± 3.5	3.1 ± 1.4
Napropamide	16.52	44.0 ± 32.0	15.90	<LOQ	<LOQ	3.2 ± 0.5	1.9 ± 1.4
Propyzamide	11.60	361.5 ± 218.4	10.60	<LOQ	<LOQ	1331.8 ± 237.3	109.0 ± 109.0
Tebuconazole	8.77	99.6 ± 62.3	7.23	11.7 ± 7.5	15.4 ± 7.7	<LOQ	<LOQ
Trifloxystrobin	25.30	153.7 ± 83.7	22.71	102.4 ± 25.6	185.5 ± 90.4	18.2 ± 4.6	35.6 ± 24.1
Cyflufenamid	8.62	305.2 ± 248.7	8.75	3.2 ± 1.8	3.7 ± 2.01	7.3 ± 2.7	ND

Table S12 continued.

Pesticide	Larvae high exposure level		Emergent adults from high exposure level		
	TWAC (µg/L)	Larvae pesticide concentration (ng/g, dw) (n =4)	TWAC (µg/L)	Adult insect pesticide concentrations (ng/g, dw)	
				Live males (n = 3)	Live females (n = 3)
Azoxystrobin	26.52	321.9 ± 119.8	24.45	253 ± 63.7	172.1 ± 74.3
Pyrimethanil	34.94	148.8 ± 81.3	34.40	<LOQ	<LOQ
Boscalid	91.92	1018.5 ± 381.6	93.28	36.1 ± 9.2	243.1 ± 52.1
Fluopyram	24.97	271.7 ± 166.3	22.08	9.8 ± 1.4	27.0 ± 13.7
Napropamide	47.11	54.6 ± 40.2	44.83	1.3 ± 0.3	7.8 ± 3.1
Propyzamide	32.75	977.5 ± 433.7	29.31	13.5 ± 1.5	3615.4 ± 1117.8
Tebuconazole	25.21	230.8 ± 135.6	21.37	4.8 ± 1.0	8.5 ± 3.5
Trifloxystrobin	72.32	308.5 ± 94.0	63.11	240.3 ± 47.1	53.8 ± 18
Cyflufenamid	24.80	605.0 ± 360.3	25.00	4.1 ± 2.1	15.8 ± 3.4

ND – Not detected. <LOQ – Measured signal below the limit of quantification.

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Appendix II

Roodt, A.P., Schaufelberger, S., Schulz, R. (2023) Aquatic-terrestrial insecticide fluxes: midges as neonicotinoid vectors. Environ. Toxicol. Chem. 42, 60–70.

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Aquatic-Terrestrial Insecticide Fluxes: Midges as Neonicotinoid Vectors

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Abstract: Exposure of freshwater ecosystems to insecticides can negatively impact the development of emerging aquatic insects. These insects serve as an important nutritional subsidy for terrestrial insectivores. Changes in insect emergence phenology (i.e., emergence success and temporal pattern) or fluxes of insecticides retained by the emerging adults have the potential to negatively impact terrestrial food webs. These processes are influenced by contaminant toxicity, lipohilicity, or metabolic processes. The interplay between emergence phenology, contaminant retention through metamorphosis, and associated contaminant flux is not yet understood for current-use insecticides. In a microcosm study, we evaluated the impacts of a 24-h pulse exposure of one of three current-use insecticides, namely pirimicarb, indoxacarb, and thiacloprid, at two environmentally realistic concentration levels on the larval development and emergence of the nonbiting midge *Chironomus riparius*. In addition, we measured insecticide concentrations in the larvae and adults using ultrahigh performance liquid chromatography coupled to tandem mass spectrometry by electrospray ionization. Exposure to pirimicarb delayed larval development and emergence, and exposure to indoxacarb reduced emergence success. The neonicotinoid thiacloprid had the greatest impact by reducing larval survival and emergence success. At the same time, thiacloprid was the only insecticide measured in the adults with average concentrations of 10.3 and 37.3 ng/g after exposure at 0.1 and 4 µg/L, respectively. In addition, an approximate 30% higher survival to emergence after exposure to 0.1 µg/L relative to a 4-µg/L exposure resulted in a relatively higher flux of thiacloprid, from the aquatic to the terrestrial environment, at the lower exposure. Our experimental results help to explain the impacts of current-use insecticides on aquatic–terrestrial subsidy coupling and indicate the potential for widespread dietary exposure of terrestrial insectivores preying on emerging aquatic insects to the neonicotinoid thiacloprid. *Environ Toxicol Chem* 2023;42:60–70. © 2022 The Authors. *Environmental Toxicology and Chemistry* published by Wiley Periodicals LLC on behalf of SETAC.

Keywords: Aquatic insect emergence; aquatic–terrestrial food web; trophic cascade

INTRODUCTION

Globally, freshwater ecosystems are at risk of contamination with a wide range of insecticides originating from agriculture (Ippolito et al., 2015; Stehle & Schulz, 2015). This includes both legacy insecticide classes that are no longer permitted for application as well as newer classes of current-use insecticides (McKnight et al., 2015; Wolfram et al., 2018). Newer classes of current-use insecticides were developed to have a reduced negative impact on the environment by being less persistent and having a more selective toxic mode of action compared with older classes (Carvalho, 2017). In the case of neonicotinoids and pyrethroids, selectivity was improved by lowering vertebrate toxicity while increasing invertebrate toxicity (Morrissey et al., 2015; Schulz et al., 2021). Alternatively, insecticides with a more selective toxicity (i.e., at the insect order level) have also been developed (Jeschke, 2016; Wing et al., 2000). Neonicotinoid insecticides, however, break away from the trend toward reduced environmental persistence and are found in freshwater ecosystems globally (Morrissey et al., 2015). The ubiquitous presence of insecticides in freshwater ecosystems, even for brief exposure periods during runoff or spray drift events, has the potential to negatively impact linked aquatic–terrestrial food webs through emerging aquatic insects (Bundschuh et al., 2022; Kraus et al., 2021; Kraus, 2019; Schulz & Liess, 2001; Tooker & Pearsons, 2021).

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Up to now aquatic–terrestrial contaminant transfer has been reported for several classes of contaminants, including metals (Chételat et al., 2008; Wesner et al., 2017), metal-based nanoparticles (Bundschuh et al., 2019), pharmaceuticals (Previšić et al., 2021), halogenated organic pollutants (Liu et al., 2018), and some fungicides and herbicides (Roodt et al., 2022). In the terrestrial environment, these contaminants can enter the food web and potentially result in detrimental effects on consumers, such as prey avoidance or sublethal effects (Koch et al., 2020; Kraus et al., 2014, 2021; Richmond et al., 2018). Furthermore, exposure to insecticides during the development of emerging aquatic insects can impact the terrestrial recipient food web through changes in insect emergence phenology and productivity. Several studies have shown aquatic insect emergence to be either reduced or the temporal emergence pattern to be altered following larval insecticide exposure (Palmquist et al., 2008; Schulz & Liess, 2001; Tada & Hatakeyama, 2000). Both effects may lead to a de-coupling of aquatic resource provision and terrestrial resource requirements (Marczak & Richardson, 2008). This can decrease the availability of a high-quality food source supporting critical lifecycle stages in terrestrial consumers, such as breeding by insectivorous birds (Twining et al., 2018), or place additional pressure on species of high conservation value, such as insectivorous bats (Sirami et al., 2013; Stahlschmidt et al., 2012).

Contaminant transfer through metamorphosis can occur for those contaminants previously bioaccumulated in aquatic larval stages. Bioconcentration, which is defined as the bioaccumulation of contaminants through only passive absorption from the surrounding water, increases with increasing lipophilicity, that is, increasing log octanol/water partition coefficients ($\log K_{ow}$), for mid-polarity pesticides ($\log K_{ow}$ 2–5) in aquatic larvae and nymphs (Katagi & Tanaka, 2016). Once accumulated, retention of fungicides, herbicides, and other mid-polarity contaminants through metamorphosis is generally negatively correlated with lipophilicity (Kraus et al., 2014; Roodt et al., 2022). For those contaminants which are retained by emerging insects, the contaminant flux is defined as the weight of contaminant transferred by emerging insects per unit area during a unit of time. Contaminant flux is therefore predicted to decrease with increasing toxicity of a contaminant to aquatic life stages due to decreased survival and emergence (Kraus, 2019). Fluxes of insecticides, including highly toxic neonicotinoids, have recently been reported from contaminated wetlands corresponding with reduced overall insect emergence (Kraus et al., 2021). However, there is a lack of information on the interplay of exposure concentration, toxicity, emergence phenology, and contaminant retention through metamorphosis on the insect-mediated flux of current-use insecticides.

Against this background, we conducted microcosm experiments in which we exposed 10-day-old larvae of the nonbiting midge, *Chironomus riparius*, to a 24-h pulse of one of three current-use insecticides at two environmentally realistic concentrations. All three insecticides, namely the selective carbamate pirimicarb, the oxadiazine pro-insecticide indoxacarb, and the neonicotinoid thiacloprid, have seen large-scale application in Europe in the last decade (Helbig, 2019). We measured endpoints related to insect development and emergence (i.e., larval mortality, emergence success, sex-specific changes in development time, and adult body wt) as well as insecticide concentrations in the larvae and the adults as an indication of insecticide flux. Our hypotheses were based on the order of increasing insecticide $\log K_{ow}$ values (thiacloprid 1.26 < pirimicarb 1.7 ≪ indoxacarb 4.65) and increasing toxicity to the aquatic larvae (i.e., 28-day no observed effect concentrations [NOECs]: pirimicarb ≫ indoxacarb > thiacloprid). We hypothesized that the bioconcentration of the insecticides in the aquatic larvae would increase with increasing insecticide lipophilicity. Retention of these accumulated insecticides across adult metamorphosis would decrease with increasing lipophilicity, similar to other contaminants within this $\log K_{ow}$ range (Kraus et al., 2014; Roodt et al., 2022). Finally, that overall insecticide flux would increase with exposure concentration and decrease with increasing toxicity to the larvae, that is, the highest concentration of pirimicarb would have the greatest flux and the highest concentration of thiacloprid would have the lowest.

MATERIALS AND METHODS

Chemicals and reagents

Analytical standards for all the pesticides were obtained from LGC Standards or HPC Standards. Solvents (liquid chromatography coupled to mass spectrometry [LC-MS] grade) were purchased from Honeywell. All other chemical reagents (>99% purity) and clay were purchased from Carl Roth. Aquarium sand and peat were obtained from Schicker Mineral and Floragard, respectively.

Microcosm experiments

The nonbiting midge, *C. riparius* (Meigen), was used in three sequential microcosm experiments for exposure to pirimicarb, indoxacarb, or thiacloprid. In each case, freshly laid egg masses were collected from an in-house laboratory culture. Egg masses ($n = 9$) were equally distributed between three aquaria (32 × 22 cm, 3.75 L) containing 2–3 cm of sediment, which was prepared in accordance with Organisation for Economic Co-operation and Development (OECD) test guideline 219 (OECD, 2004), and SAM-5S aqueous medium of depth approximately 5 cm (Borgmann, 1996). The aquaria were kept in a climate-controlled chamber at 20 °C with 70% relative humidity and a 16:8-h light:dark cycle. All aquaria were aerated during the experiment and water parameters, namely temperature (19–21 °C), pH (7–8), conductivity (550–700 µS/cm) and dissolved oxygen concentrations (5–7 ppm), were monitored during the experiments. After hatching, larvae were fed three times a week with finely ground TetraMin fish food. After 10 days of development, larvae were sieved from the sediment and randomly divided between three treatment levels (each with 225 larvae), the control, low, and high treatment levels. The concentrations of each treatment level were selected based on environmental monitoring data. Pirimicarb, indoxacarb, and thiacloprid have been widely applied in recent decades and have been reported in environmental monitoring of surface waters and sediments.

Pirimicarb can be detected at low concentrations under base flow conditions in contaminated waterbodies (Struger et al., 2016) and has been measured at concentrations up to 10 µg/L after heavy rainfall triggered runoff events (Kreuger, 1998). Despite its higher lipophilicity, indoxacarb's high application rates in viticulture result in frequent detections in impacted ground water, with some concentrations exceeding 0.1 µg/L (Herrero-Hernández et al., 2020). Average sediment concentrations of indoxacarb in vineyard-adjacent streams have been reported to be 24 µg/kg (Bereswill et al., 2012). Within the widely detected neonicotinoid class, thiacloprid has been measured at concentrations in surface waters up to 4.5 µg/L (Stehle et al., 2018). In the present study, the control treatment contained only aqueous medium, the low treatment level had a nominal concentration of 0.1 µg/L for all the insecticides, and the high treatment level had a nominal concentration of 16 µg/L for pirimicarb and indoxacarb. A lower concentration of 4 µg/L was used as the high treatment level for thiacloprid on account of its higher toxicity. The NOECs for chronic 28-day water exposure of *C. riparius* larvae to pirimicarb, indoxacarb, and thiacloprid are >1000, 1.8, and 0.2 µg/L, respectively (Lewis et al., 2016). The relative toxicity of each treatment level was calculated as the ratio of the tested concentration to the respective NOEC (Supporting Information, Table S1). The highest treatment level selected for thiacloprid is therefore considered to be approximately twice as toxic as the highest treatment level of indoxacarb and more than 10 000 times more toxic than the highest pirimicarb treatment level. The exposure period lasted for 24 h and took place in the absence of sediment or food. For this, three aquaria containing 2.5 L of aqueous medium were prepared at the relevant insecticide concentration and allowed to equilibrate for 1 h before larvae were added. The insecticides were prepared in aqueous medium from formulation products, namely, Pirimor (500 g/kg pirimicarb; Syngenta), Steward (300 g/kg indoxacarb; DuPont), and Calypso (480 g/L thiacloprid; Bayer). Concentrations of pirimicarb, indoxacarb, and thiacloprid in the medium were measured at the start of the experiment by ultrahigh performance liquid chromatography coupled to tandem mass spectrometry by electrospray ionization (UHPLC-ESI-MS/MS) to be approximately 87%, 104%, and 100% of the nominal concentrations, respectively. Details about the analysis are provided in Supporting Information, Table S2. We therefore use the nominal concentrations to refer to the treatment levels in the remaining text.

After completing the 24-h exposure period, the larvae were rinsed with clean medium. Dead larvae were identified by a lack of response to a physical stimulation with tweezers, removed and counted. In the case of pirimicarb and indoxacarb, at least 98% of larvae were alive after the 24-h insecticide exposure, regardless of insecticide or treatment level. In the case of thiacloprid, 88% and 75% of the larvae survived in the 0.1 and 4 µg/L treatment levels, respectively. Surviving larvae in each treatment level were randomly assigned to four replicate groups. A replicate group consisted of three samples: two larvae samples collected at different time points and the adult insect sample. One quarter of the larvae from each replicate group were frozen and stored at −80 °C directly after cleaning, in preparation for insecticide concentration determination. The remaining three quarters were transferred to smaller aquaria (three per replicate group, 11.5 cm diameter, 0.5 L) containing sediment and aqueous medium with depths of 2 and 5 cm, respectively. Each small aquarium contained 14 larvae in the case of pirimicarb and indoxacarb, providing at least 7.4 cm^2 surface area per larvae. The lower survival of larvae exposed to thiacloprid resulted in slightly smaller replicate groups. Each small aquarium contained 10 larvae in this case and the aquaria used to collect adult emergence were reduced to three replicates, instead of four, for the highest treatment level. The larvae were provided with 10–15 mg of fish food every second day during the post-exposure period. Individuals in the less densely populated replicates for thiacloprid therefore had less competition for resources, which potentially aided their development, reflecting sequential effects after insecticide exposure that would occur in the field. After a 72-h post-exposure period, the larvae from one of the three aquaria per treatment replicate group were collected from the sediment, rinsed, counted, and frozen at −80 °C. The remaining two aquaria per treatment replicate group were covered with a 0.6-mm polyester mesh to capture emerging adults. The emerging adults were collected daily, grouped by sex, counted, and frozen at −20 °C prior to insecticide concentration determination. Adult insects which died during emergence were excluded.

Sample preparation for pesticide analysis

Extraction of insecticides from the larvae and adults was performed by ultrasonically assisted solid–liquid extraction as previously described (Roodt et al., 2022). Briefly, midge larvae ($n = 7–14$) were pooled within each replicate and sampling time point. Similarly, adult midges ($n = 7–19$) were pooled within each replicate (consisting of two aquaria per replicate group) by sex. All samples were freeze dried and pulverized using a Tissuelyser with steel pellets (Retsch). The total dry weight of all samples was determined on a fine scale to an accuracy of 0.001 mg (Mettler-Toledo). In the case of pirimicarb and indoxacarb, subsamples of midge larvae (7.0 ± 0.5 mg), midge females (20.0 ± 0.5 mg), and midge males (6.0 ± 0.5 mg) were prepared from each replicate. In the case of thiacloprid, subsamples of midge larvae (4.0 ± 0.5 mg), midge females (13.0 ± 0.5 mg), and males (4.5 ± 0.5 mg) were prepared from each replicate. Before solvent extraction was performed, 20 µl of a 50-ng/ml solution containing the deuterated internal standards indoxacarb-D3, pirimicarb-D6 or thiacloprid-D4 was added to the samples, which were subsequently allowed to stand for 30 min at room temperature. Samples were then extracted with acetonitrile (3 × 1.5 ml) and methanol (2 × 1.5 ml). The pooled extracts were evaporated to dryness under a gentle stream of nitrogen gas and redissolved in a water/methanol (70:30 v/v) solution containing 0.1% formic acid. Aqueous medium samples were diluted with methanol to achieve a 70% aqueous solution before being centrifuged at 16 000 rpm for 5 min. All samples were then filtered through 0.2 µm polytetrafluoroethylene syringe filters prior to UHPLC-ESI-MS/MS analysis.

