



Importance of Dietary Uptake for in Situ Bioaccumulation of Systemic Fungicides Using *Gammarus pulex* as a Model Organism

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Abstract: Bioaccumulation of organic contaminants from contaminated food sources might pose an underestimated risk toward shredding invertebrates. This assumption is substantiated by monitoring studies observing discrepancies of predicted tissue concentrations determined from laboratory-based experiments compared with measured concentrations of systemic pesticides in gammarids. To elucidate the role of dietary uptake in bioaccumulation, gammarids were exposed to leaf material from trees treated with a systemic fungicide mixture (azoxystrobin, cyprodinil, fluopyram, and tebuconazole), simulating leaves entering surface waters in autumn. Leaf concentrations, spatial distribution, and leaching behavior of fungicides were characterized using liquid chromatography coupled with high-resolution tandem mass spectrometry (LC-HRMS/MS) and matrix-assisted laser desorption ionization-mass spectrometric imaging. The contribution of leached fungicides and fungicides taken up from feeding was assessed by assembling caged (no access) and uncaged (access to leaves) gammarids. The fungicide dynamics in the test system were analyzed using LC-HRMS/MS and toxicokinetic modeling. In addition, a summer scenario was simulated where water was the initial source of contamination and leaves contaminated by sorption. The uptake, translocation, and biotransformation of systemic fungicides by trees were compound-dependent. Internal fungicide concentrations of gammarids with access to leaves were much higher than in caged gammarids of the autumn scenario, but the difference was minimal in the summer scenario. In food choice and dissection experiments gammarids did not avoid contaminated leaves and efficiently assimilated contaminants from leaves, indicating the relevance of this exposure pathway in the field. The present study demonstrates the potential impact of dietary uptake on in situ bioaccumulation for shredders in autumn, outside the main application period. The toxicokinetic parameters obtained facilitate modeling of environmental exposure scenarios. The uncovered significance of dietary uptake for detritivores warrants further consideration from scientific as well as regulatory perspectives. *Environ Toxicol Chem* 2023;42:1993–2006. © 2023 The Authors. *Environmental Toxicology and Chemistry* published by Wiley Periodicals LLC on behalf of SETAC.

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INTRODUCTION

Potential systemic fungicide exposure routes for aquatic invertebrates

It is well established that pesticides from agricultural applications can enter adjacent water bodies through various pathways, with spray drift and runoff being the most significant (Schulz, 2004). The presence of pesticides in the aquatic environment increases exposure and potential uptake by organisms. Nonetheless, monitoring studies have found discrepancies between measured internal concentrations in aquatic invertebrates

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(i.e., gammarids) and predicted body burdens based on water concentrations combined with toxicokinetic laboratory experiments (Lauper et al., 2021; Munz et al., 2018). This observation was dominantly made for systemic pesticides, including fungicides. Such pesticides are mobile in the environment because of their relatively high polarity (i.e., being water-soluble), facilitating their distribution to different matrices. In addition, their systemic properties allow them to be taken up by the plant root system and translocated into plant compartments aboveground (Bundschuh et al., 2019; Erwin, 1973). Thus, off-field plants at the field margins may also take up retained systemic pesticides, as simulated by Englert, Bakanov, et al. (2017). Plant material, such as leaf litter, can enter nearby water bodies after abscission by lateral transport (wind) or vertical fall in autumn (Abelho, 2001). This pathway can cause a time-delayed aqueous exposure for aquatic biota to systemic pesticides leaching from the leaves as well as a secondary poisoning scenario by consumption of contaminated leaf litter (Englert, Bakanov, et al., 2017; Englert, Zubrod, Pietz, et al., 2017; Kreutzweiser et al., 2007). The implications of contaminated leaf litter consumption are currently understudied because most monitoring studies of pesticides focus on the pesticide application season via water sample collection and analysis (Chow et al., 2020).

Impact of systemic fungicides on decomposer–detritivore systems

Input of allochthonous organic matter such as leaf litter into aquatic systems feeds a series of detritivorous organisms, including insect larvae and crustaceans, and decomposers, such as fungi (Wallace et al., 1997). Decomposition is an important ecosystem service for nutrient cycling and aquatic ecosystem integrity (Hynes, 1975; Vannote et al., 1980). Shredders are responsible for the largest proportion of litter removal in low-order temperate streams (Graça & Canhoto, 2006; Hieber & Gessner, 2002). However, for the processing of litter, shredders rely on colonizing and conditioning by aquatic saprotrophic fungi, such as hyphomycetes (Arsuffi & Suberkropp, 1985; Bärlocher & Kendrick, 1975; Suberkropp & Klug, 1976). These decomposer–detritivore systems respond sensitively toward chemical stressors. Fungicides, many of which possess systemic properties, are specifically designed to damage fungi and thus impose adverse effects on nontarget litter-colonizing fungal communities (Bundschuh et al., 2011; Maltby et al., 2009). Furthermore, fungicides exert direct (i.e., by aqueous and dietary exposure) and indirect (i.e., decreased nutritional quality of litter) adverse effects on shredders (Baudy et al., 2017; Flores et al., 2014; Zubrod, Englert, Feckler, et al., 2015). Thus, the adverse effects on fungal communities and shredders can eventually result in decreased decomposition rates (Cornejo et al., 2020; Pascoal & Cássio, 2004; Rasmussen et al., 2012).

Current research gaps and challenges

While research on the potential direct and indirect toxic effects of systemic pesticides on decomposer–detritivore systems

has received more attention in the last decade (Bundschuh et al., 2019; Zubrod et al., 2019), there are still many knowledge gaps to be filled. For example, (1) a large proportion of studies rely on dietary exposure scenarios that are based on sorption (partitioning) of the tested pesticides to leaf material. In the following, this will be called *summer scenario* because water concentrations and consequential sorption processes are expected to be highest around the times of pesticide application (summer season). Because of the high associated effort, only in a few studies was leaf material from systemically exposed plants tested (Englert, Zubrod, Link, et al., 2017; Englert, Zubrod, Pietz, et al., 2017; Kreutzweiser et al., 2007, 2008, 2009; Newton et al., 2018), of which only Newton et al. (2018) tested systemic fungicides. This scenario describes a contamination of aquatic systems originating from previously contaminated plant material and will thus be called *autumn scenario*. (2) In addition to this research gap, the vast majority of studies (regardless of systemic or sorption-driven exposure) utilized leaves that were preserved by freezing or drying after pesticide application. Such procedures may be necessary for sample conservation, but damage the leaf structures. As a consequence, the leaching kinetics of both contaminants and leaf constituents increases compared with natural scenarios (as summarized by Consolandi et al., 2021). To the best of our knowledge, studies with undamaged systemically exposed leaf litter were only performed by Kreutzweiser et al., (2007, 2008, 2009) and focused on neonicotinoid insecticides. (3) Lastly, existing literature rarely includes determination of internal concentrations (i.e., bioaccumulation potential) of pesticides in exposed shredders (Englert, Zubrod, Pietz, et al., 2017). Therefore, implementing toxicokinetic approaches could help in improving the understanding of the mechanisms behind bioaccumulation processes and adverse effects.

Research scope

To address the described research gaps, the present study investigated the in situ relevance of dietary uptake for bioaccumulation of systemic fungicides in the amphipod species *Gammarus pulex* (Linnaeus, 1758) via the evaluation of behavioral, physiological, and chemical endpoints. For this purpose, dietary exposure experiments were designed to simulate an autumn exposure scenario by using pristine (here defined as undamaged by drying or freezing) leaf material from horse chestnut (*Aesculus hippocastanum*) trees treated with a systemic fungicide mixture (azoxystrobin, cyprodinil, fluopyram, and tebuconazole). In addition, for comparative purposes, a summer exposure scenario was created, with the main contamination source being the aqueous phase, causing fungicides to be sorbed to uncontaminated leaves.

We hypothesized that systemic fungicides could be effectively assimilated into the tissue of *G. pulex* through dietary exposure. Furthermore, we expected that the dietary uptake of systemic fungicides from contaminated leaves might be of more importance in autumn than in a summer scenario with aqueous exposure during the application period. Consequently, dietary uptake of systemic fungicides from contaminated leaf litter by

aquatic shredders, such as gammarids, could help explain the observed discrepancies between observed and predicted body burdens in the field, thus supporting bioaccumulation assessments.

MATERIALS AND METHODS

Creation of the test material

To obtain leaf material from trees exposed to systemic fungicides, specimens of *A. hippocastanum* L. (horse chestnut) were reared under field conditions (IES Landau, Siebeldingen, Germany) following Newton et al. (2018). Chestnut trees are common in European riparian vegetation (Ravazzi & Caudullo, 2016), and their leaves are commonly used to feed gammarids (Consolandi et al., 2021). Trees were treated two times (middle of May and end of June) either at the recommended field application rates of a mixture consisting of four systemic fungicides (Table 1) or with received tap water as a control. Thereby a direct overspray of off-crop areas such as vegetated buffer zones was simulated as a worst-case scenario. The fungicide selection was based on environmental relevance (i.e., high concentrations measured in water and gammarid tissue in monitoring studies; Lauper et al., 2021) and potential co-occurrence (i.e., co-application in the same culture, i.e., cereals). Further details on the tree treatment are provided in Supporting Information, A1.

Leaves were then collected from senescent trees in autumn (October 2020), stored in airtight bags in the dark at 4 °C, and used within the following month. Leaves used for mass spectrometry (MS) imaging experiments were snap-frozen as a whole in liquid nitrogen within 12 h after collection and stored at –80 °C until further analysis. For use in gammarid biotests and sorption and leaching experiments, as well as chemical analysis, leaf discs were cut using a cork borer of 20 mm in diameter.

Characterization of the test material

In addition to the determination of total fungicide concentrations (described below), the spatial distribution of fungicides within the leaf tissue was assessed to evaluate their bioavailability (presence of fungicides in the lamina tissue consumed by

shredders). MS-imaging facilitated by matrix-assisted laser desorption ionization (MALDI) was performed on cross sections obtained from control leaves, leaves from trees exposed to the systemic fungicide mixture, and leaves contaminated through sorption from a spiked test medium (Supporting Information, A2). Further details on the analyzed leaves are provided in Supporting Information, A3.

