



Concentration dependent fate of tebuconazole applied as a commercial formulation and identification of transformation products in a one-year mesocosm study

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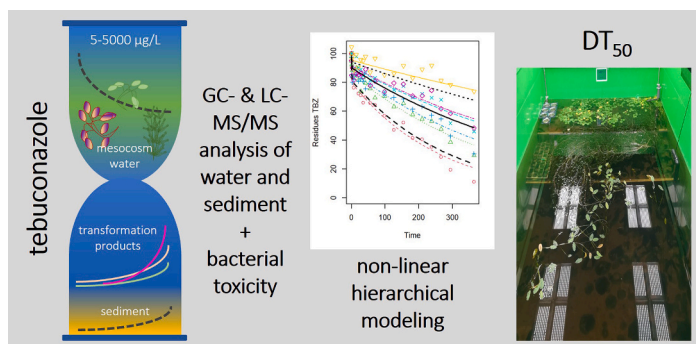
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HIGHLIGHTS

- Tebuconazole persists at environmentally relevant concentrations ($DT_{50} > 40$ days).
- DT_{50} for tebuconazole in water shows concentration dependency.
- Non-linear hierarchical kinetic model takes influence of covariate into account.
- Negligible formation of tebuconazole transformation products.
- Only minor translocation of maximum 5% from water to sediment within one year.

GRAPHICAL ABSTRACT



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ABSTRACT

Determining the half-lives of plant protection products and biocides is essential for assessing their environmental persistence and regulatory risk. However, there is no standardized approach defining how many data sets should be considered or whether influencing factors such as substance concentration must be included. For pesticides that are primarily degraded microbially, concentration can significantly influence degradation rates. Elevated levels may exert toxic effects on microorganisms, thereby slowing degradation. This is relevant for the fungicide tebuconazole, which has a broad mode of action including antimicrobial toxicity.

Within a comprehensive mesocosm study, concentration-dependent degradation of a commercial tebuconazole formulation was investigated. Eight freshwater stream-pond mesocosms containing flow-through and stagnant zones were established and dosed once with 0, 5, 50, 100, 500, 1000, and 5000 µg/L tebuconazole. Over one year, concentrations in water from all systems and in sediment layers of selected treatments were monitored using GC-MS/MS.

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Dissipation times for water, sediment, and the overall system were calculated using a newly developed hierarchical kinetic model that incorporated concentration as a covariate, alongside standard degradation models with different error models. A clear concentration dependency was observed: dissipation time increased from 176 days at the lowest concentration to 1079 days at the highest. Transfer to sediment was minimal (<5% within one year). Relevant transformation products were detected and partially quantified in water via LC-MS/MS, but their low quantities allowed them to be excluded from kinetic modeling.

1. Introduction

Though the need for a systemic change to reduce the use of- and to minimize the world's reliance on- synthetic plant protection products (PPPs) is pressing, it is unlikely that these products will be replaced completely in the near future by more sustainable farming practices. Next to herbicides and insecticides, fungicides play a key role in the strategic combat of harmful infections, particularly in the cultivation of cereals, fruits and vegetables (Jørgensen and Heick, 2021). In addition to single applications within a harvest cycle in cereal cultivation, multiple applications of fungicides are frequently carried out as spray sequences over a single harvest season, particularly in fruit cultivation. Spray drift during application and run-off after precipitation events can result in the contamination of adjacent aquatic ecosystems by these substances (Huber et al., 2000; Knäbel et al., 2014). For all PPP groups (herbicides, insecticides and fungicides), concentrations in the microgram per liter range, including exceedances of the regulatory acceptable concentrations (RAC), have been detected in European water bodies in the immediate vicinity of agricultural land, especially after rain events (Halbach et al., 2021; Szöcs et al., 2017).

The risk assessment of plant protection products and their active substances takes physico-chemical properties (e.g. persistence in the ecosystem, displacement behavior between compartments and biota) and classical ecotoxicological standard tests, representative for the terrestrial and aquatic environments (EFSA, 2013), into account. Active substances need to be approved at EU level (EU Commission) and products, containing additional co-formulants such as stabilizers or solubilizers, are then authorized at national level (in Germany by the Federal Office of Consumer Protection and Food Safety (BVL)). If the ecotoxicity of the product can be explained by the ecotoxicity of the active substance, co-formulants are not considered relevant for the risk assessment, and no separate assessment of persistence, bioavailability, translocation or degradation behavior is carried out for the finished commercial product.