Quantitative pesticide analysis

Insecticide concentrations were determined by UHPLC-ESI-MS/MS. Instrument parameters were set as previously described (Roodt et al., 2022). Details of the instrument parameters are provided in Supporting Information, Table S2. Matrix-matched standards were prepared with concentrations of 0.005, 0.01, 0.05, 0.1, 0.5, 1.0, 5.0, and 10.0 ng/ml using insects collected from the in-house culture. Two multiple reaction monitoring transitions were used for each analyte (Supporting Information, Table S3). Recoveries of internal standards were monitored for quality control, with recoveries between 70% and 120% being considered acceptable. Measurement limits of quantification (LOQs) and detection (LODs) are provided in Supporting Information, Table S4.

Data analysis and statistics

The sex-specific effects of exposure to each insecticide on larval development were quantified by calculating the time taken for 50% of the total number of successfully emerged individuals to emerge in each replicate (EmT_{50}). Bioconcentration factors (BCFs) were calculated as the larval concentration after 24-h exposure based on dry weight divided by the exposure concentration. Insect-mediated insecticide flux was calculated as the product of the average insecticide concentrations and total average dry weights of successfully emerged adult insects divided by the respective EmT_{50} when calculated for total emergence of both sexes. The pooled EmT_{50} for both sexes was chosen as a proxy time dimension of the flux calculation in the present specific microcosm study. This was done to account for changes in the overall emergence pattern resulting from changes in the development duration and emergence success of female insects. Male midges develop faster than females (Day et al., 1994), and an accelerated female development can therefore narrow the emergence window of both sexes together, while increasing the daily insecticide flux. An increase in the development times of female insects would have the opposite effect, thus decreasing the daily flux. Equations for the calculation of the flux are provided in Supporting Information, Table S6. Treatment effects on adult sex-specific EmT_{50}, sex-specific biomass of emergent adults, and total emergence success were tested for using the Kruskal–Wallis *H* test followed by a post hoc Dunn's test with Bonferroni correction when treatment level effects were detected. The significance level, α, was set at 0.05 for all tests. Statistical analyses were performed in R Ver 4.0.3 (R Core Team, 2020). All average values reported in the discussion were calculated as an arithmetic mean.

RESULTS

Survival, development, and emergence phenology

The pulse exposure of 10-day old larvae to pirimicarb had no effect on larval survival (Supporting Information, Table S5), but delayed emergence of the female insects by approximately 3–4 days regardless of exposure concentration (Figure 1). The males were not significantly affected, but a tendency toward a longer development time is apparent at the 16 µg/L treatment level. The overall emergence success was not affected by exposure to pirimicarb (Figure 2). Pulse exposure to indoxacarb did not have an effect on larval survival (Supporting Information, Table S5) and did not delay the emergence of either sex at any treatment level (Figure 1). However, total emergence success was significantly decreased by approximately 25% at the 16 µg/L treatment level, with a tendency toward lower emergence success in the 0.1 µg/L treatment level (Figure 2).

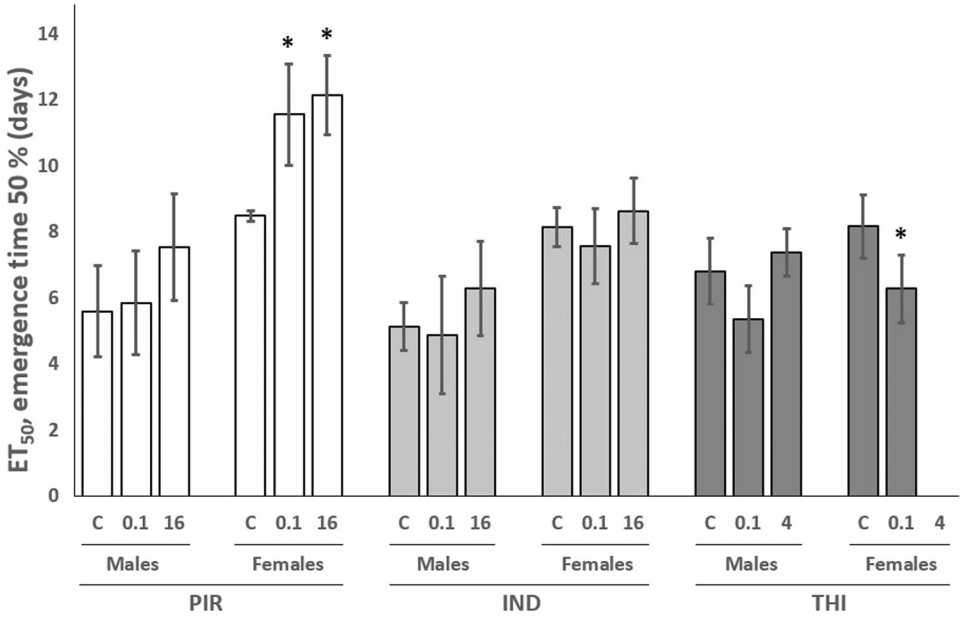

FIGURE 1: Average ($n = 4$ for all treatments, $n = 3$ for thiacloprid (THI) 4 µg/L, ±standard deviation) sex-specific time taken for 50% of adults to emerge (EmT_{50}) after pulse exposure to pirimicarb (PIR; white bars), indoxacarb (IND; light gray bars), or THI (dark gray bars) from replicate aquaria at each treatment level (control, 0.1, 4, or 16 µg/L). Asterisks indicate a significant difference to the respective control (Dunn's test, $p < 0.05$).

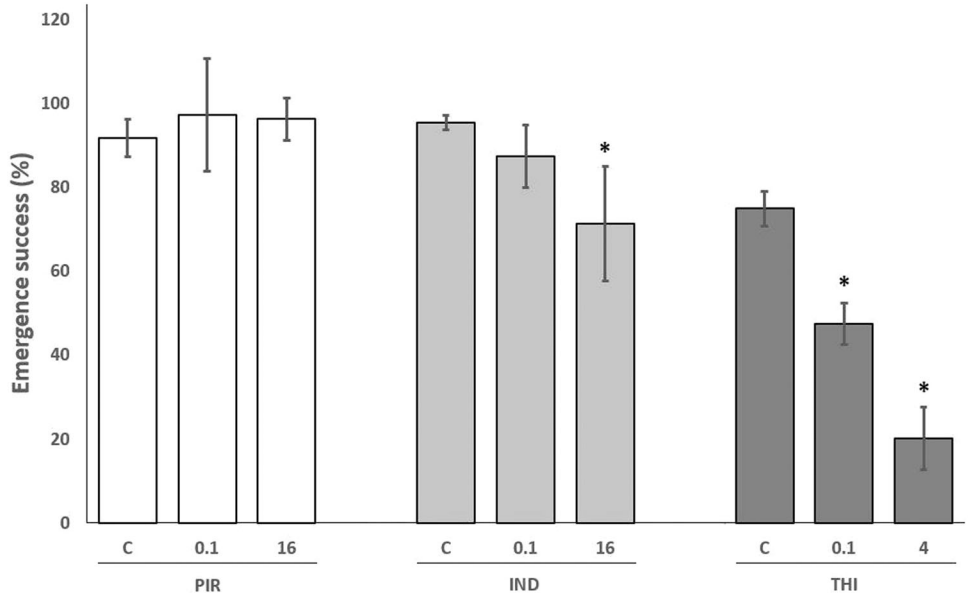

FIGURE 2: Average percentage ($n = 4$ for all treatments, $n = 3$ for thiacloprid (THI) 4 µg/L, ±standard deviation) total emergence success of adult insects after pulse exposure to pirimicarb (PIR; white bars), indoxacarb (IND; light gray bars), or THI (dark gray bars) from replicate aquaria at each treatment concentration (control, 0.1, 4 or 16 µg/L). Asterisks indicate a significant difference to the respective control (Dunn's test, $p < 0.05$).

Larvae exposed to thiacloprid were moribund after the 24-h exposure period and showed convulsive and incoherent movements in response to physical stimulation. After the 72-h post-exposure period, the larvae in the 0.1 µg/L treatment level had recovered with no difference in survival relative to the control (Supporting Information, Table S5). Larval survival in the 4 µg/L treatment level was, however, significantly reduced by approximately 50% relative to the control. Exposure to 0.1 µg/L thiacloprid reduced the EmT_{50} by approximately 1–2 days relative to the control, which was significant for the female insects (Figure 1). Exposure at 4 µg/L had no effect on the EmT_{50} of male insects, and insufficient female insects emerged at this treatment level to allow for the calculation of the EmT_{50}. Emergence success in the control of the thiacloprid experiment was lower relative to the controls for the other two insecticides, but overall emergence success was, however, still >70% and is therefore considered valid based on criteria set out for laboratory studies (OECD, 2004). Thiacloprid exposure significantly reduced overall emergence success at both treatment levels relative to the control (Figure 2). None of the insecticides affected the sex-specific average adult individual dry weight at any treatment level relative to the respective controls (Supporting Information, Figure S1).

Insecticide concentrations in larvae and adult insects

Directly after the 24-h exposure period, pirimicarb concentrations were measurable above the LOQ in the larvae from the 16 µg/L treatment level, but not the 0.1 µg/L treatment level (Figure 3). The average ($n = 4$, ±standard deviation) concentration in the higher treatment level was 31.0 ± 8.1 ng/g, with an average BCF of 1.9 L/kg. This concentration decreased to 0.4 ± 0.2 ng/g over the 72-h post-exposure period, corresponding to an approximate 99% reduction. Average larval indoxacarb concentrations, directly after the 24-h exposure period, were 415.0 ± 90.5 and 3837.9 ± 1142.1 ng/g, with average BCFs of 4150 and 240 L/kg in the 0.1 and 16 µg/L treatment levels, respectively. Over the 72-h post-exposure period, the larval concentrations decreased to below the LOD in the 0.1 µg/L treatment level, but were still measurable, at 224.7 ± 175.4, in the 16 µg/L treatment level. Thus, the concentration of indoxacarb decreased by approximately 94% over the 72-h post-exposure period. Neither pirimicarb nor indoxacarb was measured above their respective LODs in the emergent males or females, regardless of treatment level. Thiacloprid concentrations in larvae were 125.2 ± 18.3 and 287.2 ± 90.8 ng/g after 24 h of exposure to the 0.1- and 4-µg/L treatment levels, respectively. The corresponding average BCFs were 1252 and 49 L/kg. These concentrations decreased by approximately 30%–50% over the 72-h post-exposure period. Furthermore, thiacloprid was measured in the adults at an average concentration approximately 20% of that measured in the larvae 72 h post-exposure regardless of the insect sex or treatment level. The adult insects therefore transferred approximately 10–15% of the thiacloprid concentration measured in the larvae directly after exposure. Overall, the thiacloprid fluxes calculated for both sexes emerging from the microcosms were 18.6 ± 14.2 and 43.0 ± 12.4 pg/day for the 0.1 and 4 µg/L treatment levels, respectively. Parameters for the flux calculation are provided in Supporting Information, Table S6.

DISCUSSION

Effects of insecticides on larval development and emergence

All three insecticides had an effect on at least one aspect of larval development and emergence. Pirimicarb caused a delay

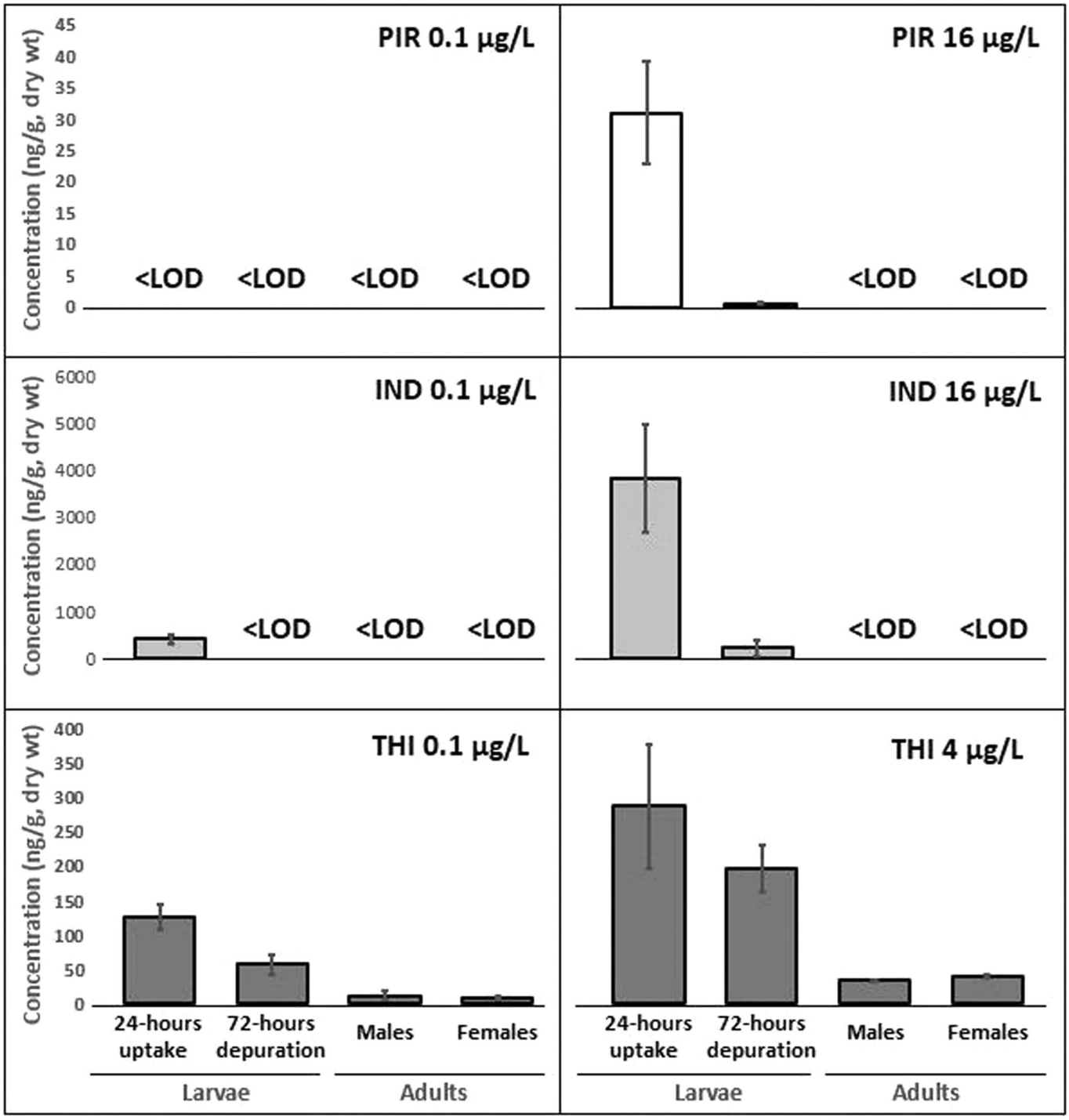

FIGURE 3: Average ($n = 4$ for all treatments, except thiacloprid (THI) 4 µg/L where $n = 3$; ±standard deviation) concentrations of pirimicarb (PIR; white bars), indoxacarb (IND; light gray bars), and THI (dark gray bars) in midge larvae sampled after 24-h exposure or 72-h depuration and in adult insects. Concentrations are based on the sample dry weights (dw). LOD, limit of detection. *y*-axis scales are different for each insecticide.

in the emergence of female midges, but had no effect on emergence success. The elimination of accumulated pirimicarb by the larvae post-exposure may incur additional energetic costs, likely resulting in an extended development time (Monteiro et al., 2019). Similar to the present study, extended development times have been reported for target pest species of aphids when exposed to sublethal concentrations of pirimicarb (Xiao et al., 2015). Contrastingly, indoxacarb did not delay emergence, but reduced emergence success. Studies investigating the chronic exposure of *C. riparius* larvae to indoxacarb reported delays in development without reducing emergence success (Monteiro et al., 2019). However, reduction of successful metamorphosis, as was observed in the present study, is known for some terrestrial pest species after exposure to indoxacarb (Gamil et al., 2011; Saryazdi et al., 2012). The toxic mode of action of indoxacarb is related to the rate of

metabolization of the parent compound to its toxic metabolite (Wing et al., 2000). Concentrations of indoxacarb still present in the larvae at the onset of metamorphosis and their conversion to its active toxic metabolite during a more vulnerable life stage may therefore explain the reduced emergence success in the highest, 16-µg/L, treatment level.

Thiacloprid reduced larval survival 72 h post-exposure, decreased overall emergence success, and accelerated the emergence of female midges. A delayed larval mortality after exposure, such as occurred in the 4-µg/L treatment level, has also been observed in a variety of freshwater macroinvertebrates exhibiting a range of sensitivities (Beketov & Liess, 2008). A microcosm study in which *C. riparius* larvae were exposed to a related neonicotinoid insecticide, imidacloprid, during development found reduced emergence success at similar concentrations to the ones used in the present study, although no effect on the timing of emergence was observed (Chandran et al., 2018). In the present study, the accelerated development and earlier emergence observed in only the lowest, 0.1-µg/L, treatment level could be interpreted as a nonmonotonic, potentially hormetic, response when considering that there was no reduction in larval density and no negative impact on the average dry weights of the emerging adults relative to the control (Steinberg, 2012). Similar accelerated development and emergence has been reported for *C. riparius*, as well as more complex insect communities, after exposure to low concentrations of pyrethroids (Goedkoop et al., 2010; Rogers et al., 2016). These authors hypothesized that stimulation of behavioural or biochemical processes by one or more of the pyrethroid stereoisomers at a low concentration resulted in the observed accelerated development. An accelerated larval development may, however, have negative consequences (e.g., decreased longevity) for the adult life stage which were not measured in the present study (Metcalfe & Monaghan, 2001).