The preparation of leaf cryosections was based on Lorensen et al. (2023). Sections were created by cutting 16-μm-thick slices of embedded (2.5% carboxymethyl-cellulose) leaves on a cryomicrotome (–16 °C; Leica CM3050S; Leica Microsystems). The sectioning was assisted by adhesive tape (Kawamoto Cryotape 2C[9], SECTION-LAB; Kawamoto & Kawamoto, 2021) to improve sample integrity and reproducibility. The sections were then attached to a standard microscope slide using a double-sided adhesive carbon tape (SPI Supplies) and dried in a vacuum desiccator prior to matrix application. A matrix solution of 7 mg mL^{–1} α-cyano-4-hydroxyconnamic acid (CHCA) in 50:50 acetonitrile:H₂O (v/v) containing 1% trifluoroacetic acid was applied using an in-house-built spray apparatus (University of Copenhagen). A quantity of 300 μL of matrix solution was sprayed at a flow rate of 30 μL min^{–1} (nebulizer gas pressure was 2 bar) from a distance of 100 mm while the sample was rotating at 600 rpm. Sample integrity and quality (i.e., homogenous thickness) as well as matrix crystals were evaluated under a light microscope at ×400 magnification using reflected light.

The MALDI-MS-imaging experiments were performed at ambient conditions using an AP-SMALDI5 ion source (TransMIT) coupled with a QExactive Orbitrap mass spectrometer (Thermo Fisher Scientific). The scans were performed in positive ion mode with a resolving power of $R = 140,000$ at a mass-to-charge ratio (m/z) of 200, a mass range of m/z 140–980, and a scan speed of 1 pixel s^{–1} in two-dimensional line mode. Matrix peaks at m/z 190.04987 (CHCA [$M + H$]⁺) and m/z 401.07440 (CHCA [$2M + Na$]⁺) were used as lock masses for internal mass calibration, ensuring a mass accuracy of 2 ppm or better. The x – y raster width was set to 30 μm. At least two replicates were analyzed for each of the three treatments (control, systemic uptake, and sorption). MS-imaging data analysis was performed as described by Lorensen et al. (2023).

TABLE 1: Applied systemic fungicides and their main biotransformation products

Compound	Class	Formula	MW (g mol ^{–1})	Log D_{OW}	FR (mmol ha ^{–1}) or AI (nmol tree ^{–1})
Azoxystrobin	Strobilurin	C ₂₂ H ₁₇ N ₃ O ₅	403.4	2.5	620
AZ_M390aa		C ₂₁ H ₁₅ N ₃ O ₅	389.4	3.5	
Cyprodinil	Pyrimidine	C ₁₄ H ₁₅ N ₃	225.3	4.0	2000
CGA 249287 ^b		C ₈ H ₁₁ N ₃	149.2	0.9	
Fluopyram	Benzamide	C ₁₆ H ₁₁ ClF ₆ N ₂ O	396.7	3.3	380
TFM-benzamide ^c		C ₈ H ₆ F ₃ NO	189.1	0.7	
Tebuconazole	Triazole	C ₁₆ H ₂₂ ClN ₃ O	307.8	3.7	490

References of the BTPs: ^aRösch et al. (2017).

^bKiefer et al. (2019).

^cWei et al. (2016).

Biotransformation pathways are illustrated in Figure 2.

MW = molecular weight; log D_{OW} = partitioning coefficient between octanol and water (log K_{OW}) of neutral species at pH 7 obtained from Pubchem; AI = active ingredient; FR = recommended field rate by the product companies.

Leaching characteristics at different leaf conditions

Leaching experiments using leaf discs of different conditions were conducted to understand the influence of leaf conditions and integrity on leaching kinetics. Leaching behavior was studied for (I) pristine leaf discs from chestnut trees exposed to the systemic fungicide mixture, (II) pristine leaf discs with gammarid feeding, (III) leaf discs from chestnut trees that were frozen before being deployed for leaching, and (IV) pristine control leaves that were contaminated by sorption from water. The leaching potential was evaluated by sampling leaf discs at different time points, analyzing fungicide residues, and calculating leaching half-life times using a one-phase decay model (GraphPad Prism, Ver 9). Leaching behavior from previously frozen leaves was estimated by comparing water concentrations in the test vessels of the leaching experiment from pristine and frozen leaves. Further descriptions of these experiments are provided in Supporting Information, A12–A14.

Test specimens

Specimens of *G. pulex* were collected during autumn 2020 (water temperature of 9–11 °C) from a pristine stream in a natural conservation area close to Zürich, Switzerland (Mönchaltorfer Aa, 47.2749°N, 8.7892°E). This population of *G. pulex* belongs to a clade distributed north of the Alps in eastern France, Switzerland, and Regensburg in Germany (National Center for Biotechnology Information sequences MF458710 and JF965940) as specified by Raths et al. (2023). Specimens were acclimated in the lab by gradually increasing the temperature to 16 °C and replacing stream water with artificial pond water (APW; Naylor et al., 1989). Gammarids were passively separated into different size classes by using a stack of sieves, exploiting their negative phototactic response (Franke, 1977). Only male gammarids—separated based on the presence of large secondary gnathopods—with a size of 12–16 mm were used to reduce variance of feeding rates caused by size and behavior (i.e., mate guarding). Gammarids with visible parasitism (i.e., acanthocephalans; Fielding et al., 2003) were excluded. A gammarid lipid content of 0.8 ± 0.1 ($n=6$) was determined gravimetrically (Raths et al., 2023) on a wet weight basis (see Supporting Information, A4). All experiments were performed at 16 ± 1 °C in the dark, thus preventing alterations of feeding behavior due to

light responses. Gammarids were fed ad libitum in all experiments. Leaf material used in biotests was soaked in the test vessels for 12 h before gammarids were inserted. Mortality during the experiments was monitored and did not exceed 15% for any experiment.

Exposure with leaves contaminated from systemic uptake (autumn scenario)

The main experiment simulated an exposure scenario where the systemic fungicides are brought into the aquatic system by leaves from previously contaminated trees in the riparian area. This exposure pathway may be especially relevant outside the main pesticide application period in autumn; thus, it is referred to as the *autumn scenario* (Figure 1A). In this case, the aqueous phase was contaminated only through leaching from the leaves.

To model bioconcentration (aqueous uptake) and biomagnification (dietary uptake) kinetics, gammarids were exposed in a glass tank (Figure 1A) filled with 6 L of APW and 1.7 g (wet wt) of contaminated leaf discs ($n=40$). First, the test system was left for 12 h for leaves to soak before gammarids (43/L) were introduced. One group (caged) of gammarids was inserted into cages built from sawed-off 50-mL Falcon tubes enclosed from both sides with a nylon net (mesh size = 1 mm), and thus had no access to the leaf discs. This group was exposed only by leaching of the systemic fungicides from the leaf discs into the medium (bioconcentration). The second group of gammarids was allowed to move freely (uncaged) within the test system, which included access to the contaminated leaf material. The uncaged group was therefore exposed from both the medium (bioconcentration) and diet (biomagnification). Gammarids were exposed to the contaminated leaves and medium for 1 day, which is generally sufficient to reach equilibrium conditions of accumulated polar organic contaminants such as the tested fungicides (Raths et al., 2023). Afterward, they were transferred for another day into an uncontaminated basin with control leaf discs fed to both groups. During the experiments, medium and gammarids were sampled at regular intervals to allow for toxicokinetic modeling. Each gammarid sample consisted of duplicates of four gammarids for every sampling point. Leaf samples were taken at the beginning and end of the exposure phase in triplicates of four leaf discs each. A mass loss control in a separate basin was used to correct for

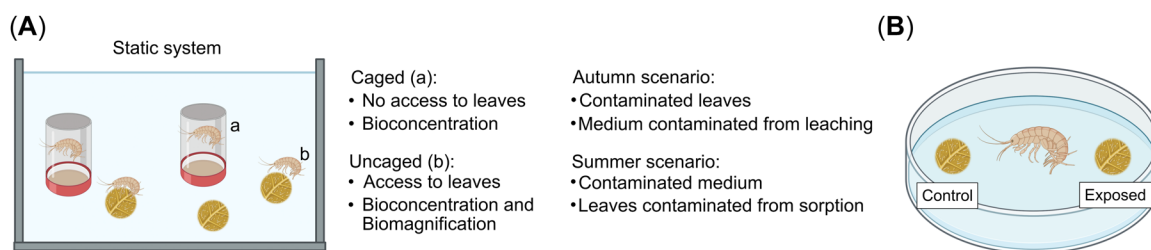


FIGURE 1: Illustration of the experimental setup of the autumn and summer scenarios (A) and the food choice assay (B). Referring to test guideline 305 (Organisation for Economic Co-operation and Development, 2012), we define bioconcentration as accumulation of fungicides following uptake from the water and biomagnification as accumulation following dietary uptake.

non-feeding-related weight loss of the leaf discs. The feeding rate was determined according to Equation 1 by using the total gammarid weight corrected by the corresponding exposure time until euthanasia.

Aqueous exposure of leaves and gammarids (summer scenario)

This subsequent experiment simulated an exposure scenario where systemic fungicides are entering the water body shortly after application (i.e., through runoff, spray drift) and leaves are only contaminated by sorption processes. Because most pesticide applications and subsequent contaminations occur in late spring or summer, this is referred to as the *summer scenario* (Figure 1A).

This experiment was conducted analogously to the autumn scenario with the difference that preexposed control leaf discs (5 days at $1\ \mu\text{g L}^{-1}$ of the parent fungicides, which equals 2.5 nM azoxystrobin, 4.4 nM cyprodinil, 2.5 nM fluopyram, and 3.2 nM tebuconazole) were used as food. At the start of the 1-day exposure phase, leaf discs and gammarids (one caged and one uncaged group) were placed into a basin contaminated with the parent fungicide mixture. Gammarid, leaf, and medium samples were only taken at the beginning and after 1 day of exposure.

Food choice assay

This experiment was designed to investigate whether gammarids would feed on contaminated leaves if alternative food sources were available. In this way, it was possible to evaluate the in situ relevance of the present exposure pathways. To investigate the feeding preferences and selectivity of gammarids, a food choice assay was conducted with modifications from a previously described setup (Zubrod, Englert, Wolfram, et al., 2015).

Prewedged pristine leaf discs from a chestnut tree exposed to systemic fungicides and control leaf discs (2 cm diameter each) were mounted in a 9-cm-diameter crystallization dish (feeding arena; Figure 1B) containing 80 mL APW. Leaf discs were left soaking for 12 h before the medium was exchanged and gammarids were inserted into the test system. Gammarids ($n=49$) were starved for 3 days in the dark, before being introduced into an individual arena. The starvation phase, chosen from previous studies (Consolandi et al., 2021), allowed for gut clearance and ensured feeding activity. Feeding arenas were set up in a randomized orientation, and specimens were allowed to feed for 24 h in the dark (16°C). At the end of the experiment, gammarids were dry-blotted, and their mass was determined as wet weight. Leaf discs were dried at 60°C overnight before the dry weight was determined. A mass loss control ($n=8$) in arenas without gammarids was used to correct for non-feeding-related mass loss of the leaf discs by leaching or degradation. Eight replicates with no detectable leaf consumption were excluded from further analysis.