To address the fate of broad-spectrum fungicides that are primarily degraded microbially but that may also affect the microbiome due to their modes of action, a comprehensive mesocosm study reflecting near-natural conditions was performed over the course of one year.

Tebuconazole as widely applied fungicide formulation was used as a model compound to examine different ecotoxicological aspects and whether degradation processes are concentration dependent in aquatic environments. Tebuconazole is considered to be photolytically and hydrolytically stable (pH 5–9 at 25 °C), and biological transformation is assumed to be the main degradation pathway. For active substances with poor water solubility (tebuconazole with 36 mg/L at 20 °C and pH 7.2, $\log K_{ow} = 3.7$, ECHA, 2013) and a tendency to adsorb to organic material ($\log K_{oc} > 2$), translocation processes between compartments such as sorption onto sediment or suspended particles, as well as resulting changes in the degradation behavior can be expected. A sediment compartment was implemented in the mesocosm to consider this aspect.

The commercially available formulation Folicur® containing the active ingredient tebuconazole and the co-formulant *N,N*-dimethyldecylamide, was selected as test substance. Tebuconazole is primarily used to protect crops from fungal diseases (Tleuova et al., 2020) but it is also included as a biocide in facade and wood paints or is used as a film preservative (EU Regulation, 2013; Jørgensen and Heick, 2021; Woo

et al., 2010). Although its domestic sales volume per year has declined since 2016 from 1000 tons to 579 in 2024, tebuconazole is still the second most sold azole fungicide in Germany (BVL, accessed December, 2025).

Even though the aquatic toxicity of tebuconazole is described as low, with macrophytes being the most sensitive non-target species with EC_{50} values of 216 and 144 µg/L tebuconazole for *Myriophyllum spicatum* and *Lemna gibba*, respectively (Mohr et al., 2025; EFSA, 2014), it is classified as hazardous to the aquatic environment, due to its possible endocrine disrupting properties (Li et al., 2019). Since aquatic fungi play a crucial role in degradation processes, be it of organic material such as leaves and wood, or other organic compounds including synthetic pesticides, these processes can be disturbed if active substances that specifically inhibit the growth of fungi, enter the aquatic environment (Carles et al., 2017; Ittner et al., 2018; Oliveira et al., 2015). As a consequence of disturbed microbial degradation processes, the persistence of hydrolytically and photolytically stable substances might be concentration dependent.

Large differences in the data on tebuconazoles half-life for water and the whole system (water and sediment) reaching from around 50 days to >1 year (EU Regulation, 2013) complicate the transferability to the aquatic ecosystem. To date, there are no precise regulations on how many data sets should be taken into account and which other influencing factors should be considered in the calculation of a reliable dissipation time (time required for the amount or concentration of a substance in a system to decrease e.g. 50% of its initial value, DT_{50}). Therefore, the aim of the present study was to determine a credible DT_{50} value for water and the water sediment system, taking the recommendations for degradation modeling in aquatic systems into account, such as sufficient sampling occasions, several test concentrations, temperature normalization, determination of known main transformation products, and the statistical comparison of different degradation kinetic models (Focus, 2014). The present study is the first study to calculate the half-life of a commercial fungicide formulation combining several data sets with a non-linear hierarchical model (NLHM, Ranke, 2023) taking the exposure concentration into account as a co-variate. This is of particular interest if the exposure concentration has a significant influence on the degradation behavior. In our case, due to the possible inhibitory effect of the active substance on underlying degradation processes (mainly microbial, EFSA, 2014), the exposure concentration was assumed to have an influence on the degradation rate. To account for this aspect, possible adverse effects on microbes were measured with a standard laboratory toxicity test for luminescent bacteria. Furthermore, the study provides precise and reliable new detection methods for tebuconazole and its transformation products in water and sediment. The fate of tebuconazole as detected in our systems is discussed in light of the existing literature and its transferability to the natural environment.

2. Material and methods

Besides determining tebuconazole's half-life, the mesocosm study also investigated its ecotoxicological effects across several taxonomic groups (not addressed in this publication). The mesocosm setup was therefore designed to adequately assess both environmental fate and ecotoxicological effects.