Bioconcentration of insecticides in larvae

Indoxacarb was the most bioconcentrated in the larvae and pirimicarb the least, thus correlating positively with their lipophilicities. This result is based on the assumption that an equilibrium between uptake and elimination was achieved over the exposure period. In this context, similar BCFs have been reported for pirimicarb in *Daphnia magna* (BCFs 31–50) and for indoxacarb in the zebra fish *Danio rerio* (BCFs 1080–1752; Kusk, 1996; Y. Li et al., 2021). Thiacloprid did not fit the predicted trend and, despite having the lowest lipophilicity of the insecticides, was more accumulative than pirimicarb but less than indoxacarb. Similar underestimation of bioconcentration potential based on the logK_{ow} has been reported for the neonicotinoid imidacloprid in an aquatic oligochaete *Lumbriculus variegatus* (Contardo-jara & Gessner, 2020). These authors reported much lower 24-h BCFs (between 20 and 70 for exposure at 0.1 µg/L) than the ones determined in the present study, but the authors reported an increasing BCF with increasing exposure time and a maximum value was not established. Underestimation of the bioconcentration potential of neonicotinoids in amphipods is also well established in the literature and may be attributed to binding of the insecticide to biomolecules and the exoskeleton of the organisms (Chen & Kuo, 2018; Lauper et al., 2022; H. Li et al., 2021). In the present study, the lower BCFs of indoxacarb and thiacloprid at the higher treatment level can result from the saturation of uptake mechanisms or changes in the physiological condition of the larvae which reduce uptake, for example reduced respiration rate and immobilization (Mackay & Fraser, 2000). In addition, surfactants which are present in the formulation products as chemical adjuvants can also reduce the bioaccumulation of mid-polarity pollutants in benthic organisms (Garcia-galan et al., 2017). Because the exact composition of the adjuvants used in each formulation is not publicly available, a comparison is not possible and the reported BCF values in the present study may therefore be specific to the formulation products used.

Insecticide retention through metamorphosis

Thiacloprid was the most toxic insecticide to the larvae and was also the insecticide best retained through development and metamorphosis. This contradicted the hypothesized trend, in which pirimicarb would have the greatest flux from the aquatic to terrestrial ecosystem because of its low lipophilicity and low toxicity to the larvae. This hypothesis was based on what has been previously reported for the retention of a range of polycyclic aromatic hydrocarbons, fungicides, and herbicides, with similar lipophilicity and low toxicity, by emerging aquatic insects (Kraus et al., 2014; Roodt et al., 2022).

In the present study, the relatively rapid and complete elimination of pirimicarb and indoxacarb in the larvae resulted in no measurable concentrations remaining in the adult insects after metamorphosis. Elimination of thiacloprid by the larvae was, however, much slower, which resulted in higher concentrations being present in the larvae at the onset of metamorphosis, generally 6–7 days post-exposure based on the median EmT_{50} values. When considering only passive uptake of insecticides from the surrounding water, metabolism by relevant enzymes is an important factor determining the resulting concentrations in aquatic organisms (Katagi, 2010). Insecticide toxicity, in turn, is related to the concentrations present in the organism in combination with the toxic mode of action (Katagi & Tanaka, 2016). The observed rates of elimination therefore correlated with the insecticides' relative toxicities (i.e., 28-day NOECs pirimicarb ≫ indoxacarb > thiacloprid). These results indicate a positive correlation between insecticide toxicity and the potential for aquatic–terrestrial transfer due to the rate of insecticide metabolism.

Thiacloprid flux from water to land

The relative thiacloprid flux was approximately 17 times higher from the 0.1-µg/L than the 4-µg/L treatment level when considering the exposure concentration (Supporting Information,

Figure S2). More specifically, the average calculated flux of thiacloprid from the 0.1-μg/L treatment level was approximately 40% of the flux from the 4-μg/L treatment level, despite two orders of magnitude difference in the exposure concentrations. This result implies that relatively significant fluxes of thiacloprid may occur even at lower aqueous-phase exposure concentrations than were used in the present study. Our results may also be relevant for other neonicotinoids. In this context, similar concentrations (2.7–47.2 ng/g) of the neonicotinoids clothianidin and imidacloprid have been reported in adult Diptera emerging from contaminated wetlands, although the exact aquatic exposure concentrations were not reported (Kraus et al., 2021). Neonicotinoids are, however, frequently detected at low concentrations as mixtures in surface waters (Schmidt et al., 2022). A compilation of neonicotinoid monitoring data from 29 studies across nine countries found a geometric mean concentration of 0.13 μg/L in water which occurred frequently and long term (Morrissey et al., 2015) and exceeds the lower thiacloprid treatment level used in the present study. Moreover, in temperate climates emerging Chironomidae (Diptera) have a very wide emergence period, discontinuing only during the coldest part of winter (Raitif et al., 2018). In addition, the proportion of Chironomidae in the aquatic insect community positively correlates with anthropogenic disturbances associated with agriculture, whereas abundances of other more sensitive taxa decrease (Raitif et al., 2018; Stenroth et al., 2015). Our results therefore indicate the potential for a widespread and near-permanent aquatic–terrestrial flux of neonicotinoid insecticides from impacted freshwater ecosystems mediated by emerging midges.

Potential impacts on terrestrial consumers

Overall, thiacloprid had the highest potential to negatively impact terrestrial consumers at environmentally realistic pulse-exposure concentrations. The resulting decrease in larval densities and emergence success has the potential to place significant pressure on higher trophic levels through reduced food availability (Tooker & Pearsons, 2021). In addition, reductions in adult aquatic insect abundance also reduce the availability of essential dietary polyunsaturated fatty acids which are not substituted by consumption of terrestrial insects (Hixson et al., 2015; Martin-creuzburg et al., 2017; Twining et al., 2016). The presence of the neonicotinoid imidacloprid in surface waters has already been linked to declining insectivorous bird populations in Europe (Hallmann et al., 2014). In addition, shifts to earlier or later emergence of aquatic insects, as found in the present study after exposure to pirimicarb or thiacloprid, has the potential to decouple aquatic subsidy availability from terrestrial insectivore life history (Marczak & Richardson, 2008), although this is likely more relevant in the case of emerging insects with longer lifecycles and fewer generations per season.

The retention of thiacloprid by adult insect results in the potential dietary exposure of terrestrial predators to thiacloprid, among other neonicotinoids, via consumption of contaminated midges (Kraus et al., 2021). This may be relevant for a wide range of predators, including birds, bats, lizards, and spiders, which obtain a large proportion of their energy requirements through consumption of emerging aquatic insects (Baxter et al., 2005). Furthermore, this may be especially relevant for predators which specialize on emerging aquatic insects as a food source, for example some species of riparian spiders (Wieczorek et al., 2015). In addition, the emerging adult insects themselves may be negatively impacted by retained neonicotinoid insecticides. The neurotoxic mode of action of neonicotinoids has been linked to a range of sublethal effects in nontarget insects, such as vision loss, reduced immune response to pathogens, and behavioural effects (Pisa et al., 2021; Tasman et al., 2021). Retention of these compounds may therefore have negative impacts on the fitness and longevity of the successfully emerged adults, with potential for further cascading impacts on terrestrial consumers through further reduced food availability in addition to dietary insecticide exposure. The impacts of dietary exposure to neonicotinoids on vertebrate predators are challenging to study at the landscape scale due to the confounding effects of multiple stressors. However, laboratory investigations have revealed sublethal effects on development, behaviour, immune function, and reproductive success for a wide variety of terrestrial insectivores, including insectivorous birds and bats (Gibbons et al., 2015; Pisa et al., 2021; Wu et al., 2020).

CONCLUSION AND IMPLICATIONS

The present study offers a detailed laboratory-scale investigation of aquatic–terrestrial insecticide fluxes propagated by emerging midges after a brief exposure to an environmentally realistic concentration. The results highlight the interplay between insecticide effects on insect emergence, the rate of elimination of the insecticide by the aquatic larvae, and insecticide retention by emerged adult insects. Exposure of the larvae to the most toxic (lowest NOEC) insecticide, thiacloprid, which was also the slowest to be eliminated, resulted in its transfer to the adults. Furthermore, relatively high fluxes were measured at the lower exposure concentration (0.1 μg/L) relative to the higher (4 μg/L) due to increased emergence success. Our results thus imply the potential for significant fluxes to take place even at lower exposure concentrations, potentially negatively affecting terrestrial insectivores hunting in adjacent terrestrial ecosystems. Our study also adds to the growing body of literature describing an underestimation of the potential for neonicotinoid bioaccumulation and persistence in food webs relative to their lipophilicities (Tooker & Pearsons, 2021). The extent and impacts of the aquatic–terrestrial transport of these insecticides by emerging aquatic insects at the field-scale is a potentially important topic of future research.

Supporting Information—The Supporting Information is available on the Wiley Online Library at https:/10.1002/etc.5495.

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Supporting Information

Table S1. Chronic 28-day no observed effect concentrations (NOECs) tested in static water with *Chironomus riparius* larvae and calculated relative toxicity factors for the treatment levels used in the microcosm experiments.

Insecticide	NOEC (µg/L)*	Treatment levels (µg/L)	Relative toxicity factors**
Thiacloprid	0.19	0.1 and 4	0.5 and 20
Indoxacarb	1.8	0.1 and 16	0.1 and 8.9
Pirimicarb	>10 000	0.1 and 16	0.01 $x10^{-3}$ and 1.6 $x10^{-3}$

*NOECs obtained from the Pesticide Properties Database (PPDB), available at http://sitem.herts.ac.uk/aeru/ppdb/en/index.htm. Accessed on: 07.06.22.

**Relative toxicity factors were calculated for each treatment level used in the present study as the ratio of the exposure concentration to the respective NOEC. In the case of pirimicarb, 10 000 µg/L was used as the NOEC.

Table S2. UHPLC-ESI-MS/MS instrument parameters used for the analyses of water and biota.

Liquid chromatography (UHPLC*) parameters:	
Instrument:	Agilent 1260 Infinity II HPLC System
Column:	ZORBAX Eclipse Plus C18 (2.1 ID x 50 mm, 1.8 micron)
Eluent A:	H_2O/MeOH (98:2), 0.1% Formic acid, 4 mM Ammonium formate
Eluent B:	H_2O/MeOH (2:98), 0.1% Formic acid, 4 mM Ammonium formate
Injection volume:	90 µL
Flow rate:	0.35 mL/min
Column temperature:	50°C
Mass Spectrometry (ESI-MS/MS**) parameters:	

Instrument:	Agilent 6495 Triple Quadrupole Mass Spectrometer
Capillary voltage:	3000 V
Nozzle voltage:	0 V
Gas flow:	11 L/min
Gas temperature:	250 °C
Sheath gas flow:	12 L/min
Sheath gas temperature:	350 °C
Nebulizer pressure:	38 psi

*Ultrahigh Performance Liquid Chromatography, **Electrospray Ionization with Tandem Mass Spectrometry

Table S3. Multiple reaction monitoring (MRM) transitions used for the identification and quantification of insecticides and deuterated internal standards by UHPLC-ESI-MS/MS.

Insecticide	Quantifier MRM (CE)	Qualifier MRM (CE)
Pirimicarb	239.1 -> 72.1 (20)	239.1 -> 182.2 (13)
Indoxacarb	528.0 -> 203.0 (40)	528.0 -> 293.0 (9)
Thiacloprid	253.0 -> 126.0 (20)	253.0 -> 90.1 (44)

Internal Standard	Quantifier MRM (CE)	Qualifier MRM (CE)
Pirimicarb-D6	245.2 -> 185.1 (15)	245.2 -> 78.2 (5)
Indoxacarb-D3	531.0 -> 293.0 (15)	531.0 -> 149.9 (15)
Thiacloprid-D4	257.0 -> 126.0 (20)	253.0 -> 90.1 (44)

CE – Collision cell energy (eV).

Table S4. Limits of detection (LODs) and quantification (LOQs) of insecticides in solvent and insect matrices.

	Solvent		Larvae		Adult females		Adult males	
Insecticide	LOD (ng/mL)	LOQ (ng/mL)	LOD (ng/g, dw)	LOQ (ng/g, dw)	LOD (ng/g, dw)	LOQ (ng/g, dw)	LOD (ng/g, dw)	LOQ (ng/g, dw)
Pirimicarb	0.004	0.01	0.3	1.3	1.2	3.7	0.8	2.6
Indoxacarb	0.02	0.06	2.2	6.9	1.2	3.8	3.3	10.1
Thiacloprid	0.01	0.04	24.0	73.0	0.9	2.9	1.9	5.9

LOD is the Limit of detection, calculated as: LOD = 3.3σ/m, and LOQ is the limit of quantification, calculated as: LOQ = 3.3σ/m, where σ is the residual standard deviation of the linear calibration regression line and m is the slope. dw – dry weight.

Table S5. Average percentage survival of larvae 72-hours post insecticide exposure.

Insecticide	Treatment	Average Percentage Survival (n=4, ± standard deviation)
Pirimicarb	Control	96.4 ± 4.1
	0.1 µg/L	83.9 ± 15.8
	16 µg/L	96.4 ± 4.1
Indoxacarb	Control	100 ± 0
	0.1 µg/L	100 ± 0
	16 µg/L	100 ± 0
Thiacloprid	Control	78.6 ± 8.2
	0.1 µg/L	82.1 ± 18
	4 µg/L	35.3 ± 20.7*

*Significant difference relative to the respective control (Dunn's test, $p < 0.05$).

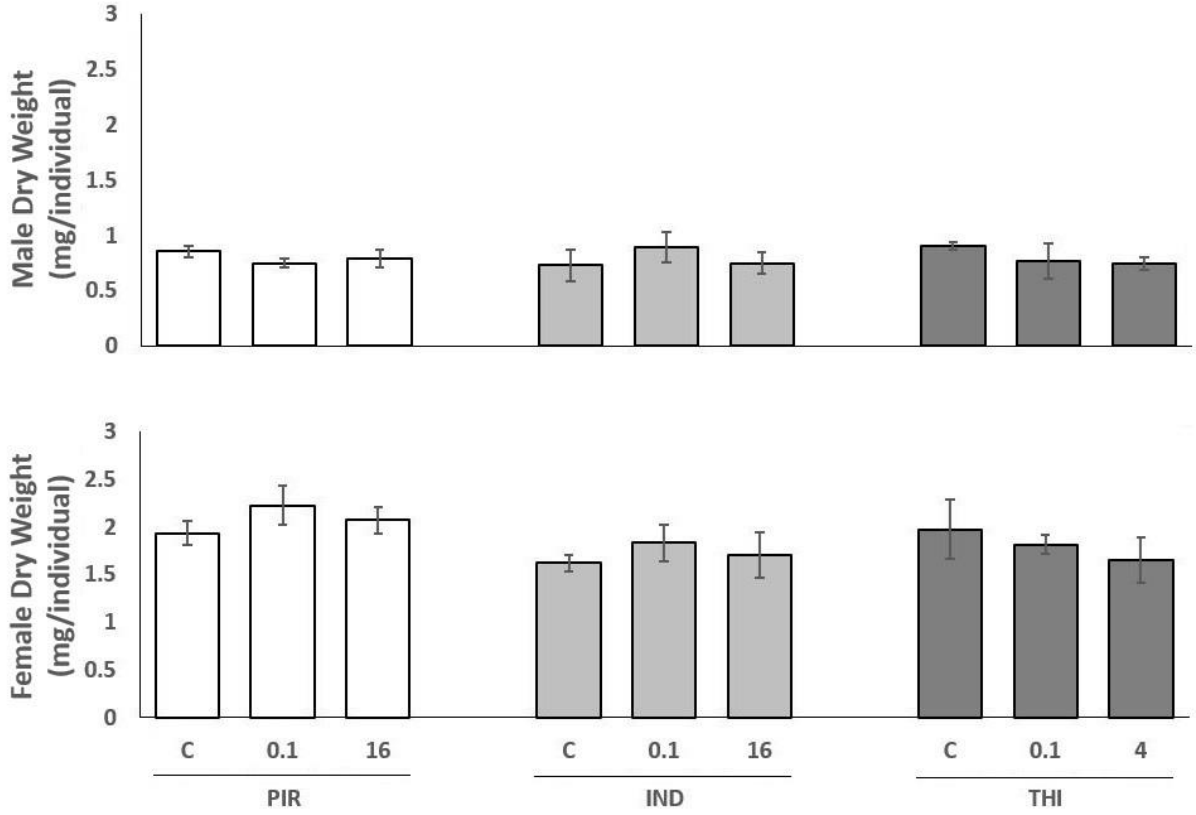

Figure S1. Average (n=4 for all treatments, n = 3 for THI 4 µg/L, ± standard deviation) dry weights of male and female insects emerging after exposure to pirimicarb (PIR), indoxacarb (IND) or thiacloprid (THI) from replicate aquaria at each treatment concentration (Control, 0.1, 4 or 16 µg/L).

Table S6. Average (± standard deviation) time for 50% of total adult emergence (EmT_{50}) and calculated thiacloprid fluxes.