The consumed leaf amount was calculated by subtracting the weight of the leaf disc at the end of the experiment, L_{end} (kg_{dw}), from the initial leaf disc weight, L_{start} (kg_{ww}), corrected by the mass ratio of the mass loss control, c ($\text{kg}_{\text{dw}}\ \text{kg}_{\text{ww}}^{-1}$). The feeding rate, k_{feed} ($\text{kg}_{\text{dw}}\ \text{kg}_{\text{ww}}^{-1}$), were determined using the consumed leaf amount; the mass of the gammarid, G (kg_{ww}); and the feeding duration, t (days):

$$k_{\text{feed}} = \frac{L_{\text{start}} \times c - L_{\text{end}}}{G \times t} \quad (1)$$

The proportions of the feeding on the two leaf discs were compared using the Wilcoxon signed-rank test and a significance level of $p=0.05$.

Dissecting

To evaluate the assimilation efficacy of the systemic pesticides, a similar exposure basin to the autumn scenario (Figure 1) was set up. However, gammarids were only sampled after 1 day of exposure but dissected into three compartments (midgut, hindgut, and carcass (see Figure 6A). Concentrations of the three compartments were determined separately.

Determination of fungicide concentrations

Liquid extraction was performed on both leaf and gammarid tissue, after adding 300 mg of 1-mm-diameter zirconia/silica beads (BioSpec Products), 500 μL of methanol, and 100 μL of isotope-labeled internal standard (250 $\mu\text{g L}^{-1}$ deuterated reference standards; Supporting Information, A6). Samples were homogenized using a FastPrep bead beater (two cycles of 15 s at $6\ \text{m s}^{-1}$; MP Biomedicals) and centrifuged (6 min, 10 000 g, 4°C). The supernatant was collected using syringes and filtered through 0.45- μm regenerated cellulose filters. Subsequently, syringes and filters were washed with another 400 μL of methanol and combined with the supernatant.

Liquid extraction from leaf disc samples was performed as described above with slight deviation of the homogenization method. Because leaf disc homogenization required dry samples, leaf discs were sampled into preweighed centrifuge vials (1.5 mL) already containing the 300 mg of silica beads, weighed (fresh wt, only for fresh, nonsoaked leaves), and freeze-dried. The dry weight was then determined by subtracting the preweight of the silica bead-containing vials. Leaf material was homogenized to dry powder using a cooled tissue lyser ($2 \times 10\ \text{s}$, $6\ \text{m s}^{-1}$, 4°C ; Bead Ruptor Elite, OMNI International). Medium samples were taken as 500 μL of medium combined with 400 μL of methanol and 100 μL of isotope-labeled internal standard. All samples were stored at -20°C until further analysis.

Chemical analysis was performed using an automated online solid-phase extraction system coupled with a reversed-phase liquid chromatography and high-resolution tandem mass spectrometer (LC-HRMS/MS; Q Exactive; Thermo Fisher Scientific). Ionization was achieved using an electrospray interface. Full scan acquisition was performed with a resolution of

70,000 (at m/z 200) in polarity-switching mode, followed by data-dependent MS/MS scans (five scans at positive mode and two at negative mode) with a resolution of 17,500 (at m/z 200) and an isolation window of 1 m/z . Further details on instrumentation, quality control parameters, and quantification are provided in Supporting Information, A6. A suspect screening on biotransformation products (BTPs) was performed based on BTPs reported in the literature for plants or aquatic invertebrates. The suspect list and corresponding literature are provided in Supporting Information, A7.

Toxicokinetic modeling

Toxicokinetic parameters of both bioconcentration and biomagnification processes were determined by applying two one-compartment first-order toxicokinetic models and the data of the autumn scenario. The models were implemented in the Matlab (R2019b)-based scripts of the Acute *Calanus* package, Ver 1.1 (Jager et al., 2017), of the Build Your Own Model platform. For bioconcentration, the tissue concentration, C_T (nmol $\text{kg}_{\text{ww}}^{-1}$), in the caged gammarids over time was described by the following ordinary differential equation:

$$\frac{dC_T(t)}{dt} = C_W(t) \times k_u - C_T(t) \times k_e \quad (2)$$

In Equation 2, C_W is the medium concentration (nM); the uptake rate, k_u ($\text{L kg}_{\text{ww}}^{-1} \text{d}^{-1}$), describes dermal and respiratory uptake; and the elimination rate, k_e (d^{-1}), integrates elimination of the parent compound by active and passive excretion as well as biotransformation.

For the biomagnification model, the concentration in the leaves, C_L (nmol $\text{kg}_{\text{dw}}^{-1}$); the experimentally determined feeding rate, k_{feed} ($\text{kg}_{\text{dw}} \text{kg}_{\text{ww}}^{-1}$); and the modeled assimilation factor, α , were used. To account for the simultaneous bioconcentration, average tissue concentrations of caged gammarids at a given time point were subtracted from the tissue concentrations of caged gammarids before these were used as a model input:

$$\frac{dC_t(t)}{dt} = C_L(t) \times k_{\text{feed}} \times \alpha - C_T(t) \times k_e \quad (3)$$

The kinetic bioconcentration and biomagnification factors (BCF_{kin} in $\text{L kg}_{\text{ww}}^{-1}$ and BMF_{kin} in $\text{kg}_{\text{dw}} \text{kg}_{\text{ww}}^{-1}$) were determined using the ratio of the uptake and elimination rates:

$$\text{BCF}_{\text{kin}} = \frac{k_u}{k_e} \quad (4)$$

$$\text{BMF}_{\text{kin}} = \frac{k_{\text{feed}} \times \alpha}{k_e} \quad (5)$$

During the uptake phase, continuous medium concentrations were estimated from measured concentrations using a linear fit. Continuous leaf concentrations were estimated by using a one-phase decay model. Corresponding model parameters are provided in Supporting Information, A11. Concentrations of both compartments were set to zero during the

elimination phase, which was confirmed by the chemical analysis. All model parameters were fitted simultaneously to the internal concentrations using the analytical solution (Ashauer & Jager, 2018). Best-fit parameters and 95% confidence intervals, using profile likelihoods, were used for further data processing.

Information on the determination of elimination half-life times, $t_{1/2}$, and time to reach 95% of the steady state, t_{ss} (equilibrium condition), are provided in Supporting Information, A8. An earlier study demonstrated the bioconcentration of the present fungicides to be independent of lipid content (Raths et al., 2023); thus, BCF_{kin} and BMF_{kin} were not lipid-normalized.

Data of the summer scenario were used for validation of the previously determined toxicokinetic model parameters. Wet weight and dry weight conversion factors were obtained over the course of the experiments. The conversion factors were 5.4 ± 0.3 (ratio wet to dry, $n=3$) for gammarids and 2.8 ± 0.2 (ratio wet to dry, $n=16$) for leaf discs and can be used to transform the generated data.

RESULTS AND DISCUSSION

Translocalization and transformation of systemic pesticides in chestnut leaves

Structures of the parent fungicides and corresponding BTPs quantified by LC-HRMS/MS are presented in Figure 2A. The residue concentrations in leafs from trees treated with systemic fungicides (Figure 2B) are presented in Figure 2C. The parent fungicide concentrations ranged over two orders of magnitude, from $260 \pm 90 \text{ nmol kg}^{-1}$ (tebuconazole) and $870 \pm 280 \text{ nmol kg}^{-1}$ (azoxystrobin) up to $22,700 \pm 1900 \text{ nmol kg}^{-1}$ (fluopyram), despite similar application rates of the four fungicides ($380\text{--}2000 \text{ nmol tree}^{-1}$; Table 1). Cyprodinil concentrations were below the limit of quantification (LOQ; 9 nmol kg^{-1}), but its main BTP, CGA 249287, was the compound with the second highest residue concentrations ($18,200 \pm 3700 \text{ nmol kg}^{-1}$). The BTPs of azoxystrobin (AZ_M390a) and fluopyram (2-trifluoromethyl benzamide) were detected in concentrations of one and two orders of magnitude lower than their corresponding parent compounds. Many further BTPs with lower intensities were tentatively identified but not quantified (Supporting Information, A7). Fungicide residues in the control leaves were below the LOQ (Supporting Information, Table S9) except for fluopyram, which was found in concentrations slightly above the LOQ but three orders of magnitude lower than the treatment. No fluopyram was detectable in gammarids fed with control leaves. Thus, fluopyram contamination in the control was considered negligible.

The high differences in leaf fungicide concentrations could indicate differences in the translaminar properties (i.e., caused by different physicochemical properties) or different biotransformation capabilities. Biotransformation may have occurred in both soil and plant tissue. However, differences in soil–leaf transfer capabilities of the fungicides persisted even if soil degradation was considered by estimating soil concentrations using half-life times in soil (Supporting Information, A9). Thus, it appears likely that the observed differences in leaf