Treatment (µg/L)	Number of replicates	Average total dry weight (mg)		Average thiacloprid concentration ($x10^{-3}$ ng/mg, dw)		Total thiacloprid transported (ng)	Average EmT_{50} (days)	Flux ($x10^{-3}$ pg/d)
		Male	Female	Male	Female	Male + Female		
0.1	4	2.2 ± 0.68	11.1 ± 2.8	11.0 ± 6.2	8.0 ± 2.4	0.13 ± 0.076	6.7 ± 1.1	18.6 ± 14.2
4	3	3.0 ± 0.97	4.1 ± 0.57	34.4 ± 1.0	40.3 ± 1.6	0.27 ± 0.066	6.2 ± 0.3	43.0 ± 12.4

Equations used for flux calculations:

$$Sex - Specific\ total\ insecticide\ transported = Average\ total\ dry\ weight * Average\ insecticide\ concentration$$

$$Flux\ = \frac{Total\ insecticide\ transported\ by\ both\ sexes}{Average\ EmT_{50}\ for\ both\ sexes}$$

dw – dry weight

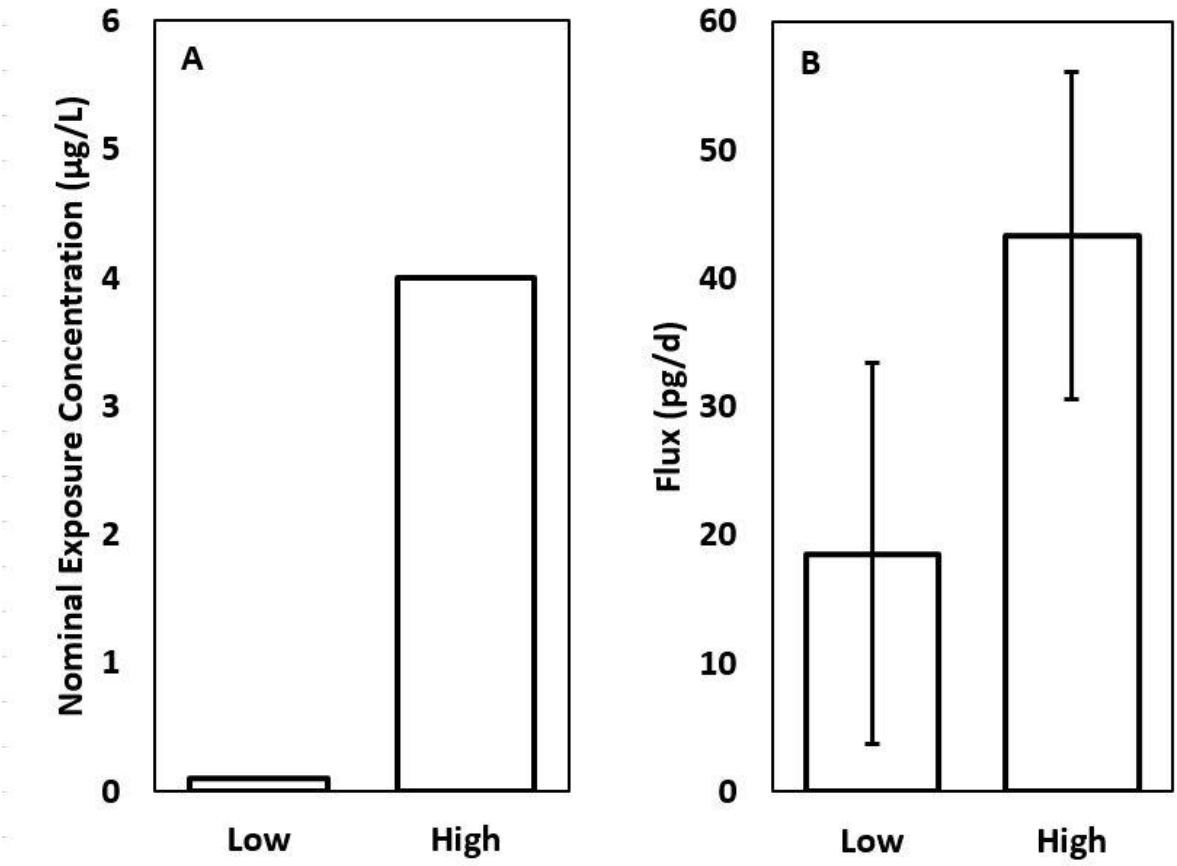

Figure S2. Thiacloprid 24-hour nominal pulse exposure concentration (A) relative to emerging midge mediated fluxes (B). Nominal exposure concentrations (A) and thiacloprid fluxes (B) are shown for a low (0.1 µg/L) and high (4 µg/L) exposure concentration. The average flux of thiacloprid from the 0.1 µg/L treatment level was approximately forty percent of the flux from the 4 µg/L treatment level, despite two orders of magnitude difference in the exposure concentrations.

Appendix III

Roodt, A.P., Huszarik, M., Entling, M.H., Schulz, R. (2023) Aquatic-terrestrial transfer of neonicotinoid insecticides in riparian food webs. *J. Hazard. Mater.* 455, 131635.

Supplementary Material – Aquatic-terrestrial transfer of neonicotinoid insecticides in riparian food webs.

Supplementary Data S1 – S7 (Note, only available in the electronic version of this thesis).

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Aquatic-terrestrial transfer of neonicotinoid insecticides in riparian food webs

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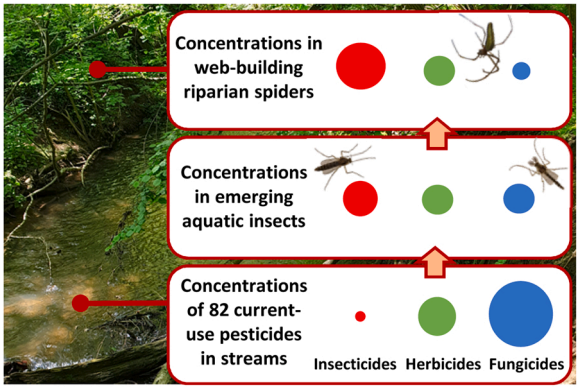
HIGHLIGHTS

- Neonicotinoids had the highest concentrations in the emerging insects and spiders.
- Concentrations of fungicides decreased between the aquatic environment and spiders.
- Riparian spiders could form a reservoir of neurotoxic insecticides in the food web.

GRAPHICAL ABSTRACT



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ABSTRACT

Current-use pesticides are ubiquitous in freshwaters globally, often at very low concentrations. Emerging aquatic insects can accumulate pesticides during their aquatic development, which can be retained through their metamorphosis into terrestrial adults. Emerging insects thus provide a potential, yet largely understudied linkage for exposure of terrestrial insectivores to waterborne pesticides. We measured 82 low to moderately lipophilic organic pesticides ($logK_{ow}$: −2.87 to 6.9) in the aquatic environment, emerging insects and web-building riparian spiders from stream sites impacted by agricultural land use. Insecticides, mainly neuro-active neonicotinoids were ubiquitous and had the highest concentrations in emerging insects and spiders ($\sum$ insecticides: 0.1–33 and 1–240 ng/g, respectively), although their concentrations in water were low, even when compared to global levels. Furthermore, neonicotinoids, although not considered to be bioaccumulative, were biomagnified in riparian spiders. In contrast, concentrations of fungicides and most herbicides decreased from the aquatic environment to the spiders. Our results provide evidence for the transfer and accumulation of neonicotinoids across the aquatic-terrestrial ecosystem boundary. This could threaten food webs in ecologically sensitive riparian areas worldwide.

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1. Introduction

Emerging aquatic insects link aquatic to terrestrial ecosystems by transporting matter and energy, supporting diverse communities of terrestrial insectivores at the land-water interface [1]. These insects are an important source of essential fatty acids which are not readily substituted by terrestrial insect prey [2–4]. Degradation of aquatic ecosystems through the introduction of micropollutants results in declines of sensitive insect orders and may negatively affect populations of insectivores, such as insectivorous birds or riparian spiders [5–7]. Recently, there has been growing interest in investigating the transport of micropollutants from contaminated surface waters to the surrounding terrestrial habitats by emerging aquatic insects [8–10]. As a result, this route of micropollutant transfer has been shown for a wide range of chemical classes, including metals [11–13], metal-based nanoparticles [14], polychlorinated biphenyls (PCBs) [15], per- and polyfluorinated alkyl substances (PFAS) [16], halogenated organic pollutants [17], pharmaceuticals [18] and pesticides [19–21]. The retention and transport of micropollutants can thus result in the dietary exposure of terrestrial insectivores, such as spiders and birds [10,19,22–25]. Among them, web-building riparian spiders are potential sentinels of aquatic pollution due to the high proportion of emerging insects in their diets [26,27].

Despite the presence of hundreds of pesticides in global surface waters, their transport by emerging insects has only been studied for a small fraction. For example, Laboratory studies of nine fungicides and herbicides found compound-specific and sex-specific effects on fungicide and herbicide concentrations in midges over their full lifecycle [20,21]. Additionally, insecticide-specific elimination rates during development affected the concentrations of three insecticides in adult emerging insects [21]. In a field study, Kraus et al. [19] detected seven pesticides and metabolites (out of targeted analyses for 16) in two taxonomic orders of emerging insects from wetlands impacted by agriculture. This study reported insecticide concentrations up to 577 ng/g and suggested the exposure of terrestrial insectivores as a consequence. The dietary exposure of terrestrial predators to pesticides through consumption of emerging insects has, however, been limited to calculations based on published consumption rates [19,20]. Empirical knowledge combining the detection of pesticides in both emerging aquatic insects and riparian predators (e.g. web-building riparian spiders) is lacking.

Systemic pesticides, characterised by high water solubility, regularly occur in aquatic environments at low concentrations [28–32]. This includes neurotoxic insecticides, among them the highly debated neonicotinoid insecticides [7,33]. Neonicotinoids often occur as mixtures and exhibit a chronic exposure profile [30,34,35]. Their mode of action results in negative impacts on aquatic invertebrate communities [35] and terrestrial food webs [36]. Despite their bioaccumulative potential being considered as low [36], bioaccumulation of neonicotinoids has recently been reported in aquatic macroinvertebrates in field studies [37,38]. Furthermore, in a laboratory study, the neonicotinoid thiacloprid was retained by emerging midges, in contrast to two other non-neonicotinoid insecticides [21], yet it remains unclear whether this applies to other neonicotinoids.

Drivers of pesticide bioaccumulation by emerging aquatic insects are not clear. Bioaccumulation and trophic magnification potential of organic molecules are related to their chemical lipophilicity (octanol-water partition coefficient K_{ow}) and metabolisation rates for moderately to highly lipophilic chemicals ($\log K_{ow} > 5$) [39]. Many currently used pesticides are, however, characterised by low to moderate lipophilicities ($\log K_{ow} < 5$). Furthermore, once accumulated by emerging insects during their aquatic development, concentrations of contaminants can be modified during metamorphosis [8]. The retention of lipophilic organic molecules ($\log K_{ow} > 5$) by emerging aquatic insects across metamorphosis potentially reflects biomagnification in food webs, correlating non-linearly with increasing lipophilicity [8]. On the other hand, small organic molecules with low to mid polarities ($\log K_{ow} < 5$) show the reverse relationship and it is unclear whether this relationship reflects the potential for trophic transfer within food webs [8,20]. These observations have, however, been based on a limited number of chemicals and chemical classes. Evaluations for a larger number of pesticides, which fall into this lipophilicity range, and how they correlate with the prevalence and concentrations in adult emerging insects and terrestrial consumers, such as riparian spiders, are lacking.

We provide new insight into the prevalence and concentrations of a larger number of currently used pesticides in aquatic habitats, emerging insects and riparian spiders. For this, we validated an analytical methodology for the measurement of 82 currently used pesticides in small-volume insect samples (30 mg) by high-performance liquid chromatography tandem to triple quadrupole mass spectrometry by electrospray ionization (HPLC-ESI-MS/MS). We then investigated the prevalence and concentrations of these pesticides in the aquatic environmental compartments (water, sediment and aquatic leaf litter) and the terrestrial biological compartment (adult *Tetragnatha* spp. riparian spiders) collected from ten stream sites differing in the degree of agricultural impact. The sampling sites were sheltered from direct impacts by agricultural activities, such as spray drift during pesticide application. We furthermore aimed to establish the link between aquatic and terrestrial compartments by quantitative and qualitative evaluation of these same pesticides in stream water, rainwater and three orders of emerging aquatic insects (namely, Diptera, Ephemeroptera and Trichoptera), which were collected from a subset of the sampling sites. We categorised pesticides based on their detection frequencies in different compartments to elucidate the potential importance of a range of pesticide properties. Finally, we test the hypothesis that the transport of thiacloprid by emerging insects occurs at low concentrations commonly found in global surface waters, and also occurs for other neonicotinoid insecticides.

2. Materials and methods

2.1. Study sites and sampling overview

Sampling took place at stream sites in the upper Rhine valley of the Palatinate region of South-West Germany (Fig. S6), an area that includes a variety of crops typically grown in Europe. In 2020, water and emerging insect samples were collected from two streams, namely the Modenbach (MB, 49°16'50.4"N 8°16'53.0"E) and Spiegelbach (SPI, 49°11'13.6"N 8°18'44.6"E). In 2021, adult riparian spiders (*Tetragnatha* spp.), sediment samples and aquatic leaf litter were collected from these two sites along with a further eight sites, namely the Katzenbach (KB, 49°16'12.0"N 7°57'58.0"E), Eußerbach (EB, 49°14'20.1"N 7°58'34.4"E), Ranschbach (RB, 49°11'57.0"N 8°04'55.0"E), Queich Site 1 (QS1, 49°12'01.0"N 8°05'40.0"E), Queich Site 2 (QS2, 49°12'04.7"N 8°08'16.1"E), Queich Site 3 (QS3, 49°12'19.1"N 8°11'32.0"E), Queich Site 4 (QS4, 49°12'39.0"N 8°13'43.0"E) and Queich Site 5 (QS5, 49°13'19.0"N 8°16'12.2"E). Two of these sites, namely KB and EB are located within a forested region with very limited agricultural activities and were therefore considered the least impacted by pesticides. The remaining eight sampling sites lie on streams which flow from West to East through a region which is characterised by intensive agriculture (Fig. S6). The sampling sites were therefore carefully selected to be sheltered from agricultural activities by areas of dense natural vegetation to minimise the potential impacts of deposition as a result of spray drift. RB, which was separated by approximately 60 m of forest from the nearest agriculturally used land, was the site nearest to agricultural activities among all sampling sites. Potential atmospheric deposition resulting from rainfall was, however unavoidable. Therefore, rainwater samples were also collected from two sites during 2021, rainwater sampler 1 was located at a site within the forest (Eußerthal Ecosystem Research Station, 49°15'15.2"N 7°57'42.3"E) and rainwater sampler 2 was located within the agricultural landscape (QS2, 49°12'04.7"N 8°08'16.1"E) (Fig. S6).

2.2. Water and emerging aquatic insect sampling in 2020

Weekly water grab samples and emerging insect samples were collected from 28.04.20 to 14.07.20. Water grab samples were collected mid-stream in clean amber glass bottles (1 L) from the two sampling sites, MB and SB (Fig. S6). Bottles were completely submerged and capped under the water surface during sample collection. All water samples were transported directly to the laboratory where 10 mL was transferred to a clear glass storage vial capped with an aluminium foil lining. The samples were then frozen and stored at - 20 °C until pesticide analysis. Three emergence traps were installed at each site to collect emerging aquatic insects. Floating emergence traps were constructed based on previously published designs [40]. Briefly, each trap covered a surface area of 0.25 m^2. A pyramid-shaped mesh tent on top of the floating base had an opening where a polypropylene sampling bottle was attached to collect flying insects. Of the three traps at each sampling site, two had a bottle with 125 mL of trapping liquid (30% propylene glycol, 70% deionized water by volume, 1 mL/L dish soap and 10 mg/L denatonium benzoate) to capture and preserve adult emergent insects, while the third contained no fluid. Emerging insects caught in the trapping fluid were collected weekly, while those in the bottles without fluid were collected after 24–48 h. Live insect samples were frozen and stored at − 80 °C. Samples in trapping fluid were stored at 4 °C. Both live-caught and fluid-caught insects were identified to the order level [41,42]. The frozen samples of the live caught insects were kept on ice during identification and sorting to prevent degradation. Order-specific biomass was estimated by measuring the body length of each insect caught with catching fluid to the nearest millimetre. Dry biomass was then calculated based on order-specific reference values using the method of Sabo et al. [43]. The frozen insect samples were then freeze dried and weighed using an MT5 analytical microbalance (d = 0.001 mg, Mettler-Toledo GmbH, Gießen, Germany) before being stored at − 80 °C. In order to obtain the required biomass for the pesticide measurements, insects were pooled by order which resulted in 15 samples with final sample weights of 26.49 ± 7.19 mg. Six replicate dipteran samples were obtained from MB, but only one from SB. One ephemeropteran sample was obtained for MB and three for SB, while both sites had sufficient trichopteran biomass to yield two samples each.

2.3. Water, sediment, aquatic leaf litter and riparian spider sampling in 2021

All ten sampling sites were sampled twice during 2021 (Fig. S6). Once in June (21.06.21 to 25.06.21) and once in July (12.07.21 to 16.07.21), which covered the summer pesticide application period. Sediment samples were collected by scooping the surface layer of the sediment using a square shovel (10 ×10 ×2 cm). Five sediment samples were randomly collected at each site and pooled to create a composite sample. Aquatic leaf litter, which serves as a habitat for many macroinvertebrates, was collected from the streambed at each site. Between 10 and 20 sexually mature *Tetragnatha* spp. spiders were collected from vegetation or from their webs directly overhanging the water surface at each site. A daily grab water sample was collected from QS2 (Fig. S6) for 47 consecutive days covering the sampling period (07.06.21 to 23.07.21). Rainwater samples were collected using in-house constructed samplers consisting of a stainless-steel funnel (diameter 30 cm) fixed to a brown glass bottle (1 L) housed in a styrofoam insulated box, which was installed approximately 1.5 m above ground level away from overhanging vegetation. Rainwater in the forest (Rainwater sampler 1) was sampled for 13 days in June (09.06.21 to 22.06.21), which coincided with the first round of field site sampling. Rainwater at QS2 (Rainwater sampler 2) was sampled during both rounds of field sampling in June (21.06.21 to 24.06.21) and July (09.07.21 to 14.07.21). Additionally, the volume of precipitation at rainwater sampler 1 was recorded by an MWS10-Weather station (Reinhardt System- und Messelectronic GmbH, Dießen am Ammersee, Germany) and at rainwater sampler 2 by udometer.

Water, sediment and leaf litter samples were all frozen after collection and stored at − 20 °C before processing for pesticide measurements. The live spiders were kept individually in plastic containers covered with a 1 mm nylon mesh at 20 °C for 72 h before being frozen and stored at − 80 °C prior to further processing and pesticide measurements. The 72-hour waiting period after collection allowed the spiders to clear their gut content while ensuring a high survival rate. Spiders that died during the 72-hour depuration period were not included in pesticide measurements. Frozen samples of each sample type were freeze dried. Aquatic leaf litter samples were then checked for macroinvertebrates, which were removed before the samples were ground and homogenised using a mortar and pestle. Both sediment and ground leaf litter samples were sieved to 1 mm. Separate subsamples of each sediment and leaf litter (n = 20 each) were weighed on a Sartorius CP225D balance (d = 0.01 mg, Sartorius Lab Instruments GmbH & Co. KG, Göttingen, Germany) for pesticide analysis. Subsamples of sediment and leaf litter had average weights (± standard deviation) of 5.04 ± 0.03 and 1.02 ± 0.01 g, respectively. Spiders were weighed on an MT5 analytical microbalance. Overall, female spiders were more abundant and had greater dry weights than male spiders across all 10 sites. In order to obtain suitable samples for pesticide measurements, individual spiders were pooled by sex and sampling site. This resulted in a total of 45 samples of female spiders, from all ten sites, each containing three to nine individuals with an average weight of 29.80 ± 2.60 mg. Similarly, a total of 34 samples of male spiders containing one to four individuals with an average weight of 6.39 ± 1.61 mg were prepared. Overall, six to eleven spider samples were analysed for pesticides per sampling site.

2.4. Pesticide concentration measurements

Pesticides were measured in all samples by HPLC-ESI-MS/MS. Analytical standards were obtained from Restek (Bad Homburg, Germany). Solvents (LC-MS Grade) were purchased from Honeywell (Seelze, Germany). Instrument parameters used for the measurements are provided in the supplementary information (Table S5).

2.4.1. Pesticides measurements in sediment and aquatic leaf litter samples

The methods used for the extraction and analyses of sediment and aquatic leaf litter have been reported elsewhere [44]. Briefly, samples of sediment and leaf litter were spiked with 50 μL of deuterated internal standards (pirimicarb-D6, thiacloprid-D4 and thiamethoxam-D3 in acetone) to achieve a final concentration of 2 μg/kg in the measured extract. The samples were air dried for 30 min before either 5 or 1 g of ammonium formate was added to the extraction tube for sediment and leaf litter samples, respectively. Subsequently, 10 mL of acetonitrile containing 2.5% formic acid was added and the samples were shaken for 60 min in an overhead shaker. Samples were then centrifuged for 6 min at 3000 rpm. The supernatants from sediment samples were then filtered through a 0.2 μm PTFE filter prior to pesticide measurements. The supernatants from leaf litter samples were first transferred to a vial containing graphitised carbon black (GCB) powder 7.5 mg/mL and vortexed for 30 s before centrifugation and filtering prior to pesticide measurements.

2.4.2. Pesticides measurements in water samples

A direct-injection HPLC-ESI-MS/MS method was validated and used for measurements of pesticides in water samples. Details of the validation method and pesticide LOQs are provided in the supplementary materials. Frozen water samples were defrosted and centrifuged at 16000 rpm at 20 °C for 10 min, after which 350 μL was transferred to an amber-glass vial. Each sample was diluted with 150 μL of methanol containing a mixture of deuterated internal standards (pirimicarb-D6, thiacloprid-D4 and thiamethoxam-D3, 3 μg/L) and 0.3% formic acid. Water samples were always measured directly after being prepared. A calibration series with 11 concentrations covering the concentration

range from 0.3 to 2000 ng/L was prepared using MS-grade water, in addition to solvent blanks.

2.4.3. Pesticides measurements in insects and spider samples

The extraction method was previously optimised in our laboratory (results not shown here). The method was validated using criteria published by the International Council for Harmonisation, guideline Validation of Analytical Procedures: Text and MethodologyQ2(R1) [45]. Details of the validation method and pesticide LOQs are provided in the supplementary materials.

Samples of freeze-dried insects and spiders were pulverised using a Tissuelyzer (Retsch MM 301, Haan, Germany) and 2.5 mm diameter steel pellets. Samples of dry insect material were weighed into 2 mL polypropylene tubes using an MT5 analytical balance (d = 0.001 mg). Internal standards (pirimicarb-D6, indoxacarb-D3 and thiacloprid-D4) were added to each sample for a final extract concentration of 0.48 ng/mL. Extractions were performed with 1 mL of acetonitrile containing 0.1% formic acid. Samples were vortexed for 30 s, after which they were sonicated for 5 min and centrifuged for a further 5 min at 16000 rpm. Subsequently, a dispersive solid phase extraction clean-up was performed by pipetting 850 μL of the extract to a new sample tube containing 24 mg of Z-Sep+ and primary-secondary amine (PSA). The mixing, sonication and centrifugation steps were repeated as before. After centrifugation, 700 μL of the extract was pipetted into a glass vial which was placed under a gentle stream of nitrogen gas until all the solvent had evaporated. The residues were then dissolved in 500 μL of a mixture of water and methanol (70:30, v/v) containing 0.1% formic acid and 0.5 ng/mL thiamethoxam-D3 as an internal standard. Matrix and sample-weight matched calibration series were prepared with ten concentrations ranging between 0.01 and 16 ng/g dw, in addition to matrix blanks.

2.5. Data evaluation and statistics

Order-specific biomass of emerging insects was calculated by multiplying the number of individuals in each sample by the estimated dry biomass calculated from their length measurements (Table S1). The site-specific average weekly pesticide flux was then estimated by multiplying the average weekly emergence flux by the average total pesticide concentration (Table S2). Biota-water accumulation factors (BWAFs) were calculated for each measured pesticide concentration in individual insect samples by dividing the concentration by the respective site-specific median concentration in the weekly water samples. Differences in BWAFs between orders of emerging insects (Fig S4), or concentrations of pesticides in spiders and emerging insects were tested for significance using a Kruskal-Wallis rank sum test with post hoc Dunn's test using Bonferroni correction when differences were detected ($p < 0.05$). A principle component analysis was performed on pesticides that had been categorised according to their frequency of detection in abiotic (sediment, aquatic leaf-litter and water) and biotic compartments (Emerging insects and spiders). Pesticides were categorised as either "transferred" or "not-transferred". The categorisation was performed using frequency data for sediment, leaf litter and spider samples from the sites QS1 to QS5, MB and SB because these sites were similarly contaminated by agriculture (as opposed to the less impacted upstream sites KB and EB). Additionally, representative frequencies of pesticide detection in water were available for these sites. Pesticides that were frequently detected in the abiotic compartments (>70% detection frequency in at least one compartment), but had no detections in spider samples, or emerging insects in the case of MB and SB, were categorised as "not transferred". Similarly, pesticides that satisfied these criteria, but were consistently detected in spider samples (and emerging insects at MB and SB) were categorised as "transferred". This process yielded eleven pesticides which were categorised as "transferred" and seven as "not-transferred" (Data S7). A principle component analysis was performed on values representing physicochemical properties, toxicity and environmental persistence of these pesticides (Table S3). These parameters were chosen because they have the potential to impact the transport and bioaccumulation of pesticides across the aquatic-terrestrial food web. The parameters included the logarithmically transformed values for the Henry's law constant (HLC), aqueous solubility (S), topological polar surface area (TPSA), monoisotopic mass (MIM) octanol-water partition coefficient (K_{ow}), first dissociation constant (pK_{a1}), water-phase half-life (DT50) and the chronic 28-day no observed effects concentration for *Chironomus riparius* (MidgeNOEC28). For neutral pesticides, the pK_{a1} was assigned the value 14. Additionally, pesticides for which no appropriate NOEC was available, the proxy value of 100 mg/L was used. Statistical analyses were performed in R version 4.2.2. [46].

3. Results

3.1. Prevalence and relative concentrations of pesticides in riparian spiders and the adjacent aquatic environment

Twenty-nine pesticides were detected in spider samples across all ten sampling sites (Data S1). This included thirteen fungicides and five herbicides at relatively low concentrations, with sum average concentrations (SACs): < 6.2 and < 1.6 ng/g, respectively (Fig. 1 A and B). Eleven insecticides were measured at higher concentrations, with SACs: 2.1–94.2 ng/g (Fig. 1 C). In contrast, aquatic leaf litter and sediment samples frequently contained many more fungicides (20–23 at the eight most impacted sites, with median detection frequencies of 88% and 38% in leaf litter and sediment, respectively (Data S2). This included all the fungicides detected in the spiders. The fungicide concentrations were also the highest of the three pesticide classes (SACs: 60.8–340.4 ng/g in leaf litter and 2.1–85.5 ng/g in sediments, Fig. 1. A). Up to six herbicides were detected in leaf litter and sediment with median detection frequencies of 70% and 25%, respectively (Data S2). The herbicides were present at lower concentrations (SACs: 1.9 – 18.3 ng/g in leaf litter and < 0.1 ng/g in sediments, Fig. 1. B), two of which were also present in spider samples (Data S1). Eight insecticides were detected in sediment and leaf litter with the lowest frequency of all classes (median detection frequencies of 25% and 13% in leaf litter and sediment, respectively, Data S2). They also had the lowest concentrations of any class (Fig. 1. C, SACs: < 12.7 and < 0.6 ng/g in leaf litter and sediments, respectively). Five of the insecticides detected in the leaf litter and sediment were also detected in spider samples (Data S1).

Insecticides were the largest and most frequently detected group of pesticides in spider samples from individual sites (Fig. 2, Data S1). Spider samples collected from the two least impacted sites, KB and EB, contained up to five insecticides, while those collected from the remaining eight most impacted sites contained up to ten (Data S1). The insecticides included four neonicotinoids, namely acetamiprid, clothianidin, imidacloprid and thiacloprid; fipronil and its sulfone metabolite; two ryanoid insecticides, namely, chlorantraniliprole and cyantraniliprole; the butanolide insecticide, flupyradifurone; tebufenozide and spinosad. The four neonicotinoids were among the most frequently detected insecticides and were present in 16 – 100% of spider samples across all ten sites (Data S1). These four neonicotinoids also had the highest site-specific average concentrations (up to 46 ng/g) compared to other insecticide classes (up to 14.9 ng/g, Fig. S1). Moreover, the overall median concentrations of acetamiprid, clothianidin, imidacloprid and thiacloprid (3.2, 0.9, 14.4 and 12.6 ng/g, respectively) were all higher than the median concentration of other (non-neonicotinoid) insecticides (0.5 ng/g, Fig. 2 A). Concentrations of individual fungicides and herbicides were also comparatively low compared to the neonicotinoids (site-specific average concentrations < 7 ng/g dw, Fig. S2) and overall median concentrations of 0.4 and 0.5 ng/g, respectively (Fig. 2 A).

Water samples taken at three out of the ten sites accounted for pesticides that were present in spiders but not present in leaf litter or sediment (Data S1–3). Complex mixtures of between 15 and 32, 9 – 35

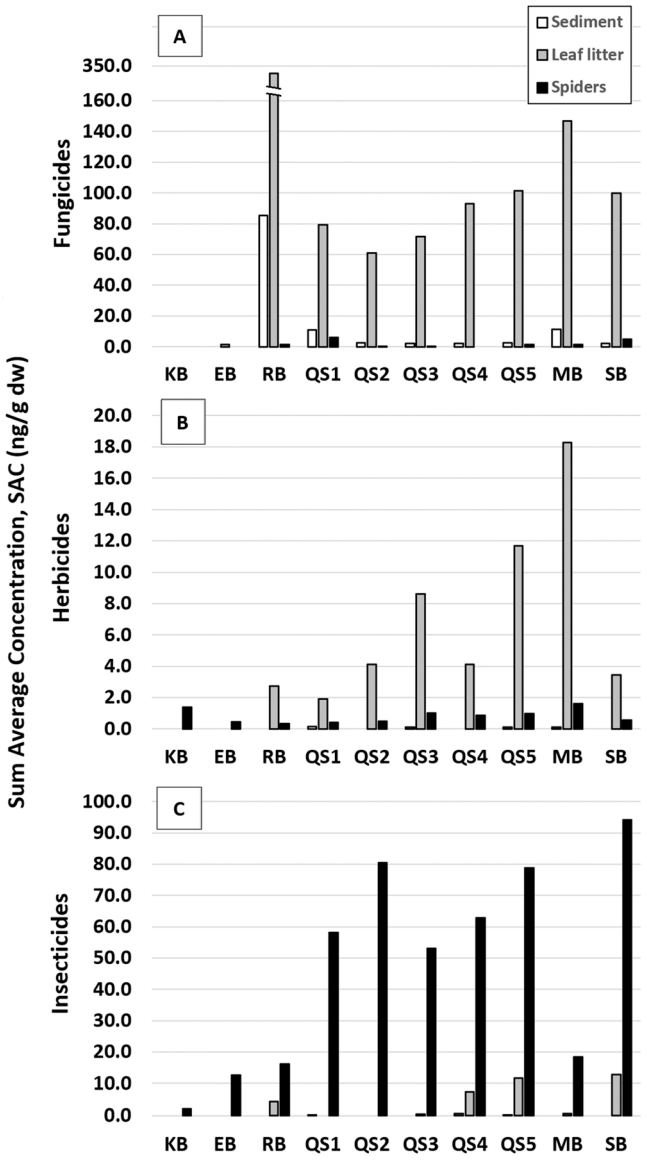

Fig. 1. Sum average concentrations (SACs) of fungicides (A), herbicides (B) and insecticides (C) in sediment (white bars), leaf litter (grey bars) and spiders (black bars) from ten sampling sites with differing degree of agricultural impact.

and 26 – 36 pesticides were detected in individual water samples collected from QS2, MB and SB, respectively (Data S3). Up to twenty fungicides were chronically present at all three sites (median detection frequencies: 73%, 77% and 65% at QS2, MB and SB, respectively) with the highest median concentration (0.01 ng/mL, Fig. 2B.) of the three pesticides classes. Individual sampling sites had moderate to high concentrations (∑median concentrations: 0.9, 0.1 and 0.2 ng/mL at QS2, MB and SB, respectively, Data S3). Up to eleven herbicides were frequently detected with low to high concentrations (median detection frequencies: 70%, 27% and 92% and ∑median concentrations: 0.04, 0.8 and 0.1 ng/L at QS2, MB and SB, respectively, Data S3) and an overall median concentration of 0.004 ng/mL (Fig. 2 B). Up to nine insecticides had the lowest overall median concentration of 0.003 ng/mL and were measured with moderate to high frequency and low to moderate concentrations at individual sites (median detection frequencies: 40%, 45% and 85% and ∑median concentrations: 0.05, 0.04 and 0.06 ng/mL at QS2, MB and SB, respectively, Data S3).

Mixtures of up to five neonicotinoid insecticides were chronically present in water at low concentrations (Data S3). Thiacloprid was detected in 100% of samples collected from all three sites. Moreover, acetamiprid and imidacloprid were detected in 100% and thiamethoxam was detected in 85% and 94% of samples from SB and QS2, respectively (Data S3). Clothianidin was only detected in five water samples overall but is the product of thiamethoxam metabolism in organisms [47]. This is consistent with the observation that only clothianidin was measured in the spiders and not thiamethoxam. The water concentrations of these two neonicotinoids were thus plotted together in Fig. 2 B. Overall, mixtures of neonicotinoids accounted for between 13% and 100% of total insecticide concentrations in each sample across all three sites. Furthermore, both the 50th and 90th percentiles of the individual neonicotinoid concentrations measured in the present study were considerably lower than what has been reported recently for global surface water concentrations (Table 1)).

Rainwater deposited small amounts of between 28 and 41 pesticides (Data S4). This included fourteen of the pesticides detected in the spiders collected from the least impacted sampling sites, KB and EB (Data S1 and S4). However, amounts of pesticides deposited via rainfall were extremely small during the sampling period (0.002–0.6, 8×10^{-5} to 0.3 and 8×10^{-5} to 0.04 ng/cm^2 for individual fungicides, herbicides and insecticides, respectively) and therefore do not provide a likely explanation for high concentrations of especially neonicotinoids in spider samples (Data S4).

3.2. *Aquatic-terrestrial pathway of pesticides via emerging insects to spiders*

Eleven fungicides, eight herbicides and eight insecticides were detected in at least one emerging insect sample (Fig. 3; Fig. S3; Data S5). Individual concentrations of fungicides, herbicides and insecticides ranged from 0.02 to 3.7, 0.05–1.9 and 0.02–23.2 ng/g, respectively (Fig. S3). The majority, 83%, of concentrations were below 1 ng/g, but two neonicotinoids, thiacloprid and imidacloprid, had consistently higher concentrations, which were up to 23.2 and 6.7 ng/g, respectively (Fig. S3). The neonicotinoids, acetamiprid and thiacloprid were the most frequently detected pesticides, found in 90–100% of all samples from each site. Of the 19 fungicides and herbicides detected in the emerging insects, 11–16 were detected in the aquatic leaf litter and sediment samples (Data S5). However, only two of the insecticides detected in the emerging insects were also detected in the aquatic leaf litter and sediment; water samples contained the pesticides detected in the insects but not in sediment or leaf litter (Data S5). Biota-water accumulation factors (BWAF) for the emerging insects covered a range of approximately 1.8 – 12300, with 84% of the values lying between 10 and 1000 (Fig. 3). The neonicotinoid, thiacloprid, had the highest BWAFs (up to 12300, Fig. 3).

Emerging dipterans made the greatest contribution to the flux of insect biomass (70–90%) and pesticides (94–96%) at both sampling sites (Table S1). Dipterans also had significantly higher BWAFs for two insecticides, namely thiacloprid and dimethoate, compared to the other insect orders (Fig. S4). Average total pesticide concentrations were the lowest in trichopterans at both sites compared to the other two orders (Table S2). Thus, despite contributing approximately 10–25% to the emerging biomass (Table S1), they contributed similarly to the weekly pesticide flux as the ephemeropterans, which contributed only approximately 1–5% of the biomass. The concentrations and prevalence of fungicides and most herbicides were greater in emerging insects than in spiders, whereas the opposite was true for insecticides (Data S5).