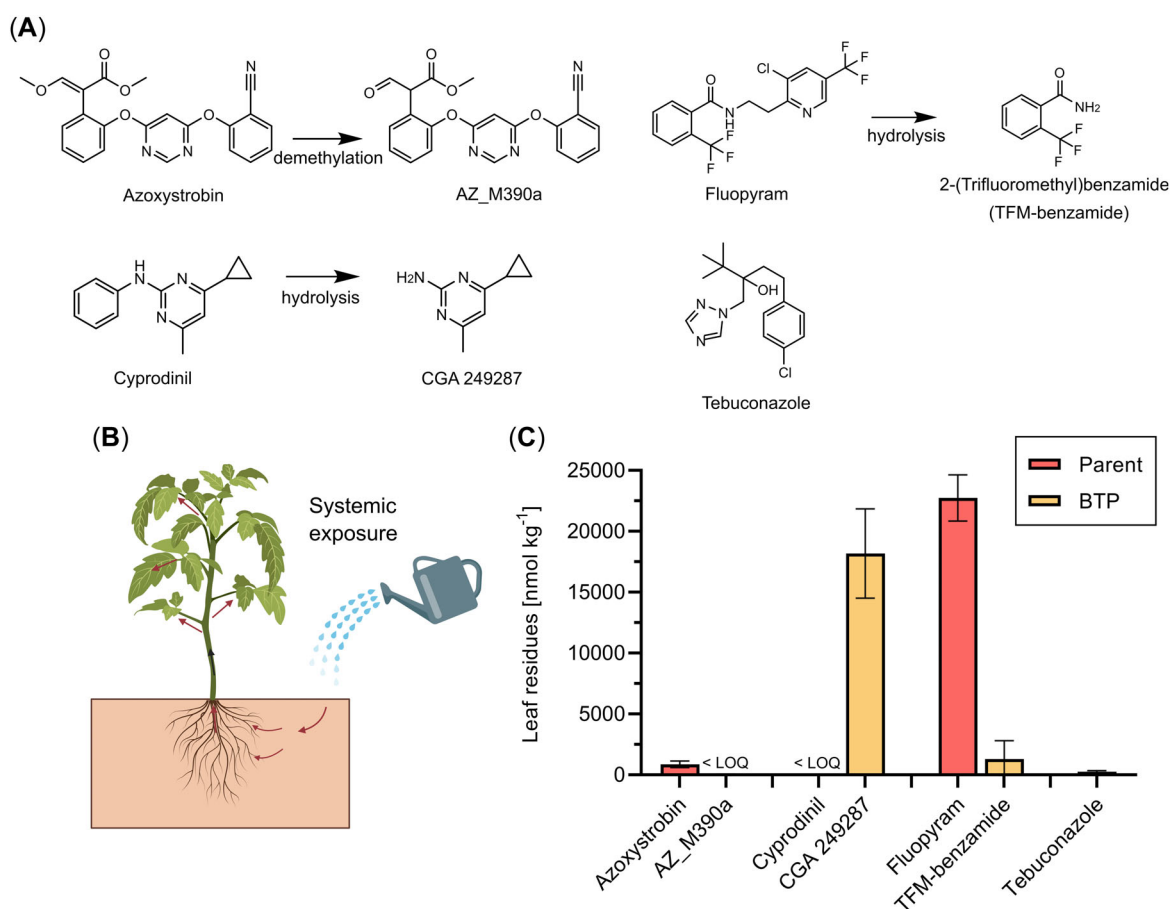


FIGURE 2: Structures of the tested systemic fungicides and their identified main biotransformation products (BTPs) and pathways found in chestnut leaves (A). Illustration of the systemic exposure procedure (B). Leaf residue concentrations (dry wt) of the four field rates applied systemic fungicides (red) and the most abundant BTPs (yellow) in chestnut leaves (C). Presented as mean \pm SD ($n = 3$). Underlying values, including concentrations in the control and the 10 times field rate treatment that was used in the mass spectrometric imaging, are provided in Supporting Information, B1. LOQ = limit of quantification.

concentrations were caused by toxicokinetics within the trees, rather than by soil degradation. The main BTPs were hydrolysis or dealkylation products and still contained the active moiety of the parent compound. Both transformations are common Phase I detoxification processes in plants (Bártíková et al., 2015). Further, strong biotransformation of the tested systemic fungicides in plants has been observed before (Gautam et al., 2018; Lv et al., 2017; Matadha et al., 2019; Robatscher et al., 2019; Sapp et al., 2004; Wei et al., 2016) and thus can be an important mechanism for detoxification and controlling leaf residues.

The MS-imaging of chestnut leaves exposed to systemic fungicides revealed a uniform distribution of the BTP CGA 249287 throughout the whole leaf cross section, similar to the membrane lipid phosphatidylcholine PC(32:0) which served as an orientation within the MS-image (Figure 3). However, fluopyram and tebuconazole were detected only in the laminar tissue and not in the vascular tissue of the veins. To an extent, both compounds were also affected by slight delocalization (detection outside of the sample area), potentially caused by leaching from leaf tissue into the embedding matrix. The other fungicides and BTPs could not be detected even when using leaves from trees exposed to 10 times the field rate. This was due to lower concentrations in the leaf tissue but also

lower response factors of azoxystrobin, fluopyram, and trifluoromethyl (TFM)-benzamide compared with the other compounds. In the leaves exposed to highly contaminated water, all fungicides (parents and BTPs), except for TFM-benzamide, could be detected because of the much higher leaf concentrations (Supporting Information, Figures S3 and S4). In leaves contaminated by sorption, all compounds showed the same uniform distribution, similar to PC(32:0). Distinct distributions between lamina and vascular tissue of other xenobiotics in arboreal leaf cross sections have been observed before (Villette et al., 2019), but underlying mechanisms remain unexplained. The comparison of systemically and sorption-exposed leaf cross sections indicates that plant physiology, such as biotransformation, transport, and deposition mechanisms in the trees, is driving the spatial distribution.

Regarding gammarid exposure, MS-imaging of the leaf material validated the accessibility of the incorporated fungicides. Because shredders are known to feed on lamina tissue of leaf litter but avoid higher lignified structures such as the vascular tissue of the veins (Arsuffi & Suberkropp, 1985), gammarids may have even been exposed to slightly higher local fluopyram and tebuconazole concentrations than estimated from whole leaf extracts.

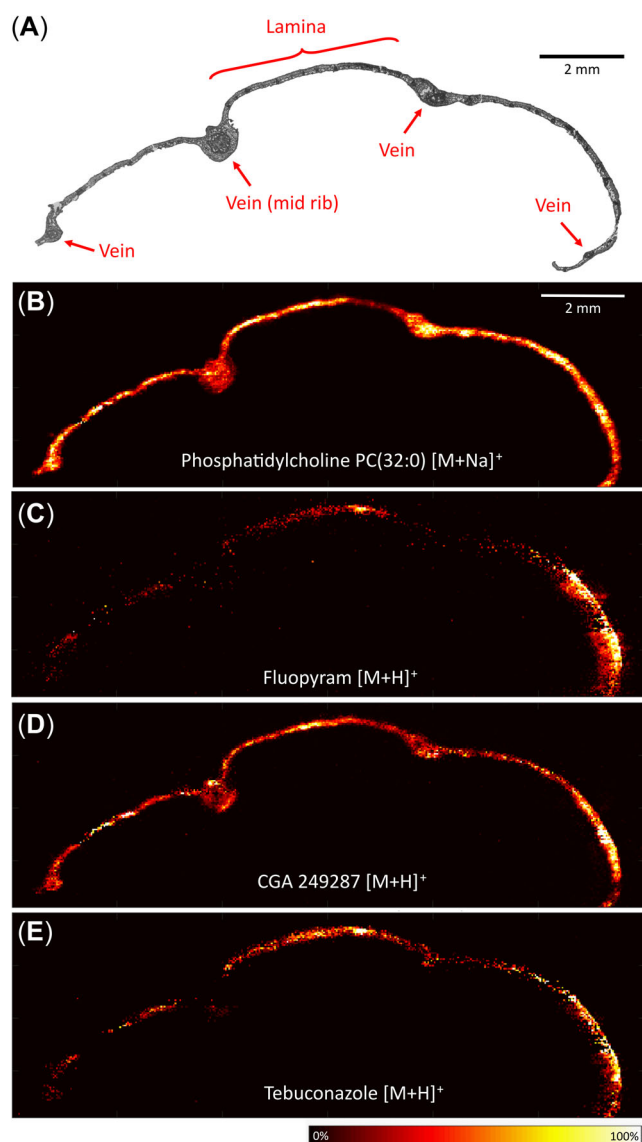


FIGURE 3: Matrix-assisted laser desorption/ionization-mass spectrometric images of chestnut leaf cross sections. (A) Microscopic image, (B) mass-to-charge ratio (m/z) 756.5513 = phosphatidylcholine PC(32:0) $[M+Na]^+$, (C) m/z 397.0537 = fluopyram $[M+H]^+$, (D) m/z 150.1026 = CGA 249287 $[M+H]^+$, (E) m/z 308.1524 = tebuconazole $[M+H]^+$. Color scale indicates the relative total ion current (TIC) normalized intensity. The pixel size was 30 μm . Additional replicates and controls, as well as leaves exposed via sorption, are presented in Supporting Information, A3.

Impact of leaf conditions on leaching behavior

The determined leaching half-life times for leaf discs of different conditions are shown in Table 2. All half-life times were in the range of 1 up to several days, indicating considerable fungicide losses within the time frame of gammarid exposure. Half-life times of systemic fungicides were highest in leaf discs from pristine leaves (I). They were much lower for leaf discs with reduced tissue integrity (II and III) or that were contaminated through sorption (IV). For instance, the leaching half-life of fluopyram decreased by approximately 70% through gammarid feeding or in leaf discs that were previously frozen.

TABLE 2: Leaching half-life time ($t_{1/2}$) of (I) fungicides from pristine leaves from trees exposed to systemic fungicides by soil application (field rate [FR]; Table 1; Supporting Information, A13), (II) pristine leaves with gammarid feeding (Supporting Information, A11), (III) frozen and thawed pristine leaves (FR \times 10; Supporting Information, Table S2, and A13), and (IV) pristine control leaves contaminated by sorption of fungicides from the aqueous phase (Supporting Information, A14)

Leaf condition	Pristine (I) $t_{1/2}$ (days)	Feeding (II) $t_{1/2}$ (days)	Frozen (III) ^a $t_{1/2}$ (days)	Sorption (IV) $t_{1/2}$ (days)
Azoxystrobin	9.2 (4.5–47)	2.5 (1.3–9.8)	4.0 (2.0–6.1)	3.0 (2.0–5.1)
CGA 249287	3.2 (1.9–6.2)	0.8 (0.6–1.3)	n.a.	n.a.
Fluopyram	3.6 (1.8–8.5)	1.3 (0.9–1.8)	1.0 (0.5–1.5)	1.6 (1.2–2.3)
Tebuconazole	8.1 (4.7–20)	3.2 (1.1–n.a.)	3.4 (2.1–4.6)	2.6 (1.9–3.9)

^aThe half-life times from III were extrapolated from the difference in medium concentration between pristine and frozen leaf discs and the pristine leaf leaching model.

The 95% confidence intervals are provided in parentheses. An expanded version of this table is presented in Supporting Information, A13.

$t_{1/2}$ = half-life time; n.a. = not available, because the biotransformation product was not identified or tested at the stage of the corresponding experiments.

This observation may be explained by damages to the leaf structure by feeding activity or consumption of more highly contaminated leaf compartments by gammarids or damage to the leaf structure caused by freezing and thawing (explained by Consolandi et al., 2021). The half-life of fluopyram in leaves contaminated through sorption of fungicides from the aqueous phase (IV) was less than half that of fluopyram incorporated into leaves by systemic uptake (I). The difference may be caused by an incorporation of fungicides into leaf compartments such as the vacuole or cell wall of leaves (Bártíková et al., 2015).

It was demonstrated that the leaf contamination pathway, as well as leaf condition could influence the fungicide dynamics in the gammarid test systems and should be considered when designing and evaluating feeding experiments. The decisions on the used leaf conditions most likely shaped the outcome of the presented experiments. With the use of leaf discs from pristine leaves, we created a realistic worst-case scenario for dietary exposure.