Biomagnification in spiders was observed for three neonicotinoids and one herbicide (Fig. 4). Significantly higher concentrations (factor of 6–15) of the neonicotinoids acetamiprid, imidacloprid and thiacloprid, were observed in female spiders compared to the emerging insect samples collected from SB. Male spiders from this site had concentrations that were a factor of 3–5 times higher than found in the emerging insects, although not statistically significant. Acetamiprid concentrations were significantly higher (factor 15–32) in both spider sexes at MB, as well as for the herbicide propyzamide in male spiders (by factor 7).

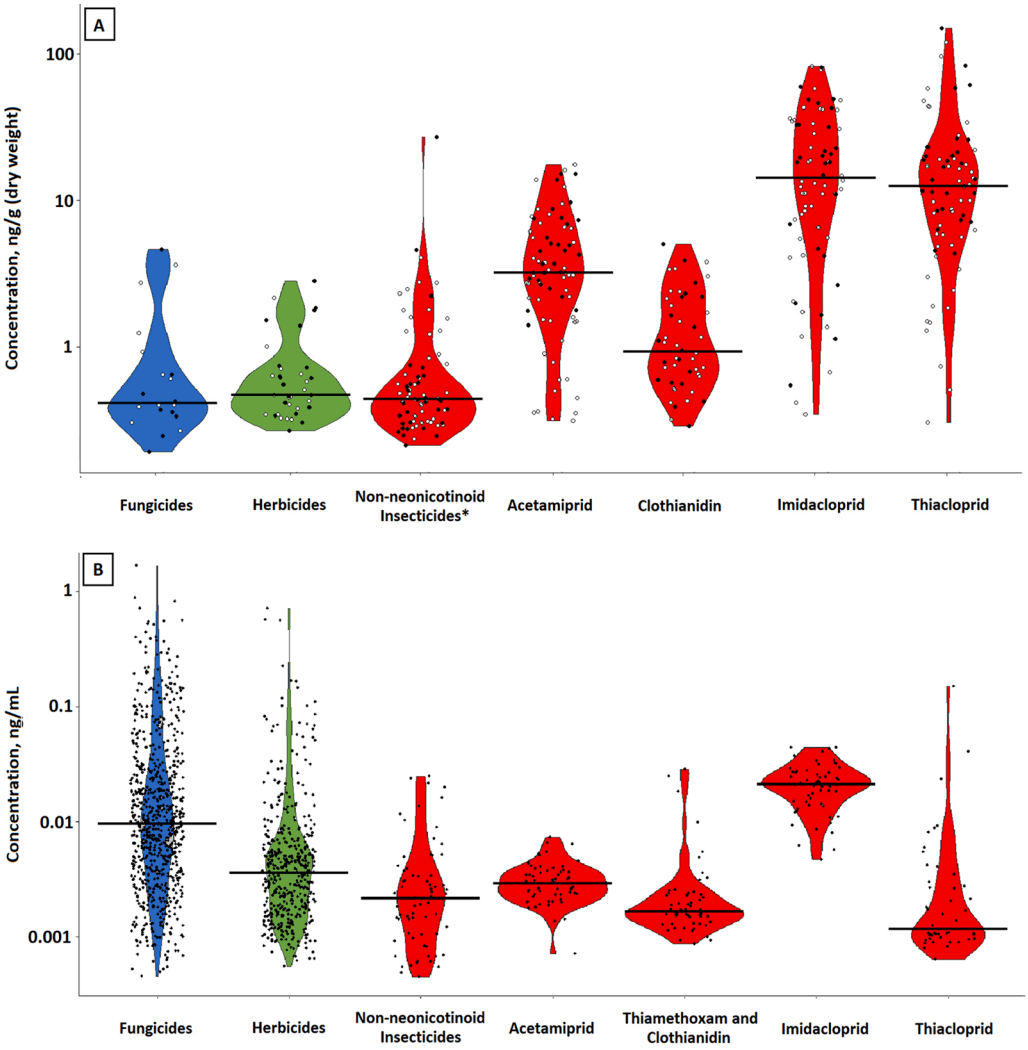

Fig. 2. Pesticide concentrations in spider and water samples from stream sites. (A) Pesticide concentrations are reported for individual spider samples (n = 79 samples) collected from ten stream sites. Points indicate individual detections above the analytical limits of quantification for fungicides (n = 20 detections), herbicides (n = 38 detections), other (non-neonicotinoid) insecticides (n = 77 detections) and the neonicotinoid insecticides; acetamiprid (n = 77 detections), clothianidin (n = 49 detections), imidacloprid (n = 74 detections) and thiacloprid (n = 76 detections). Spider samples collected from the three sites where water samples were also collected are indicated with solid black points. (B) Pesticide concentrations in daily water samples from QS2 and weekly water samples from MB and SB (n = 71 pooled from all three sites). Horizontal lines indicate the median concentrations. Violin plot colours indicate the pesticide class (blue – fungicides, green – herbicides and red – insecticides). *Non-neonicotinoid insecticides in spider samples includes concentrations for fipronil's sulfone metabolite.

Table 1
Concentrations of neonicotinoids in water samples compared to global values. Global values are calculated from data in a recent meta-analysis by Stehle et al. [30].

Neonicotinoid insecticide	50th percentile concentration (ng/L) in field samples	Equivalent global concentration percentile	90th percentile concentration (ng/L) in field samples	Equivalent global concentration percentile	Number of field measurements	Number of reference measurements
Acetamiprid	2.2	22.5	3.6	30.7	61	272
Clothianidin	5.2	29.8	10.0	44.3	6	951
Imidacloprid	15.0	47.6	27.0	60.6	62	1305
Thiacloprid	0.9	10.0	8.4	66.5	47	246
Thiamethoxam	1.2	8.8	2.3	19.2	55	785

Principle component analysis of eight parameters associated with physicochemical properties, toxicity and stability of 18 pesticides (Table S3), which were grouped according to their frequency of detection in abiotic and biotic compartments (Data S7) did not yield a separation of groups (Fig. S5, Table S4).

4. Discussion

4.1. Pesticides in riparian spiders and the adjacent aquatic environment

Sum average concentrations (SACs) of insecticides in spiders were four orders of magnitude greater than fungicides and herbicides (Fig. 1). Furthermore, the insecticide SACs were composed of 78–100% by four neonicotinoids, out of a total of eleven insecticides detected in the spiders (Fig. 2 A, Fig. S1, Data S1), revealing a selective bioaccumulation. Insecticide SACs in the present study (Fig. 1 C) were at least a factor of approximately 10–20 times lower than what has been reported for sum per- and polyfluorinated alkyl substances (PFAS) and sum polychlorinated biphenyls (PCBs) in tetragnathid spiders feeding on emerging insects, but similar to concentrations of pharmaceuticals and endocrine disrupting substances [16,48,49]. As far as the authors are aware, no data on pesticide concentrations in riparian spiders exists. Due to their neurotoxic mode of action and potential for synergistic and cumulative toxicity, mixtures of neonicotinoids may have a high potential to disrupt food webs through sublethal effects even when accumulated at lower concentrations compared to more bioaccumulative

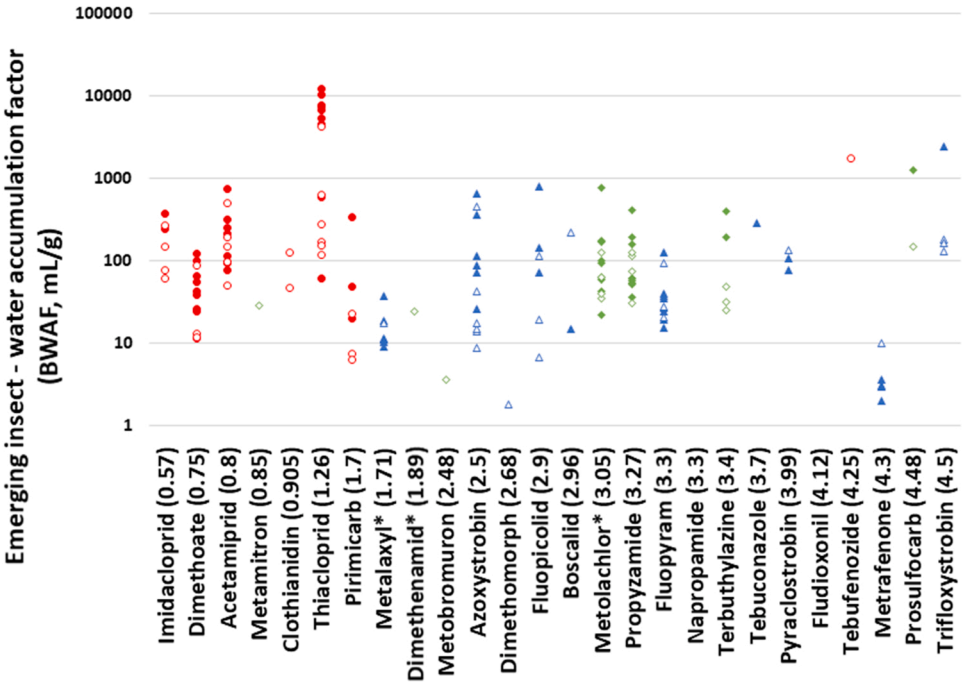

Fig. 3. Biota-water accumulation factors (BWAF) for pesticides in emerging aquatic insects. Values shown for fungicides (blue triangles), herbicides (green diamond) and insecticides (red circles) in samples of emerging insects (including Diptera, Ephemeroptera and Trichoptera) collected from two stream sites affected by agricultural land use, namely the Modenbach (MB, solid shapes) and the Spiegelbach (SB, outlined shapes). Pesticides are arranged from left to right in order of increasing lipophilicity ($logK_{ow}$ values are provided in brackets). *BWAFs are reported for the sum of isomers.

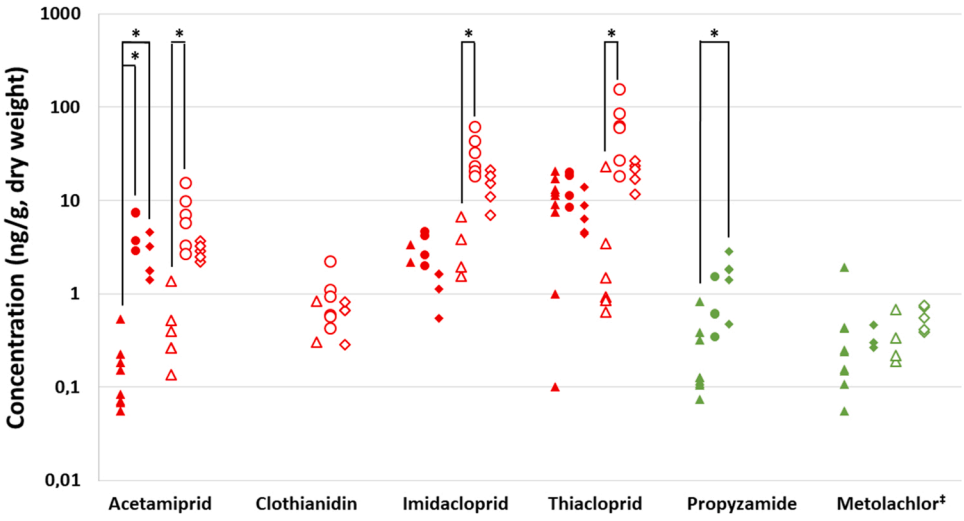

Fig. 4. Pesticide concentrations in emerging insects and riparian spiders (adult *Tetragnatha* spp.) Samples were collected from two stream sites impacted by agricultural activities (Modenbach, MB – solid black shapes and Spiegelbach, SB – outlined shapes). Pesticide concentrations in emerging aquatic insect samples, comprised of dipterans, ephemeropterans and trichopterans, are indicated by triangles, while concentrations in female and male spiders are indicated by circles and diamonds, respectively. Insecticide concentrations are shown in red and herbicides in green. Asterisks indicate a significant difference in concentrations between groups (Kruskal-Wallis rank sum test with post hoc Dunn's test using Bonferroni correction, $p < 0.05$). ‡Concentrations are reported as the sum of isomers.

chemical classes [35,36,50].

Mixtures of neonicotinoids in water samples were chronically present at low concentrations, which are typically exceeded in agricultural surface waters worldwide (Fig. 2 B, Table 1 and Data S3). In fact, the 90th percentiles of individual neonicotinoid concentrations detected in the present study corresponded with the 19th to 67th percentiles of neonicotinoid concentrations detected globally (Table 1). This hydrophilic class of insecticides (log K_{ow}: 0.57–1.26) represented a substantial proportion of insecticide usage (approximately 20%, contributed primarily by thiacloprid and acetamiprid) in Germany during the period 2019–2021 [51]. Their ubiquitous presence at the sampling sites likely results from their preemptive use, high solubility and resulting high mobility in soils [36]. It should also be noted that the neonicotinoids clothianidin, thiamethoxam and imidacloprid were banned for outdoor use in the entire EU by the end of 2018 [52–54], yet were found in water and spiders collected in 2021. This implies that either the half-life times in the environment are longer than those used during the regulatory risk assessment [55], considerably higher concentrations were present until the end of 2018 or illegal pesticide use took place. The fungicides and herbicides measured were all more lipophilic ($logK_{ow}$: 1.7–4.5) compared to the neonicotinoids, which correlated with their higher prevalence and concentrations in aquatic sediment and leaf litter (Fig. 1 A and B). Chronic low to medium concentrations of many different fungicides are common in aquatic environments globally, due to their prophylactic but mixed applications to prevent outbreaks while controlling for pest resistance [29].

4.2. *Aquatic-terrestrial transfer via emerging insects to spiders*

Emerging insects contained a broad range of pesticides, including those found in the spiders and were the mediators between low insecticide concentrations in the aquatic environment and high concentrations in riparian spiders. Fungicides, in contrast, were more prevalent and showed higher concentrations in emerging insects compared to

spiders (Data S5). In the present study, developmental stages of aquatic insects were exposed to insecticides with low lipophilicity ($logK_{ow} < 1.7$) at very low concentrations primarily through water, while other pesticide classes with higher lipophilicity ($logK_{ow}$ 1.7–4.5) had higher concentrations in water, sediment and leaf litter (Data S5). However, despite an exposure- and lipophilicity-gradient, BWAFs remained within a similar range for 84% of measurements (Fig. 3). This might be explained by a combination of bioaccumulation and retention processes over the emerging insect life cycle. Under laboratory conditions, bioconcentration of low to medium-polarity pesticides ($logK_{ow}$: 2–5) generally increases with pesticide lipophilicity in aquatic larvae [56], thus favouring fungicides and herbicides in the present study. Retention of accumulated pesticides across metamorphosis, however, generally follows the opposite pattern, decreasing with increasing lipophilicity [8,20], and thus favours the hydrophilic insecticides. Moreover, the rate of pesticide-specific elimination by developmental stages can determine concentrations in the adults. For example, a slow elimination rate was responsible for the selective transport of the neonicotinoid, thiacloprid, over more rapidly eliminated insecticides by emerging insects in a previous laboratory study [21]. The results from the present study indicate that this is also true for other neonicotinoids. Thus, chronic sub-lethal concentrations of neonicotinoids in the aquatic environment have a higher than expected propensity to be retained and transported to riparian spiders by emerging aquatic insects. The generally more lipophilic fungicides and herbicides, however, appear to be more easily eliminated by the emerging insects, resulting in lower concentrations in adult insects even when exposure takes place at persistently higher concentrations compared to the neonicotinoids.

Individual neonicotinoids share a propensity to be transported by emerging insects and bioaccumulated by riparian spiders. The tendency for pesticides to be transported by emerging insects could, however, not be explained by evaluation of their physicochemical properties, toxicity or persistence (Fig. S5, Table S4). The majority of pesticides measured in the present study have systemic properties strongly linked to their solubility, which facilitates their dispersal and movement through plants and insects [57]. It is therefore unsurprising that differences in the physicochemical properties of pesticides were not sufficient to differentiate between those pesticides with a high or a low propensity to be transported by emerging insects and detected in spiders. A similar selective bioaccumulation of neonicotinoids in the presence of complex mixtures of pesticides, as in the present study, has been reported in earthworms under laboratory conditions [58]. The neonicotinoids used in Chevillot et al. [58] as well as those from the present study, are first- and second-generation neonicotinoids, which share a common structural backbone and stearic conformations that are essential to their systemic behaviour and mode of toxic action [59,60]. Specific binding of neonicotinoids to proteins or other large biomolecules has been put forward by several authors to explain the differences between the predicted and measured toxicokinetics of neonicotinoids in aquatic crustaceans [38,61]. A mechanism involving specific binding is further supported by the enantioselective bioaccumulation rates in earthworms reported for dinotefuran, the only neonicotinoid containing a stereocenter [62]. Furthermore, flupyradifurone, which is a newer generation butenolide insecticide [63] structurally related to the neonicotinoids, was frequently detected at low concentrations in spiders in the present study. It was, however, not detected in the water or emerging insects (Data S1 and S5). The volume of this insecticide applied was < 1% of the total neonicotinoids applied during the sampling period [51], which could potentially have resulted in these concentrations lying below the analytical detection limits [64]. A mechanism of biomagnification similar to the neonicotinoids could explain the results in spiders, it can, however, only be speculated from the current data.

The bioaccumulation and biomagnification of neurotoxic insecticides across the aquatic-terrestrial ecosystem boundary have potential negative impacts on terrestrial food webs. Emerging dipterans contributed the most to both the overall pesticide flux and transport of specific insecticides in the present study (Fig. 4). This result is relevant for the exposure of terrestrial insectivores considering that dipterans have a very wide emergence window, subsiding only during the coldest months of the year [65]. Furthermore, communities of emerging aquatic insects disturbed by agricultural activities shift toward dominance by more tolerant dipterans, often with an increase in overall emergence biomass [4]. This implies the potential for a near-constant flux of neurotoxic insecticides from contaminated surface waters. Moreover, this could potentially include sites with very low insecticide input, as seen at the upstream forested sites in the present study (Data S1), where atmospheric deposition is assumed to contribute to concentrations in the aquatic environment (Data S4). Furthermore, spiders are fairly tolerant towards neonicotinoids in comparison to insects [66] and could create a reservoir for these insecticides in the food web. Both emerging aquatic insects and web-building riparian spiders can serve as prey for small birds and bats, the latter of which can consume 25–100% of their body weight in a single night especially in times of peak energy requirement (e.g. reproduction) [48,67]. Dietary exposure to neurotoxic insecticides can cause several sublethal effects in vertebrates [68,69]. These sublethal effects include, for example, reduced fecundity, raised stress hormone concentrations, reduced immune response, disorientation and other behavioural effects [37,68], which may threaten insectivores in riparian food webs.

5. Conclusion

Our results provide new evidence for the transfer of a broad range of neonicotinoids by aquatic insects emerging from agriculturally impacted surface waters to web-building riparian spiders preying on these insects, and the persistence of neonicotinoids at this higher trophic level. The results are based on the measurement of a large number of pesticides in a large number of insect and spider samples, despite biomass often being a limiting factor in similar studies [19]. The results do, however, carry some uncertainties because the study comprises only a restricted number of ten sites from a small geographic region and the entire set of water, emerging insect and spider samples were only collected at two sites. However, neonicotinoids are one of the most used groups of insecticides worldwide [30] and the patterns of neonicotinoid concentrations in water samples were compared to a large global data set. The results of this comparison show that neonicotinoid concentrations detected in the present study were at the lower end of neonicotinoid levels reported in the published literature. Furthermore, the emerging insects and web-building riparian spiders are not endemic to this particular region, but are widely studied in similar studies internationally [26]. We therefore assume that the results from this study and most notably, the transfer of neonicotinoids to riparian spiders is a relevant pathway in many other impacted surface waters worldwide. Future studies need to evaluate the importance of the pesticide biomagnification reported here for the viability of populations of terrestrial predators. A more detailed mechanistic study of molecule characteristics is also needed to further elucidate the mechanisms involved in the observed biomagnification of neonicotinoids. Overall, the importance of systemic insecticides, particularly neonicotinoids, for terrestrial consumers preying on emerging insects or riparian spiders and the ecological integrity of exposed riparian ecosystems as a whole requires further attention.

Environmental implication

Statement of environmental implication in support of the submission of an original research paper with the title Neonicotinoid insecticides at low concentrations in surface waters are mediated by emerging insects to high concentrations in riparian spiders. Neonicotinoid insecticides are in terms of use the most important group of insecticides in agriculture. They have a high potential to negatively impact food webs due to their invertebrate toxicity. Furthermore, sub lethal effects of neonicotinoid

exposure have been reported for vertebrate insectivores, such as birds and bats. Neonicotinoids are, however, not considered bioaccumulative in organisms, although their bioaccumulation in earth worms has recently been reported. Our results, which document the bioaccumulation and biomagnification of neonicotinoids across the aquatic-terrestrial ecosystem boundary, therefore provide new information on the risks associated with surface water contamination by these insecticides.

CRediT authorship contribution statement

Alexis P. Roodt: Conceptualization, Methodology, Investigation, Formal analyses, Visualization, Writing – original draft. **Maike Huszarik:** Conceptualization, Methodology, Investigation, Writing – review & editing. **Martin Entling:** Funding acquisition, Conceptualization, Supervision, Writing – review & editing. **Ralf Schulz:** Funding acquisition, Conceptualization, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Alexis Pieter Roodt reports financial support was provided by German Research Foundation. Maike Huszarik reports financial support was provided by German Research Foundation.

Data Availability

Data will be made available on request.

Acknowledgments

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2023.131635.

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Supplementary Materials for

Aquatic-terrestrial transfer of neonicotinoid insecticides in riparian food webs

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This PDF file includes:

Figs. S1 to S6
Tables S1 to S5
Validation method for the measurement of pesticides in water and biological matrices by HPLC-ESI-MS/MS

Other Supplementary Materials for this manuscript include the following:
Data S1 to S7

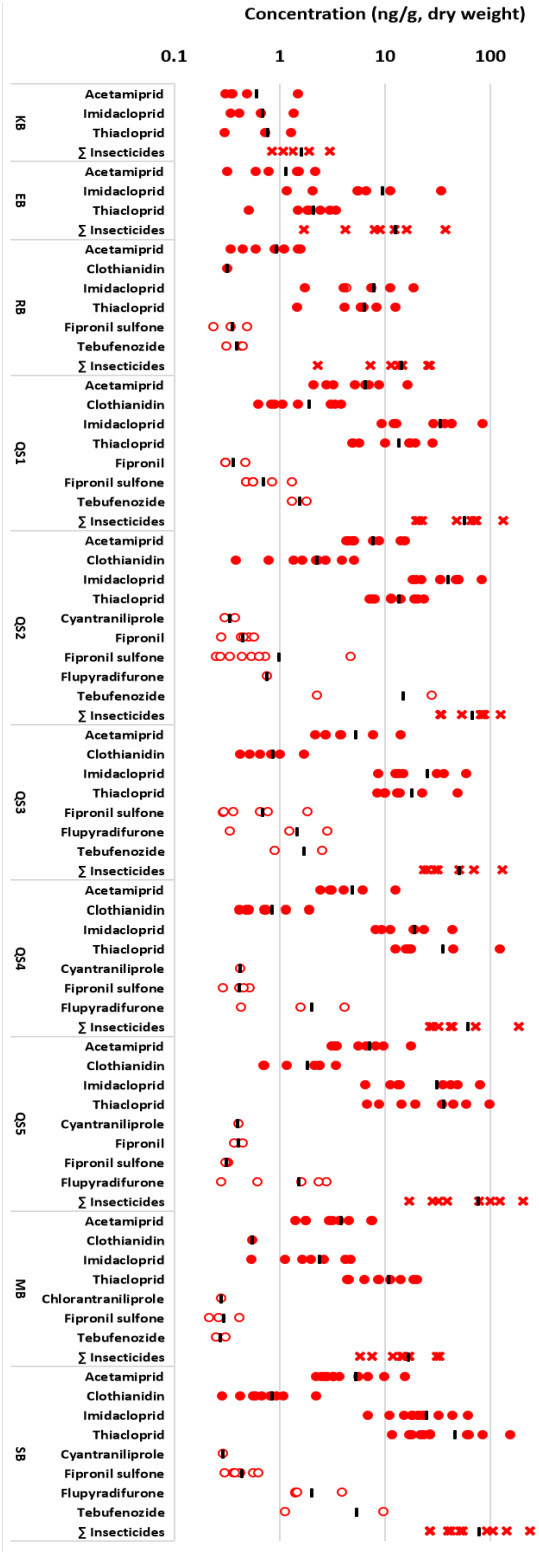

Fig. S1. Measured insecticide concentrations in spider samples from ten sampling sites. Concentrations of neonicotinoid insecticides (solid red circles) and other insecticide classes (outlined red circles) are shown for individual samples. Average concentrations are indicated by black dashes. Sum insecticide concentrations indicated by red crosses.

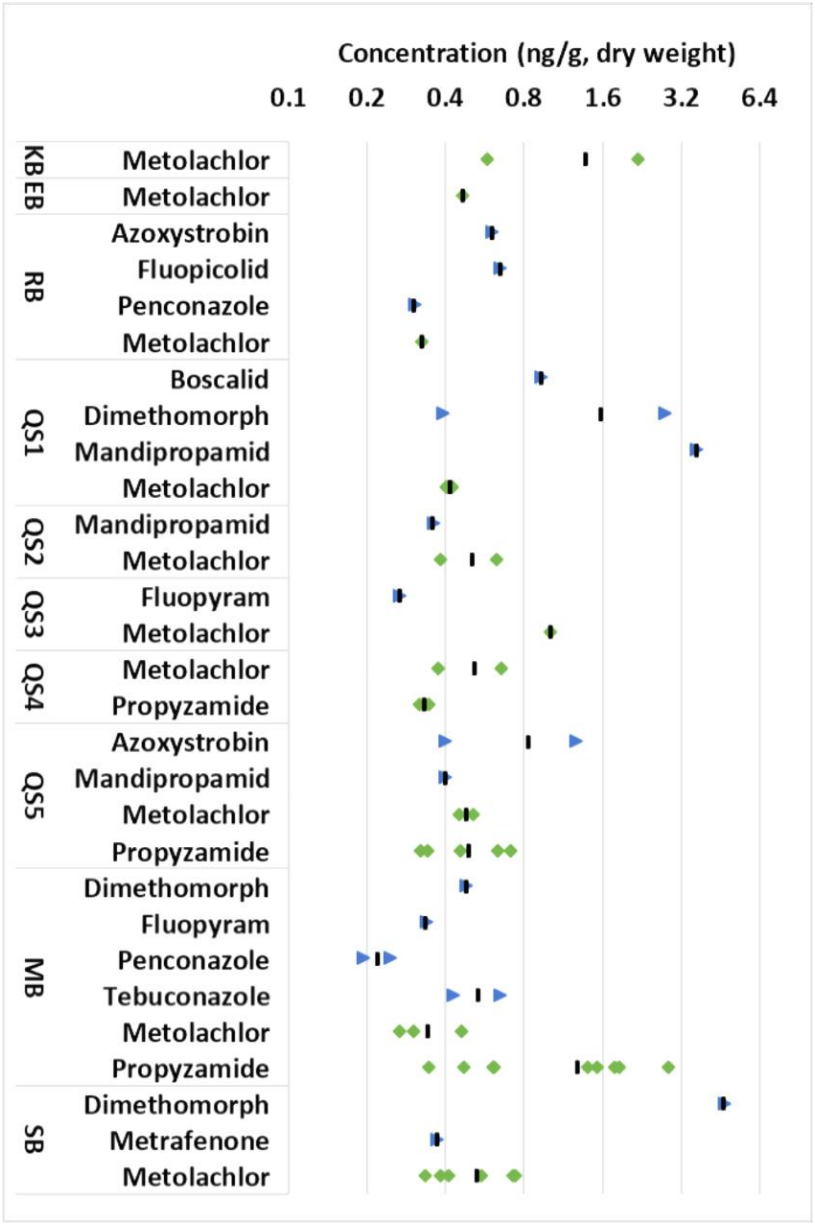

Fig. S2. Measured fungicide and herbicide concentrations in spiders from ten sampling sites. Concentrations of fungicides (blue triangles) and herbicides (green diamonds) are shown for individual samples. Average concentrations are indicated by black dashes. Metolachlor concentrations are reported as the sum of isomers.

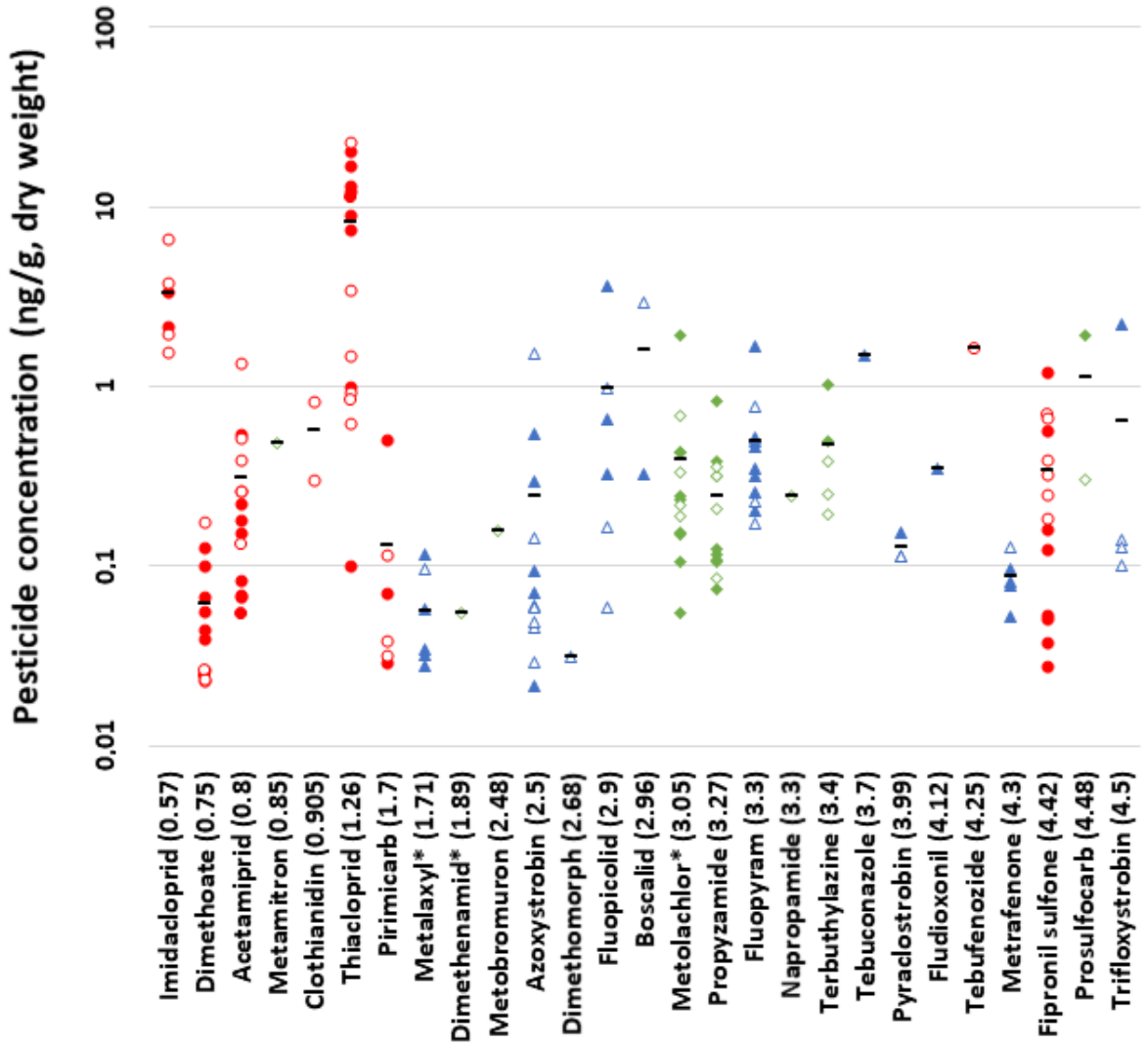

Fig. S3. Pesticide concentrations in emerging aquatic insects from two sampling sites. Concentrations of fungicides (blue triangles), herbicides (green diamond) and insecticides or insecticide metabolite (red circles) in samples of emerging insects (including Diptera, Ephemeroptera and Trichoptera) collected from two stream sites affected by agricultural land use, namely the Modenbach (MB, solid shapes) and the Spiegelbach (SB, outlined shapes). Pesticides are arranged from left to right in order of increasing lipophilicity ($\log K_{ow}$ values are provided in brackets). Overall average concentrations for both sampling sites are indicated by black dashes. *Concentrations are reported for the sum of isomers.

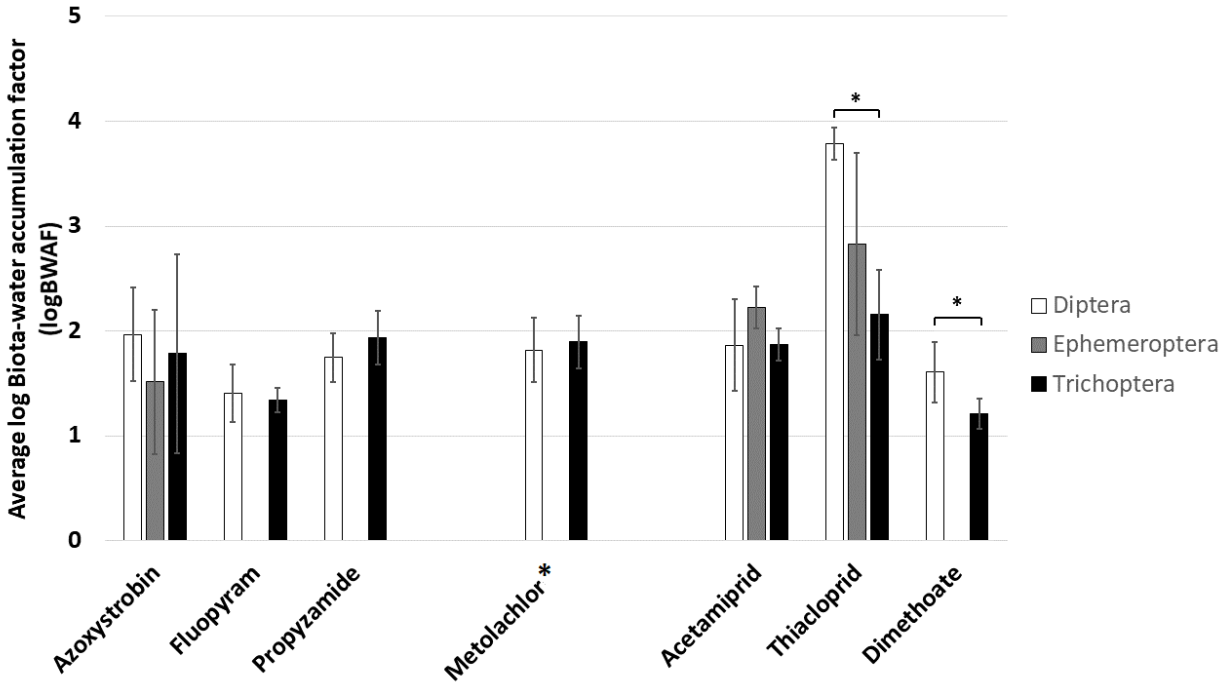

Fig. S4. Arithmetic means of log biota-water accumulation factors (log BWAFs) for pesticides which were detected in more than 50 % of samples of adult emerging insects from three orders (n = 6 for Diptera, n = 4 for Ephemeroptera and n = 4 for Trichoptera). Error bars indicate the standard deviations. Asterisks indicate a significant difference between orders (Kruskal-Wallis rank sum test with post hoc Dunn's test using Bonferroni correction, $p < 0.05$). *Concentration is reported for the sum of isomers.