Sorption and leaching parameters of 26 common organic contaminants (including systemic fungicides and insecticides as well as pharmaceuticals) are provided in Supporting Information, A14, and may help decision-making in future experimental designs.

Dietary uptake drives fungicide bioaccumulation in the autumn scenario

Because of high differences in fungicide concentrations in the leaves (Figure 2), only fluopyram and CGA 249287 were detected throughout all compartments of the autumn scenario. Concentrations of the other fungicides and BTPs were below the LOQ for most medium and gammarid samples, which did not allow for toxicokinetic modeling and biomagnification assessment. The dynamics of the determined exposure

concentrations in the autumn scenario (Figure 1) were driven by leaching from the leaf material (Figure 4A). The leaf concentrations of fluopyram and CGA 249287 were decreasing during the exposure phase, with half-life times of 1.3 and 0.8 days, respectively (Table 2). Consequently, the medium concentration of both compounds increased from 0.9 to 2.2 nM. The concentrations in the medium and leaf material of the elimination phase remained below the LOQ. Model fits for the determination of medium and leaf concentrations are provided in Supporting Information, A11.

The time-resolved tissues analysis in the autumn scenario revealed much higher internal concentrations in uncaged gammarids compared with caged gammarids (Figure 4B). By the end of the uptake phase of the autumn scenario, the uncaged gammarids (bioconcentration and biomagnification) had three to nine times (fluopyram) and seven to eight times (CGA 249287) higher tissue concentrations than the caged gammarids (bioconcentration). The increased tissue concentrations in uncaged gammarids may be caused by a combination of fungicides assimilated from the diet and contaminated leaf material in the intestine (Figure 6B). The toxicokinetics in caged gammarids could be very well described by the applied bioconcentration model. However, the internal concentrations in gammarids with access to the leaf material showed a very high variance, with duplicates differing by up to a factor of 2.5. Consequently, many measured values were outside the range of the confidence intervals of the toxicokinetic model that included biomagnification. Because the provided leaf material had a rather low variance in leaf concentrations (SD ~10%, all leaf discs originated from the same tree), the high variance was most likely caused by variation in the individual feeding rates, as observed in the food choice assay. Furthermore, feeding rates of amphipods are known to vary not only on the individual level but also on a temporal scale. In both cases, feeding rates are also affected by abiotic parameters (i.e., light and temperature), leaf condition, physiological state (i.e. starvation),

and interactions with other organisms or contaminants (Consolandi et al., 2021; Götz et al., 2021; Maltby et al., 2002). Thus, the assessment of feeding rates may be challenging in modeling biomagnification processes with amphipods because feeding cannot be as controlled as in more standardized guidelines with other organisms (e.g., test guideline 305 using fish; Organisation for Economic Co-operation and Development, 2012). In this context, the variability in feeding rates could be addressed by averaging out over a longer period or by pooling a larger number of animals for tissue analysis. In the present study, the toxicokinetic rates and BCFs (BCF_{kin}) for fluopyram were slightly higher than reported previously (Raths et al., 2023). No literature data were available for bioconcentration of CGA 249287 or biomagnification of the tested compounds. The parameters of the bioconcentration and biomagnification model calibration are provided in Table 3.

Despite the different uptake processes of bioconcentration (filtration and diffusion) and biomagnification (feeding and assimilation), similar elimination rates were observed. Studies investigating the mathematical relationship between BCFs and BMFs generally observe that BCFs are three to four orders of magnitude higher than the corresponding BMFs in fish (Grisoni et al., 2018; Inoue et al., 2012). In our study, the BCFs of fluopyram and CGA 249287 were 2900 and 800 times higher than the BMFs and fit into the lower range of the reported relationships.

Aqueous uptake drives fungicide bioaccumulation in a summer scenario

Medium concentrations in the summer scenario remained stable with <10% deviation from the nominal concentration. The measured medium concentration of fluopyram was 2.2 nM and thus similar to the medium concentration at the end

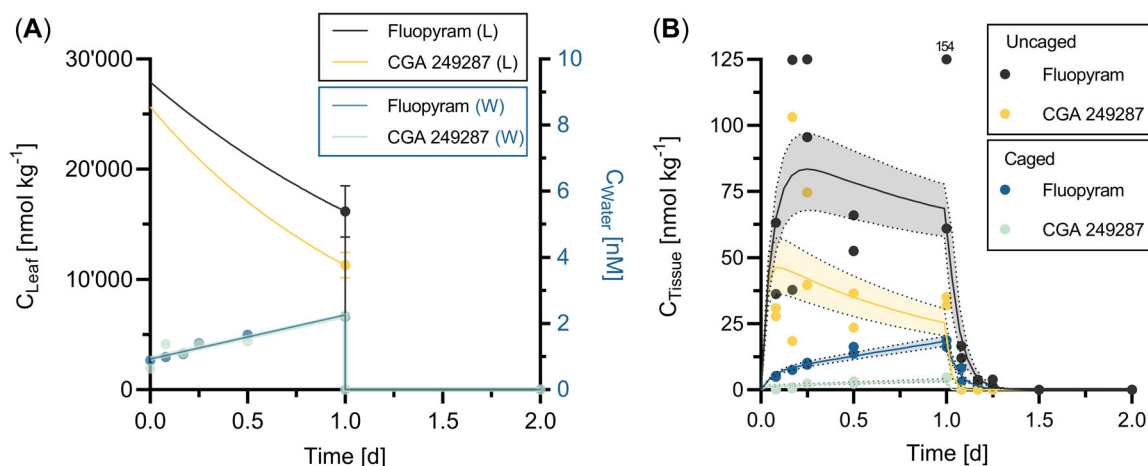


FIGURE 4: Exposure concentrations (L = leaf; W = water) of the autumn scenario (A) presented as modeled (lines) and measured (dots; mean \pm SD, $n = 3$) values. Water concentrations of fluopyram and CGA 249287 were similar so that the fits overlay. Tissue concentrations (B) in gammarids of the autumn scenario are presented as measured internal concentrations (dots) and toxicokinetic model fits (lines \pm 95% confidence intervals) for fluopyram and CGA 249287 in caged (bioconcentration) and uncaged (bioconcentration and biomagnification) gammarids. Dot labeled 154 = concentration in the replicate was outside of the plotted y-axis range. Model parameters are provided in Table 3 (gammarids) and Supporting Information, A11 (exposure concentrations). Underlying data are provided in Supporting Information, B4.

TABLE 3: Toxicokinetic parameters estimated (Equations 3–5) from the “autumn” scenario separating bioconcentration and biomagnification processes

Bioconcentration		k_u (L kg _{ww} ^{−1} day ^{−1})	k_e (day ^{−1})	BCF _{kin} (L kg _{ww} ^{−1})	R^2	
CGA 249287	Present study	184.8 (79–1900)	114 (50→1000)	1.6 (1.4–1.9)	0.95	
Fluopyram	Present study	128.5 (105–155)	15.0 (12.8–17.6)	8.6 (7.7–9.5)	0.97	
Fluopyram	Raths et al. (2023)	92.0 (83–103)	11.7 (10.7–12.9)	7.9 (7.5–8.3)	0.99	
Biomagnification		k_{feed} (kg _{dw} kg _{ww} ^{−1} day ^{−1})	α	k_e (day ^{−1})	BMF _{kin} (kg _{dw} kg _{ww} ^{−1})	R^2
CGA 249287	0.085	1 (0.5→1)	45.3 (22.3–57.7)	0.0019 (0.0015–0.0024)	0.88	
Fluopyram	0.085	0.6 (0.3–1)	15.9 (10.2–29.0)	0.0030 (0.0024–0.0037)	0.86	

The 95% confidence intervals are provided in parentheses.
 k_u = uptake rate; ww, wet weight; k_e = elimination rate; BCF_{kin} = kinetic bioconcentration factor; k_{feed} = feeding rate; BMF_{kin} = kinetic biomagnification factor; dw = dry weight.

of the autumn scenario. The equilibrated leaf concentration of fluopyram was 370 ± 10 nmol kg^{−1} and approximately 40 times lower than in the autumn scenario. Data for the three other fungicides are provided in Supporting Information, A12.

The measured tissue concentrations of fluopyram under equilibrium conditions were 20.1 ± 1.7 in the caged and 22.5 ± 2.8 nmol kg^{−1} in the uncaged gammarids (Figure 5). Because of similar medium concentrations, the internal fluopyram concentrations of caged gammarids were similar to those of caged gammarids in the autumn scenario. However, the internal concentration in uncaged gammarids of the summer scenario was approximately three to seven times lower than the measured tissue concentrations in gammarids of the autumn scenario. The toxicokinetic models calibrated on the autumn scenario could predict the internal concentration in both groups of the summer scenario properly, which validated the model parameters. Dietary uptake accounted for only 10% of the total tissue concentration in uncaged gammarids in the summer scenario, whereas dietary uptake accounted for >60% in the autumn scenario. This observation occurred despite the feeding rate being 1.8 times higher than in the autumn scenario (0.15 kg_{dw} kg_{ww}^{−1} d^{−1}). The reported contribution of the dietary uptake to the total tissue concentration of gammarids in equilibrated systems ranged from 10% (azoxystrobin, fluopyram; present study), 30% (cyprodinil, tebuconazole; present study), and 30%–40% (lead and brominated diphenyl ether 47; Hadji et al., 2016; Lebrun et al., 2014) up to 60% (4-nonylphenol; Gross-Sorokin et al., 2003) and increased with sorption-driven partitioning (log K_{ow}) toward the leaves.

With regard to in situ bioaccumulation of systemic fungicides in gammarids, the present results demonstrate that the concentration ratio of the two compartments, diet and water, determines the importance of their contribution to the whole body burden. For polar compounds, this ratio is usually in favor of bioconcentration. However, as observed in the autumn scenario, this is not necessarily the case when pesticides incorporated into the diet (i.e., leaves from a buffer stripe) are the initial contamination source of a system.

Behavioral bioavailability of contaminated leaves

Unexpectedly, *G. pulex* displayed a significant preference (Supporting Information, Figure S5; Wilcoxon's, $p > 0.001$) for leaf discs from exposed chestnut trees in the food choice assay. The median relative food consumption was 0.37 for the control versus 0.63 for the contaminated leaf discs. The average absolute feeding rate was 0.21 ± 0.09 kg_{dw} kg_{ww}^{−1} d^{−1}, but individual feeding rates showed a large variance, ranging from 0.05 to 0.46 kg_{dw} kg_{ww}^{−1} d^{−1} (Supporting Information, Figure S6). A high variance of individual feeding rates is common for feeding experiments because they strongly depend on individual physiological state and behavior (Consolandi et al., 2021; Götz et al., 2021; Maltby et al., 2009). In addition, this variance may have been increased by uncertainties in the leaf weight correction by mass loss and dry weight controls. Furthermore, the soaking time of 12 h was

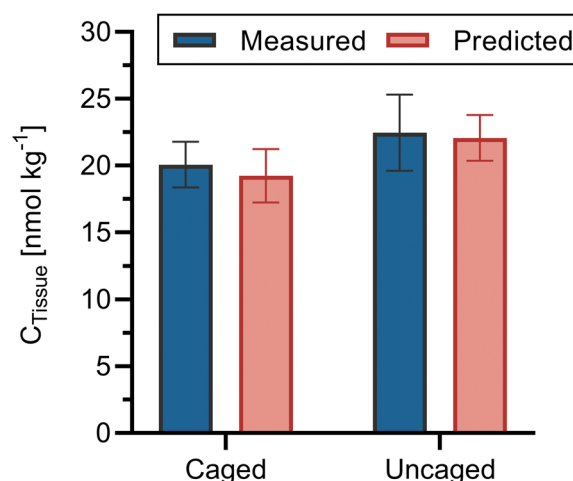


FIGURE 5: Internal concentrations under equilibrium conditions (mean ± SD, $n = 3$) and predicted concentrations (±95% confidence interval) for fluopyram in caged and uncaged gammarids of the summer scenario. Underlying data are provided in Supporting Information, B5.

rather short and may have resulted in lower feeding rates compared with longer soaking periods and thus increased leaf palatability (Consolandi et al., 2021). However, a short pre-soaking period was chosen to minimize the contaminant loss by leaching. The observed inability of gammarids to discriminate contaminated from uncontaminated leaf discs has been reported for systemically exposed material before (Englert, Zubrod, Link, et al., 2017; Kreutzweiser et al., 2009; Newton et al., 2018). Consequently, it is likely that gammarids are also not able to avoid contaminated leaves in situ, despite uncontaminated food sources being available.

Physiological bioavailability (assimilation) of fungicides from contaminated leaves

Absolute fungicide concentrations in the gammarid carcass were two (CGA 249287) to four (fluopyram) times higher in uncaged than in caged gammarids. The highest absolute fungicide concentrations were found in the intestine of gammarids, with up to seven times and 21 times higher concentrations compared with the carcass in caged and uncaged gammarids, respectively.

The determined tissue contributions to the total recovered body burden (Figure 6B) also showed a high contribution of the intestinal compartments to the total tissue concentrations. In caged gammarids, the intestinal compartment contributed 20%–34% to the total recovered fungicide amount. The relative contributions of the intestinal compartments to the total tissue concentrations were approximately 7%–10% for midgut and 23%–45% for hindgut in gammarids with access to contaminated leaves. A higher proportion of CGA 249287 was associated with the intestine, in comparison with fluopyram.

In conclusion, dissection of caged and uncaged gammarids revealed that CGA 249287 and fluopyram were bioavailable and efficiently assimilated into surrounding tissue from contaminated leaf material in the intestine, indicated by higher carcass concentrations in uncaged gammarids. These findings are further supported by the high assimilation factors (0.6 and

1) obtained from the biomagnification models presented above (Table 3). Overall, the higher concentrations in uncaged gammarids with access to leaves would be of toxicological relevance. In addition, it was demonstrated that the intestine tissue plays an important role in accumulation of waterborne organic contaminants in gammarids, as previously observed by Nyman et al. (2014).

Implications for risk assessment

At the end of the autumn scenario study, the medium concentrations were 0.9 and 0.3 $\mu\text{g L}^{-1}$ (2.3 and 2.2 nM) for fluopyram and CGA 249287, respectively. The water concentrations of CGA 249287 remobilized from leaf material exceeded the chronic environmental quality standard (EQS) of the parent cyprodinil (0.2 $\mu\text{g L}^{-1}$; Moschet et al., 2014). And CGA 249287 still contains the active moiety of cyprodinil, and thus may exert similar toxicological effects. For fluopyram, data for the EQS determination for surface waters are scarce because it was just introduced on the European market in 2013 (European Food Safety Authority, 2013). Li et al. (2020) indicated potential chronic EQS values for fluopyram in the 100 $\mu\text{g L}^{-1}$ range.

Because EQS values determined for surface waters do not consider other exposure routes such as contaminated diet, a conversion of the gammarid tissue concentration to the equivalent water concentration may be applied to evaluate the corresponding risk (Inostroza et al., 2016). In this case internal concentrations of gammarids with access to leaves from trees exposed to systemic fungicides (autumn scenario) would be equivalent to 7.1 and 7.0 $\mu\text{g L}^{-1}$ for fluopyram and CGA 249287, respectively. These hypothetical water concentrations are higher than most concentrations measured for fluopyram and cyprodinil during a summer monitoring study in a low-order agricultural stream (Lauper et al., 2021).

The ratio of leaves and water in the autumn scenario was approximately 5% of a typical amount reported for a first-order stream in central Germany (Benfield, 1997). This ratio was

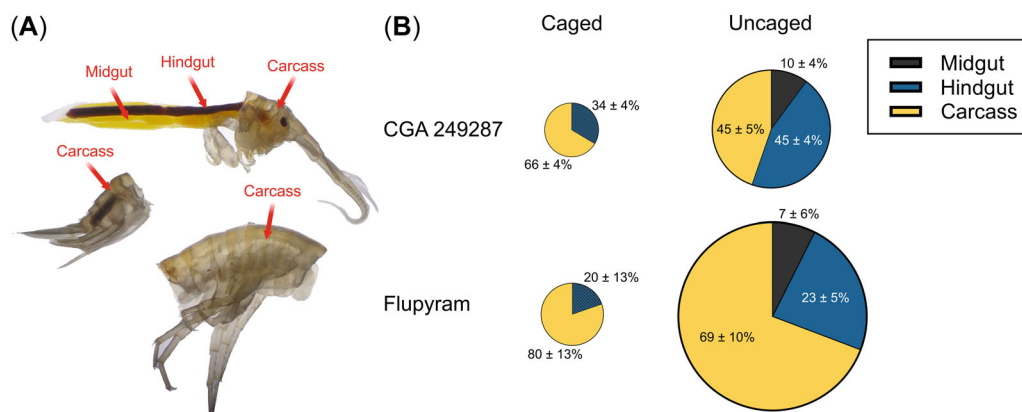


FIGURE 6: Dissected gammarid (A) and corresponding recovered fungicide proportions (B) in caged (no access to leaves) and uncaged (access to leaves) gammarids. Both intestine compartments were pooled for caged gammarids. Circle sizes are relative to the total tissue concentrations. Hatched blue indicates that midgut and hindgut were not separated for caged gammarids. Underlying data are provided in Supporting Information, B3.

applied in a leaching study by Englert, Bakanov, et al. (2017; 600 g m^{-2}), who demonstrated a strong effect of systemic pesticide remobilization from leaves of exposed trees. Thus, remobilization effects of fungicides from leaf material in the field could be potentially higher than observed in the present study. However, it is important to note that we tested a static system. Running water in streams may dilute the leached contaminants but also transport them to other, less contaminated sites. Thus, leaching from foliage may be an overlooked water contamination pathway because most monitoring studies focus on late spring and summer (Chow et al., 2020; Phillips & Bode, 2004).

Consequently, the present study indicates a potential seasonal extension of aquatic invertebrate exposure to systemic fungicides toward autumn. The exposure scenarios from the present study illustrate a pathway that may bypass riparian buffer stripes by retained fungicides entering the water body incorporated into leaves in autumn. Acute toxicity for systemic fungicides from dietary exposure appears unlikely, but chronic effects from secondary poisoning could be expected. Despite the common assumption that dietary uptake may only be relevant for less polar compounds, because they show higher sorption behavior toward organic matter, such as leaves, the present study revealed that systemic fungicides may be important dietary contaminants because of their translaminal properties. Even though the present scenarios covered a range of uptake mechanisms that are specific for the field (i.e., systemic uptake of the fungicides by trees, food selectivity), no monitoring studies have focused on such particular questions. Studies of this nature would be important to evaluate the risk originating from the elucidated mechanisms of seasonal exposure shift and the bypassing of riparian buffer stripes.

CONCLUSION

In the present study, we provided a deep insight into different contamination pathways of allochthonous food sources (leaf litter) and evaluated the relevance of the dietary uptake pathway for bioaccumulation across seasons. We conclude that the dietary uptake of systemic fungicides is generally of relatively low relevance, unless previously contaminated plant material enters a stream. Our study brings an important perspective to environmental risk assessment by illustrating a potential mechanism for systemic fungicides to bypass riparian buffer stripes. However, further research, such as monitoring studies, is needed to understand the consequences of the interconnectivity of terrestrial and aquatic ecosystems for systemic pesticide fluxes and risk of exposure.

Supporting Information—The Supporting Information is available on the Wiley Online Library at <https://doi.org/10.1002/etc.5615>.

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Author Contributions Statement—**Johannes Raths**: Conceptualization; Data curation; Investigation; Formal analysis; Methodology; Visualization; Writing—original draft. **Jacob Schnurr**: Conceptualization; Investigation; Methodology. **Mirco Bundschuh**: Conceptualization; Supervision; Writing—review & editing. **Fernanda E. Pinto**: Methodology; Supervision. **Christian Janfelt**: Supervision; Writing—review & editing. **Juliane Hollender**: Conceptualization; Funding acquisition; Supervision; Writing—review & editing.



This article has earned an Open Data and an Open Materials badge for making publicly available the digitally-shareable data necessary to reproduce the reported results. The data is available in the Eawag Research Data Institutional Collection (ERIC) at <https://doi.org/10.25678/0007RT>. Provided are SI A (main SI section) and the excel sheet B (holds measured concentrations and model parameters). Learn more about the Open Practices badges from the Center for Open Science: <https://osf.io/tyxz/wiki>.