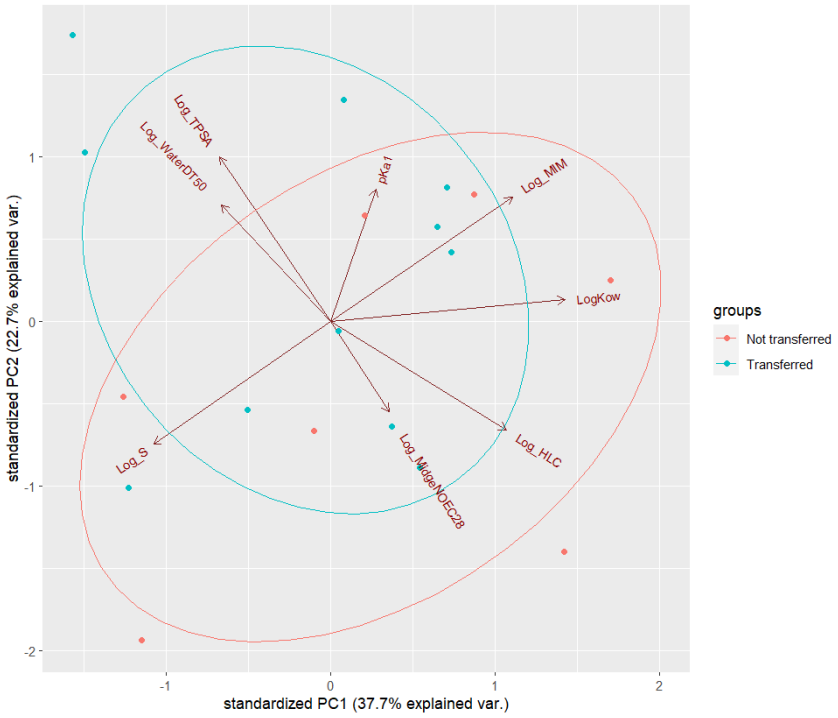

Fig. S5. Principle component analysis of the values of physicochemical properties, toxicity and environmental persistence for pesticides which were categorised as "not transferred" (detected in abiotic compartments only) or "transferred" (detected in abiotic and biotic compartments). The logarithmically transformed values of the pesticide properties were: Henry's law constant (HLC), aqueous solubility (S), topological polar surface area (TPSA), monoisotopic mass (MIM) octanol-water partition coefficient (Kow), first dissociation constant (pK_{a1}), water-phase half-life (DT50) and the chronic 28-day no observed effects concentration for *Chironomus riparius* (MidgeNOEC28).

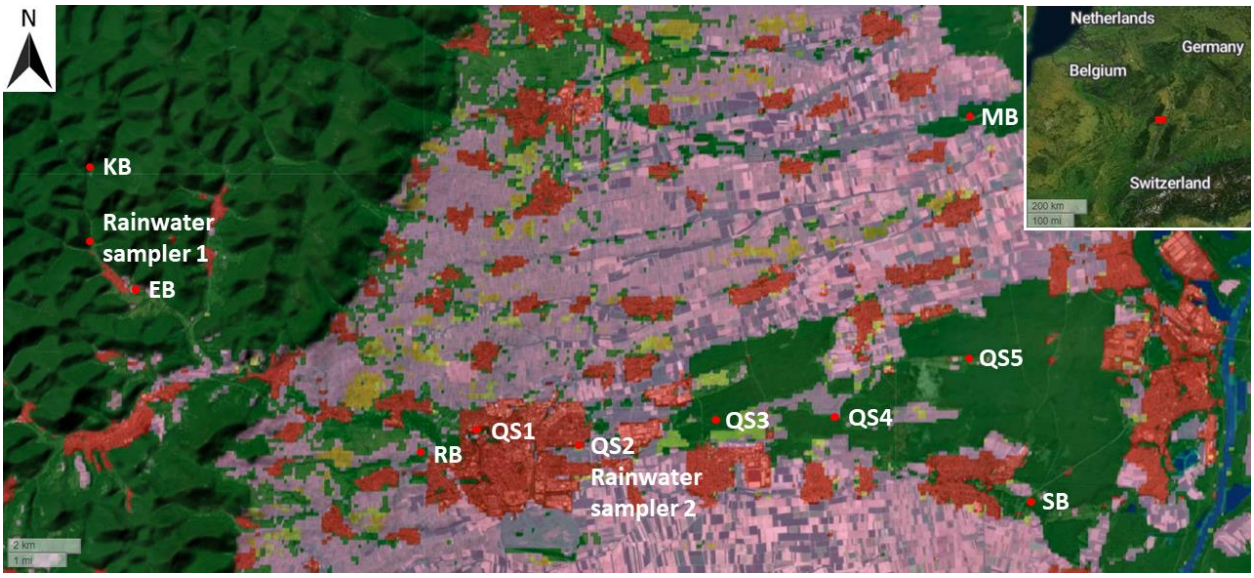

Fig. S6. Map of ten sampling sites and surrounding land use. On the map, dark green indicates forested areas, yellow indicated herbaceous vegetation, light pink indicates agricultural cropland, blue indicated permanent waterbodies and red indicates built-up areas. Samples were collected from ten sampling sites (red points) with differing levels of agricultural impact, namely; Katzenbach (KB, 49°16'12.0"N 7°57'58.0"E), Eusserbach (EB, 49°14'20.1"N 7°58'34.4"E), Ranschbach (RB, 49°11'57.0"N 8°04'55.0"E), Queich Site 1 (QS1, 49°12'01.0"N 8°05'40.0"E), Queich Site 2 (QS2, 49°12'04.7"N 8°08'16.1"E), Queich Site 3 (QS3, 49°12'19.1"N 8°11'32.0"E), Queich Site 4 (QS4, 49°12'39.0"N 8°13'43.0"E) and Queich Site 5 (QS5, 49°13'19.0"N 8°16'12.2"E), Modenbach (MB, 49°16'50.4"N 8°16'53.0"E) and Spiegelbach (SPI, 49°11'13.6"N 8°18'44.6"E). Map source: Buchhorn, M.; Smets, B.; Bertels, L.; Lesiv, M.; Tsendbazar, N.-E.; Masiliunas, D.; Linlin, L.; Herold, M.; Fritz, S. (2020). Copernicus Global Land Service: Land Cover 100m: Collection 3: epoch 2019: Globe (Version V3.0.1) [Data set]. Zenodo. DOI: 10.5281/zenodo.3939050

Table S1. Total emergence biomass estimation and average weekly emergence fluxes of insects and pesticides

Sampling Site	Modenbach (MB)			Spiegelbach (SB)		
Order	Total estimated dry weight (mg)	Average weekly emergence flux (mg/m^2·week)	Estimated weekly pesticide flux (ng/ m^2·week)*	Total estimated dry weight (mg)	Average weekly emergence flux (mg/m^2·week)	Estimated weekly pesticide flux (ng/ m^2·week)*
Diptera	9155.7	763.0	10.5	2860.9	238.4	9.6
Ephemeroptera	109.9	9.2	0.3	181.1	15.1	0.1
Trichoptera	936.3	78.0	0.4	1026.2	85.5	0.3

* Estimated weekly pesticide flux was calculated as the average weekly emergence flux multiplied by the average total pesticide concentration (Table S2).

Table S2. Average total concentrations of fungicides, herbicides and insecticides in Diptera, Ephemeroptera and Trichoptera collected from Modenbach (MB) and Spiegelbach (SB) sampling sites.

Diptera		
Sampling site (number of replicates)	MB (n=6)	SB (n=1)
Average total fungicides (ng/g dw)	0.6	5.8
Average total herbicides (ng/g dw)	0.4	2.3
Average total insecticides (ng/g dw)	12.8	32.4
Average total pesticides (ng/g dw)	13.8	40.5
Ephemeroptera		
Sampling site (number of replicates)	MB (n=1)	SB (n=3)
Average total fungicides (ng/g dw)	5.8	0.1
Average total herbicides (ng/g dw)	4.7	0.2
Average total insecticides (ng/g dw)	21.6	4.9
Average total pesticides (ng/g dw)	32.1	5.2
Trichoptera		
Sampling site (number of replicates)	MB (n=2)	SB (n=2)
Average total fungicides (ng/g dw)	2.8	0.8
Average total herbicides (ng/g dw)	1.1	0.8
Average total insecticides (ng/g dw)	0.7	1.8
Average total pesticides (ng/g dw)	4.6	3.4

dw – dry weight

Table S3. Pesticide properties used in PCA analysis.

Pesticide	Log TPSA	Log S	Log MIM	Log Kow	Log HLC	pKa1	Log_Midge NOEC28	Log_Water DT50
Metalaxyl (Sum of isomers)	1.7	4.4	2.4	1.7	-4.5	14	2.0	1.4
Fluopyram	1.6	1.2	2.6	3.3	-4.5	14	0.1	1.3
Dimethomorph	1.7	1.5	2.6	2.7	-4.6	-1	0.6	1.0
Metolachlor (Sum of isomers)	1.5	2.7	2.5	3.0	-2.7	14	0.9	1.0
Propyzamide	1.5	1.0	2.4	3.3	-8.1	10	-0.5	1.3
Acetamiprid	1.7	3.5	2.3	0.8	-7.3	1	-2.3	0.7
Thiacloprid	1.9	2.3	2.4	1.3	-9.3	14	-3.7	3.0
Imidacloprid	1.9	2.8	2.4	0.6	-9.8	14	-2.7	1.5
Azoxystrobin	2.0	0.8	2.6	2.5	-8.1	14	-0.1	0.8
Fluopicolid	1.6	0.4	2.6	2.9	-4.4	14	1.7	2.0
Mandipropamid	1.8	0.6	2.6	3.2	-4.0	14	2.0	2.0
Iprovalicarb	1.8	1.3	2.5	3.2	-5.9	14	2.0	1.7
Propamocarb	1.6	6.0	2.3	0.8	-3.8	10	2.0	1.1
Difenconazole	1.8	1.2	2.6	4.4	-6.0	14	-1.8	0.5
Cyflufenamid	1.7	-0.3	2.6	4.7	-1.6	12	0.0	0.6
Isoproturon	1.5	1.8	2.3	2.5	-4.8	14	2.0	1.6
Fenpropimorph	1.1	0.6	2.5	4.5	-3.6	7	-0.9	0.4
Chloridazon	1.8	2.6	2.3	1.2	-9.3	3	2.0	1.7

Abbreviations: Henry's law constant (HLC), aqueous solubility (S), topological polar surface area (TPSA), monoisotopic mass (MIM) octanol-water partition coefficient (Kow), first dissociation constant (pKa1), water-phase half-life (DT50) and the chronic 28-day no observed effects concentration for Chironomus riparius (MidgeNOEC28). With the exception of TPSA values, all pesticide properties were retrieved from the Pesticide Properties Database (PPDB), University of Hertfordshire. Accessed: 03.11.2022. Available at: http://sitem.herts.ac.uk/aeru/ppdb/en/index.htm. Values for TPSA were retrieved from the National Institute of Health (Maryland, USA) PubChem open chemistry database. Accessed: 03.11.2022. Available at: https://pubchem.ncbi.nlm.nih.gov/

Table S4. Summary of principle Table S4. Principle component analysis of pesticides categorised as either "transported" or "not transported" by their frequency of detection in abiotic and biotic compartments.

Variables	PC1	PC2	PC3	PC4	PC5	PC6
Eigenvalue	3.01	1.82	1.31	0.76	0.65	0.30
Standard deviation	1.74	1.35	1.14	0.87	0.81	0.55
Proportion of variance	0.38	0.23	0.16	0.09	0.08	0.04
Cumulative proportion of variance	0.38	0.60	0.77	0.86	0.94	0.98
Loadings						
Log_TPSA	-0.26	0.50	0.05	-0.63	0.17	-0.01
Log_S	-0.42	-0.37	0.18	-0.14	0.43	-0.23
Log_MIM	0.43	0.38	-0.07	-0.40	-0.02	-0.30
LogKow	0.55	0.07	-0.06	0.16	-0.01	0.15
Log_HLC	0.41	-0.33	0.31	-0.10	0.20	-0.62
pKa1	0.11	0.40	0.50	0.36	0.60	0.24
Log_MidgeNOEC28	0.14	-0.27	0.65	-0.40	-0.34	0.45
Log_WaterDT50	-0.26	0.35	0.44	0.33	-0.52	-0.44

Abbreviations: Henry's law constant (HLC), aqueous solubility (S), topological polar surface area (TPSA), monoisotopic mass (MIM) octanol-water partition coefficient (Kow), first dissociation constant (pK_{a1}), water-phase half-life (DT50) and the chronic 28-day no observed effects concentration for *Chironomus riparius* (MidgeNOEC28).

Table S5. Instrument parameters.

High performance liquid chromatography (HPLC) parameters

Instrument:	Agilent 1260 Infinity II HPLC System	
Column:	ZORBAX Eclipse Plus C18 (3.0 ID x 150 mm, 2.7 micron)	
Eluent A:	H_2O/MeOH (98:2), 0.1% Formic acid, 4 mM Ammonium formate	
Eluent B:	H_2O/MeOH (2:98), 0.1% Formic acid, 4 mM Ammonium formate	
Injection volume (water and biological samples):	100 µL	
Flow rate:	0.5 mL/min	
Column temperature:	45°C	
Elution gradient:	Time (min)	% Eluent A
	0	98
	1	50
	4	35
	14	0
	20	0
	20.1	98

Electrospray ionization (ESI) and triple quadrupole mass spectrometry (MS/MS) parameters

Instrument:	Agilent 6495 Triple Quadrupole Mass Spectrometer with an iFunnel Jet Stream ESI
Capillary voltage:	3000 V
Nozzle voltage:	0 V
Gas flow:	11 L/min
Gas temperature:	250 °C
Sheath gas flow:	12 L/min
Sheath gas temperature:	350 °C
Nebulizer pressure:	38 psi
iFunnel High pressure RF	+/- 150 V
iFunnel Low pressure RF	+/- 60 V
Cycle time	900 ms

Method validation for the measurement of pesticides in water and biological matrices by HPLC-ESI-MS/MS

Pesticides in water

A direct-injection HPLC-ESI-MS/MS method was validated and used for measurements of pesticides in water samples. A calibration series with 11 concentrations covering the concentration range 0.3 to 2000 ng/L was prepared in triplicate using MS-grade water, in addition to solvent blanks. The method performance was evaluated for specificity and repeatability in order to set the limit of quantification (LOQ) for each analyte. At least two multiple reaction monitoring (MRM) transitions were measured for each analyte (Data S6). The MRM transition with the greatest response or the least background interference was selected as the quantifier signal. At least one qualifier-MRM signal with a signal intensity between 70 to 130% of the quantifiers' signal, in addition to chromatographic peak retention time which deviated by less than 0.05 min from the highest-concentration standard were required for positive identification. The limit of quantification (LOQ) of each analyte was determined as the lowest concentration in the calibration series which satisfied the above criteria and also had a signal-area relative standard deviation below 15% (%RSD) between the three replicate analyses (Data S6). Internal standard recoveries were required to be between 70 to 120% for results to be acceptable in samples.

Pesticides in biological samples

Large volumes of uncontaminated insect material for method validation are difficult and time consuming to obtain from field collected samples, we therefore used larvae of the aquatic insect *Chironomus riparius* from an in-house laboratory culture as a representative sample matrix. insects were freeze dried and pulverised using a Tissuelyzer (Retsch MM 301, Haan, Germany) and 2.5 mm diameter steel pellets. Replicate samples of dry insect material (30 mg) were weighed into 2 mL polypropylene tubes using a MT5 analytical balance (d = 0.001 mg). To test the repeatability and recoveries of the pesticides, three concentrations of a mixture containing all analytes and internal standards were prepared in methanol. Twenty-five microlitres of each mixture was added to three replicate samples in order to achieve final concentrations of 0.01, 0.05 and 0.1 ng/g dw. Extractions were performed with 1 mL of acetonitrile containing 0.1% formic acid. Samples were vortexed for 30 seconds to prevent clump formation, after which they were sonicated for 5 minutes and centrifuged for a further 5 minutes at 16000 rpm. Afterwards, a dispersive solid phase extraction clean-up was performed by pipetting 850 µL of the extract to a new sample tube containing 24 mg of Z-Sep+ and primary-secondary amine (PSA). The mixing, sonication and centrifugation steps were repeated as before. After centrifugation, 700 µL of the extract was pipetted into a glass vial which was placed under a gentle stream of nitrogen gas until all the solvent had evaporated. The residues were then dissolved in 500 µL of a mixture of water and methanol (70:30, v/v) containing 0.1 % formic acid and 0.5 ng/mL thiamethoxam-D3 as an internal standard. Samples were analysed by HPLC-ESI-MS/MS (Table S5) and analytes which had average recoveries in the spiked samples between 70 to 120% of the expected concentration and a signal RSD% < 15, were considered acceptable for quantitation (Data S6). The LOQs were defined as the lowest calibration concentration at which both acceptable recoveries were achieved and the

criteria used to determine specificity were achieved (as defined above in the water validation). LOQs determined in the 30 mg larvae matrix were used for the quantification of insect and spider samples (Data S6). Because qualitative data was also valuable for the present work, we defined analyte concentrations with recoveries between 30 to 70% or 120 to 160% and %RSD < 15 as acceptable for qualitative use, i.e. <LOQ. In the case of actual samples, the recoveries of the internal standards were monitored to provide continuous quality control.