REFERENCES

- Abelho, M. (2001). From litterfall to breakdown in streams: A review. *The Scientific World Journal*, 1, 656–680. <https://doi.org/10.1100/tsw.2001.103>
- Arsuffi, T. L., & Suberkropp, K. (1985). Selective feeding by stream caddisfly (Trichoptera) detritivores on leaves with fungal-colonized patches. *Oikos*, 45(1), 50–58. <https://doi.org/10.2307/3565221>
- Ashauer, R., & Jäger, T. (2018). Physiological modes of action across species and toxicants: The key to predictive ecotoxicology. *Environmental Science: Processes & Impacts*, 20(1), 48–57. <https://doi.org/10.1039/c7em00328e>
- Bärlocher, F., & Kendrick, B. (1975). Leaf-conditioning by microorganisms. *Oecologia*, 20(4), 359–362. <https://doi.org/10.1007/BF00345526>
- Bártíková, H., Skálová, L., Stuchlíková, L., Vokřál, I., Vaněk, T., & Podlipná, R. (2015). Xenobiotic-metabolizing enzymes in plants and their role in uptake and biotransformation of veterina. *Drug Metabolism Reviews*, 47(3), 374–387. <https://doi.org/10.3109/03602532.2015.1076437>
- Baudy, P., Zubrod, J. P., Konschak, M., Weil, M., Schulz, R., & Bundschuh, M. (2017). Does long-term fungicide exposure affect the reproductive performance of leaf-shredders? A partial life-cycle study using *Hyalella azteca*. *Environmental Pollution*, 222, 458–464. <https://doi.org/10.1016/j.envpol.2016.11.079>
- Benfield, E. F. (1997). Comparison of litterfall input to streams. *Journal of the North American Benthological Society*, 16(1), 104–108. <https://doi.org/10.2307/1468242>

- BioRender [Computer software]. BioRender. <https://biorender.com>
- Build Your Own Model [Computer software]. MathWorks. <https://www.debtbox.info/byom.html>
- Bundschuh, M., Zubrod, J. P., Kosol, S., Maltby, L., Stang, C., Duester, L., & Schulz, R. (2011). Fungal composition on leaves explains pollutant-mediated indirect effects on amphipod feeding. *Aquatic Toxicology*, 104(1), 32–37. <https://doi.org/10.1016/j.aquatox.2011.03.010>
- Bundschuh, R., Bundschuh, M., Otto, M., & Schulz, R. (2019). Food-related exposure to systemic pesticides and pesticides from transgenic plants: Evaluation of aquatic test strategies. *Environmental Sciences Europe*, 31(1), Article 87. <https://doi.org/10.1186/s12302-019-0266-1>
- Chow, R., Scheidegger, R., Doppler, T., Dietzel, A., Fenicia, F., & Stamm, C. (2020). A review of long-term pesticide monitoring studies to assess surface water quality trends. *Water Research X*, 9, Article 100064. <https://doi.org/10.1016/j.wroa.2020.100064>
- Consolandi, G., Ford, A. T., & Bloor, M. C. (2021). Feeding behavioural studies with freshwater *Gammarus* spp.: The importance of a standardised methodology. In P. de Voogt (Ed.), *Reviews of environmental contamination and toxicology* (Vol. 253, pp. 1–41). Springer International. https://doi.org/10.1007/398_2019_36
- Cornejo, A., Pérez, J., Alonso, A., López-Rojo, N., Monroy, S., & Boyero, L. (2020). A common fungicide impairs stream ecosystem functioning through effects on aquatic hyphomycetes and detritivorous caddisflies. *Journal of Environmental Management*, 263, Article 110425. <https://doi.org/10.1016/j.jenvman.2020.110425>
- Englert, D., Bakanov, N., Zubrod, J. P., Schulz, R., & Bundschuh, M. (2017). Modeling remobilization of neonicotinoid residues from tree foliage in streams—A relevant exposure pathway in risk assessment? *Environmental Science & Technology*, 51(3), 1785–1794. <https://doi.org/10.1021/acs.est.6b05213>
- Englert, D., Zubrod, J. P., Link, M., Mertins, S., Schulz, R., & Bundschuh, M. (2017). Does waterborne exposure explain effects caused by neonicotinoid-contaminated plant material in aquatic systems? *Environmental Science & Technology*, 51(10), 5793–5802. <https://doi.org/10.1021/acs.est.7b00827>
- Englert, D., Zubrod, J. P., Pietz, S., Stefani, S., Krauss, M., Schulz, R., & Bundschuh, M. (2017). Relative importance of dietary uptake and waterborne exposure for a leaf-shredding amphipod exposed to thiacloprid-contaminated leaves. *Scientific Reports*, 7(1), Article 16182. <https://doi.org/10.1038/s41598-017-16452-9>
- Erwin, D. C. (1973). Systemic fungicides: Disease control, translocation, and mode of action. *Annual Review of Phytopathology*, 11(1), 389–422. <https://doi.org/10.1146/annurev.py.11.090173.002133>
- European Food Safety Authority. (2013). Conclusion on the peer review of the pesticide risk assessment of the active substance fluopyram. *EFSA Journal*, 11(4), Article 3052. <https://doi.org/10.2903/j.efsa.2013.3052>
- Fielding, N. J., MacNeil, C., Dick, J. T. A., Elwood, R. W., Riddell, G. E., & Dunn, A. M. (2003). Effects of the acanthocephalan parasite *Echinorhynchus truttae* on the feeding ecology of *Gammarus pulex* (Crustacea: Amphipoda). *Journal of Zoology*, 261(3), 321–325. <https://doi.org/10.1017/S0952836903004230>
- Flores, L., Banjac, Z., Farré, M., Larrañaga, A., Mas-Martí, E., Muñoz, I., Barceló, D., & Elosegi, A. (2014). Effects of a fungicide (imazalil) and an insecticide (diazinon) on stream fungi and invertebrates associated with litter breakdown. *Science of the Total Environment*, 476–477, 532–541. <https://doi.org/10.1016/j.scitotenv.2014.01.059>
- Franke, U. (1977). Experimentelle untersuchungen zur respiration von *Gammarus fossarum* in abhängigkeit von temperatur. *Sauerstoffkonzentration und Wasserbewegung*, 3(4), 369–411.
- Gautam, M., Elhiti, M., & Fomsgaard, I. S. (2018). Maize root culture as a model system for studying azoxystrobin biotransformation in plants. *Chemosphere*, 195, 624–631. <https://doi.org/10.1016/j.chemosphere.2017.12.121>
- Götz, A., Imhof, H. K., Geist, J., & Beggel, S. (2021). Moving toward standardized toxicity testing procedures with particulates by dietary exposure of gammarids. *Environmental Toxicology and Chemistry*, 40(5), 1463–1476. <https://doi.org/10.1002/etc.4990>
- Graça, M. A. S., & Canhoto, C. (2006). Leaf litter processing in low order streams. *Limnetica*, 25(1), 1–10. <https://doi.org/10.23818/limn.25.01>
- Grisoni, F., Consonni, V., & Vighi, M. (2018). Detecting the bioaccumulation patterns of chemicals through data-driven approaches. *Chemosphere*, 208, 273–284. <https://doi.org/10.1016/j.chemosphere.2018.05.157>
- Gross-Sorokin, M. Y., Grist, E. P. M., Cooke, M., & Crane, M. (2003). Uptake and depuration of 4-nonylphenol by the benthic invertebrate *Gammarus pulex*: How important is feeding rate? *Environmental Science & Technology*, 37(10), 2236–2241. <https://doi.org/10.1021/es020092n>
- Hadiji, R., Urien, N., Uher, E., Fechner, L. C., & Lebrun, J. D. (2016). Contribution of aqueous and dietary uptakes to lead (Pb) bioaccumulation in *Gammarus pulex*: From multipathway modeling to in situ validation. *Ecotoxicology and Environmental Safety*, 129, 257–263. <https://doi.org/10.1016/j.ecoenv.2016.03.033>
- Hieber, M., & Gessner, M. O. (2002). Contribution of stream detritivores, fungi, and bacteria to leaf breakdown based on biomass estimates. *Ecology*, 83(4), 1026–1038. [https://doi.org/10.1890/0012-9658\(2002\)083\[1026:COSEDA\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2002)083[1026:COSEDA]2.0.CO;2)
- Hynes, H. B. N. (1975). The stream and its valley: With 4 figures and 2 tables in the text. *SIL Proceedings*, 1922–2010, 19(1), 1–15. <https://doi.org/10.1080/03680770.1974.11896033>
- Inostroza, P. A., Vera-Escalona, I., Wicht, A.-J., Krauss, M., Brack, W., & Norf, H. (2016). Anthropogenic stressors shape genetic structure: Insights from a model freshwater population along a land use gradient. *Environmental Science & Technology*, 50(20), 11346–11356. <https://doi.org/10.1021/acs.est.6b04629>
- Inoue, Y., Hashizume, N., Yoshida, T., Murakami, H., Suzuki, Y., Koga, Y., Takeshige, R., Kikushima, E., Yakata, N., & Otsuka, M. (2012). Comparison of bioconcentration and biomagnification factors for poorly water-soluble chemicals using common carp (*Cyprinus carpio* L.). *Archives of Environmental Contamination and Toxicology*, 63(2), 241–248. <https://doi.org/10.1007/s00244-012-9761-8>
- Jager, T., Øverjordet, I. B., Nepstad, R., & Hansen, B. H. (2017). Dynamic links between lipid storage, toxicokinetics and mortality in a marine copepod exposed to dimethylnaphthalene. *Environmental Science & Technology*, 51(13), 7707–7713. <https://doi.org/10.1021/acs.est.7b02212>
- Kawamoto, T., & Kawamoto, K. (2021). Preparation of thin frozen sections from nonfixed and undecalcified hard tissues using Kawamoto's film method (2020). In M. J. Hilton (Ed.), *Skeletal development and repair: Methods and protocols* (pp. 259–281). Springer. https://doi.org/10.1007/978-1-0716-1028-2_15
- Kiefer, K., Müller, A., Singer, H., & Hollender, J. (2019). New relevant pesticide transformation products in groundwater detected using target and suspect screening for agricultural and urban micropollutants with LC-HRMS. *Water Research*, 165, Article 114972. <https://doi.org/10.1016/j.watres.2019.114972>
- Kreutzweiser, D., Good, K., Chartrand, D., Scarr, T., & Thompson, D. (2007). Non-target effects on aquatic decomposer organisms of imidacloprid as a systemic insecticide to control emerald ash borer in riparian trees. *Ecotoxicology and Environmental Safety*, 68(3), 315–325. <https://doi.org/10.1016/j.ecoenv.2007.04.011>
- Kreutzweiser, D. P., Good, K. P., Chartrand, D. T., Scarr, T. A., & Thompson, D. G. (2008). Are leaves that fall from imidacloprid-treated maple trees to control Asian longhorned beetles toxic to non-target decomposer organisms? *Journal of Environmental Quality*, 37(2), 639–646. <https://doi.org/10.2134/jeq2007.0278>
- Kreutzweiser, D. P., Thompson, D. G., & Scarr, T. A. (2009). Imidacloprid in leaves from systemically treated trees may inhibit litter breakdown by non-target invertebrates. *Ecotoxicology and Environmental Safety*, 72(4), 1053–1057. <https://doi.org/10.1016/j.ecoenv.2008.09.017>
- Lauper, B. B., Anthamatten, E., Rath, J., Arlos, M., & Hollender, J. (2021). Systematic underestimation of pesticide burden for invertebrates under field conditions: Comparing the influence of dietary uptake and aquatic exposure dynamics. *ACS Environmental*, 2(2), 166–175. <https://doi.org/10.1021/acsenvironau.1c00023>
- Lebrun, J. D., Leroy, D., Giusti, A., Gourlay-Francé, C., & Thomé, J.-P. (2014). Bioaccumulation of polybrominated diphenyl ethers (PBDEs) in *Gammarus pulex*: Relative importance of different exposure routes and multipathway modeling. *Aquatic Toxicology*, 154, 107–113. <https://doi.org/10.1016/j.aquatox.2014.05.015>
- Li, C., Yuan, S., Jiang, F., Xie, Y., Guo, Y., Yu, H., Cheng, Y., Qian, H., & Yao, W. (2020). Degradation of fluopyram in water under ozone enhanced microbubbles: Kinetics, degradation products, reaction mechanism, and toxicity evaluation. *Chemosphere*, 258, Article 127216. <https://doi.org/10.1016/j.chemosphere.2020.127216>
- Lorensen, M. D. B. B., Bjamholt, N., St-Pierre, B., Heinicke, S., Courdavaud, V., O'Connor, S., & Janfelt, C. (2023). Spatial localization of monoterpenoid indole alkaloids in *Rauvolfia tetraphylla* by high resolution

- mass spectrometry imaging. *Phytochemistry*, 209, 113620. <https://doi.org/10.1016/j.phytochem.2023.113620>
- Lv, T., Carvalho, P. N., Casas, M. E., Bollmann, U. E., Arias, C. A., Brix, H., & Bester, K. (2017). Enantioselective uptake, translocation and degradation of the chiral pesticides tebuconazole and imazalil by *Phragmites australis*. *Environmental Pollution*, 229, 362–370. <https://doi.org/10.1016/j.envpol.2017.06.017>
- Maltby, L., Brock, T. C. M., & van den Brink, P. J. (2009). Fungicide risk assessment for aquatic ecosystems: Importance of interspecific variation, toxic mode of action, and exposure regime. *Environmental Science & Technology*, 43(19), 7556–7563. <https://doi.org/10.1021/es901461c>
- Maltby, L., Clayton, S. A., Wood, R. M., & McLoughlin, N. (2002). Evaluation of the *Gammarus pulex* in situ feeding assay as a biomonitor of water quality: Robustness, responsiveness, and relevance. *Environmental Toxicology and Chemistry*, 21(2), 361–368. <https://doi.org/10.1002/etc.5620210219>
- Matadha, N. Y., Mohapatra, S., Siddamallaiha, L., Udupi, V. R., Gadigeppa, S., & Raja, D. P. (2019). Uptake and distribution of fluopyram and tebuconazole residues in tomato and bell pepper plant tissues. *Environmental Science and Pollution Research*, 26(6), 6077–6086. <https://doi.org/10.1007/s11356-018-04071-4>
- Moschet, C., Wittmer, I., Simovic, J., Junghans, M., Piazzoli, A., Singer, H., Stamm, C., Leu, C., & Hollender, J. (2014). How a complete pesticide screening changes the assessment of surface water quality. *Environmental Science & Technology*, 48(10), 5423–5432. <https://doi.org/10.1021/es500371t>
- Munz, N. A., Fu, Q., Stamm, C., & Hollender, J. (2018). Internal concentrations in gammarids reveal increased risk of organic micropollutants in wastewater-impacted streams. *Environmental Science & Technology*, 52(18), 10347–10358. <https://doi.org/10.1021/acs.est.8b03632>
- Naylor, C., Maltby, L., & Calow, P. (1989). Scope for growth in *Gammarus pulex*, a freshwater benthic detritivore. *Hydrobiologia*, 188(1), 517–523. <https://doi.org/10.1007/BF00027819>
- Newton, K., Zubrod, J. P., Englert, D., Lüderwald, S., Schell, T., Baudy, P., Konschak, M., Feckler, A., Schulz, R., & Bundschuh, M. (2018). The evil within? Systemic fungicide application in trees enhances litter quality for an aquatic decomposer–detritivore system. *Environmental Pollution*, 241, 549–556. <https://doi.org/10.1016/j.envpol.2018.05.069>
- Nyman, A.-M., Schirmer, K., & Ashauer, R. (2014). Importance of toxicokinetics for interspecies variation in sensitivity to chemicals. *Environmental Science & Technology*, 48(10), 5946–5954. <https://doi.org/10.1021/es5005126>
- Organisation for Economic Co-operation and Development. (2012). Test No. 305: Bioaccumulation in fish: Aqueous and dietary exposure. *OECD guidelines for the testing of chemicals*. https://www.oecd-ilibrary.org/environment/test-no-305-bioaccumulation-in-fish-aqueous-and-dietary-exposure_9789264185296-en
- Pascoal, C., & Cássio, F. (2004). Contribution of fungi and bacteria to leaf litter decomposition in a polluted river. *Applied and Environmental Microbiology*, 70(9), 5266–5273. <https://doi.org/10.1128/AEM.70.9.5266-5273.2004>
- Phillips, P. J., & Bode, R. W. (2004). Pesticides in surface water runoff in south-eastern New York State, USA: Seasonal and stormflow effects on concentrations. *Pest Management Science*, 60(6), 531–543. <https://doi.org/10.1002/ps.879>
- Prism (Version 9) [Computer software]. GraphPad Software.
- Rasmussen, J. J., Wiberg-Larsen, P., Baattrup-Pedersen, A., Monberg, R. J., & Kronvang, B. (2012). Impacts of pesticides and natural stressors on leaf litter decomposition in agricultural streams. *Science of the Total Environment*, 416, 148–155. <https://doi.org/10.1016/j.scitotenv.2011.11.057>
- Raths, J., Švara, V., Lauper, B., Fu, Q., & Hollender, J. (2023). Speed it up: How temperature drives toxicokinetics of organic contaminants in freshwater amphipods. *Global Change Biology*, 29(5), 1390–1406. <https://doi.org/10.1111/gcb.16542>
- Ravazzi, C., & Caudullo, G. (2016). *Aesculus hippocastanum* in Europe: Distribution, habitat, usage and threats. In J. San-Miguel Ayanz, D. de Rigo, G. Caudullo, T. H. Durrant, & A. Mauri (Eds.), *European Atlas of Forest Tree Species* (p. 60). Publication Office of the European Union.
- Robatscher, P., Eisenstecken, D., Innerebner, G., Roschatt, C., Raifer, B., Rohregger, H., Hafner, H., & Oberhuber, M. (2019). 3-Chloro-5-trifluoromethylpyridine-2-carboxylic acid, a metabolite of the fungicide fluopyram, causes growth disorder in *Vitis vinifera*. *Journal of Agricultural and Food Chemistry*, 67(26), 7223–7231. <https://doi.org/10.1021/acs.jafc.8b05567>
- Rösch, A., Gottardi, M., Vignet, C., Cedergreen, N., & Hollender, J. (2017). Mechanistic understanding of the synergistic potential of azole fungicides in the aquatic invertebrate *Gammarus pulex*. *Environmental Science & Technology*, 51(21), 12784–12795. <https://doi.org/10.1021/acs.est.7b03088>
- Sapp, M., Ertunç, T., Bringmann, I., Schäffer, A., & Schmidt, B. (2004). Characterization of the bound residues of the fungicide cyprodinil formed in plant cell suspension cultures of wheat. *Pest Management Science*, 60(1), 65–74. <https://doi.org/10.1002/ps.787>
- Schulz, R. (2004). Field studies on exposure, effects, and risk mitigation of aquatic nonpoint-source insecticide pollution: A review. *Journal of Environmental Quality*, 33(2), 419–448. <https://doi.org/10.2134/jeq2004.4190>
- Suberkropp, K., & Klug, M. J. (1976). Fungi and bacteria associated with leaves during processing in a woodland stream. *Ecology*, 57(4), 707–719. <https://doi.org/10.2307/1936184>
- Vannote, R. L., Minshall, G. W., Cummins, K. W., Sedell, J. R., & Cushing, C. E. (1980). The river continuum concept. *Canadian Journal of Fisheries and Aquatic Sciences*, 37(1), 130–137. <https://doi.org/10.1139/f80-017>
- Villette, C., Maurer, L., Wanko, A., & Heintz, D. (2019). Xenobiotics metabolization in *Salix alba* leaves uncovered by mass spectrometry imaging. *Metabolomics*, 15(9), Article 122. <https://doi.org/10.1007/s11306-019-1572-8>
- Wallace, J. B., Eggert, S. L., Meyer, J. L., & Webster, J. R. (1997). Multiple trophic levels of a forest stream linked to terrestrial litter inputs. *Science*, 277(5322), 102–104. <https://doi.org/10.1126/science.277.5322.102>
- Wei, P., Liu, Y., Li, W., Qian, Y., Nie, Y., Kim, D., & Wang, M. (2016). Metabolic and dynamic profiling for risk assessment of fluopyram, a typical phenylamide fungicide widely applied in vegetable ecosystem. *Scientific Reports*, 6(1), Article 33898. <https://doi.org/10.1038/srep33898>
- Zubrod, J. P., Bundschuh, M., Arts, G., Bruhl, C. A., Imfeld, G., Knabel, A., Payraudeau, S., Rasmussen, J. J., Rohr, J., Scharmuller, A., Smalling, K., Stehle, S., Schulz, R., & Schafer, R. B. (2019). Fungicides: An overlooked pesticide class. *Environmental Science & Technology*, 53(7), 3347–3365. <https://doi.org/10.1021/acs.est.8b04392>
- Zubrod, J. P., Englert, D., Feckler, A., Koksharova, N., Konschak, M., Bundschuh, R., Schnetzer, N., Englert, K., Schulz, R., & Bundschuh, M. (2015). Does the current fungicide risk assessment provide sufficient protection for key drivers in aquatic ecosystem functioning? *Environmental Science & Technology*, 49(2), 1173–1181. <https://doi.org/10.1021/es5050453>
- Zubrod, J. P., Englert, D., Wolfram, J., Wallace, D., Schnetzer, N., Baudy, P., Konschak, M., Schulz, R., & Bundschuh, M. (2015). Waterborne toxicity and diet-related effects of fungicides in the key leaf shredder *Gammarus fossarum* (Crustacea: Amphipoda). *Aquatic Toxicology*, 169, 105–112. <https://doi.org/10.1016/j.aquatox.2015.10.008>