2.1. Experimental design

For the present study, eight limnetic mesocosms (Fig. 1) with varied flow characteristics in different regions of the systems, i.e. flow-through areas (stream simulation) and stagnant areas (pond simulation) were set up four months prior to the start of the experiment (establishing phase) at the artificial stream and pond facility (FSA) of the German Environment Agency (UBA). In the following, the systems are referred to as stream-pond mesocosms. Two stream-pond mesocosms were used as untreated controls and in six stream-pond mesocosms, the tebuconazole formulation Folicur® (Bayer AG) was dosed once in concentrations of 5, 50, 100, 500, 1000 and 5000 µg/L. The treatments were randomly assigned to the eight stream-pond mesocosms. The two lower treatment concentrations represented environmentally relevant concentrations, whereas the higher treatment concentrations were included to investigate potential effects on microbial activity. On the one hand, bacterial growth might be promoted (tebuconazole as carbon source) while, on the other hand, microbial inhibition could occur due to the substance's toxic properties. Both mechanisms could potentially affect dissipation and, consequently, the half-life of tebuconazole.

2.1.1. Establishment phase

The stream-pond mesocosms were divided into a 40 m long flow-through channel section (1 m width) with one bend, driven by a screw pump at a flow velocity of 0.1 m/s, and a pond section ($6.9 \times 3.25 \times 2.5$ m) with moderate flow at the in- and outflow, and stagnant conditions in the shore area of the pond (Fig. 1). The shore area was separated into two terraced steps of equal size (3.3 m^2 each), which were divided by sheet pile walls. All constructional elements except the stainless-steel screw pumps and stainless-steel sheet pile walls, consisted of inert glass fiber reinforced polyester. Four months prior to TBZ application, the stream-pond mesocosms were set up with sand and fine organic sediment to a height of 15 cm in four of the 11 channel segments, as well as in the pond section to a height of 2.4 and 2.1 m in the first and second shore area, respectively, and to a height of 1.6 m at the pond bottom (15.9 m^2). Under the top 5 cm layer of pure sand, a 5 cm layer consisting

of a mixture of fine organic sediment from a reference pond and sand (3:1) was applied to provide a natural supply of nutrients. The stream-pond mesocosms were filled with a mixture of processed ground water and de-ionized water with a final conductivity of 520 µS/cm (total water volume 30.83 m^3), to a water depth of 45 cm in the sediment-filled channel segments, 60 cm in the non-sediment-filled channel segments, 35 cm and 85 cm in shore areas 1 and 2, respectively, and 135 cm at the pond bottom. Throughout the establishment phase, the eight systems were interconnected via bulkheads (Fig. 1). To promote circulation and homogenous mixing of the water between the individual mesocosms, airlift pumps close to the bulkheads and close to the shore area were operated continuously in this phase. Two weeks before fungicide dosing, the systems were decoupled by closing the bulkheads.

In the pond area, each system was illuminated with six 4-channel LED lights (Neptun®, Roschwege) with $150 \text{ µmol/m}^2/\text{s}$ photosynthetically active radiation (PAR) measured at the water surface (400–700 nm, two white channels), 200 µW/cm^2 ultraviolet radiation (350–425 nm, UV channel) and 30 µW/cm^2 far red radiation (650–800 nm, FR channel). Pond illumination included a twilight phase of 1 h each at dawn and dusk and was adjusted monthly to the seasonal day length. In the stream section, systems were illuminated with fluorescent tubes (Biolux L 58 W/965, Osram) resulting in a PAR of $80 \text{ µmol/m}^2/\text{s}$ at the water surface, using a similar lighting regime, but without the twilight phase.

For the biological set-up of the systems, the two shore areas and the adjacent pond bottom area were planted at ten pre-marked positions each with *Nymphoides peltatum* (shore area 1, 1 cutting per position), *Myriophyllum spicatum* (shore area 2, 5 cuttings per position) and *Potamogeton natans* (pond bottom, 3 cuttings per position). Mesocosms were inoculated with periphyton and plankton communities from two different mesotrophic lakes (Lake Britzer Garten and Teufelssee, Berlin, Germany), as well as with different developmental stages of aquatic fungi and macrozoobenthic organisms originating from a reference stream (Lieberoser Mühlenfließ, Brandenburg, Germany). For biological sampling, racks for periphyton and aquatic fungi (stainless steel racks with glass tiles, leaf litter enclosures respectively), as well as eclector

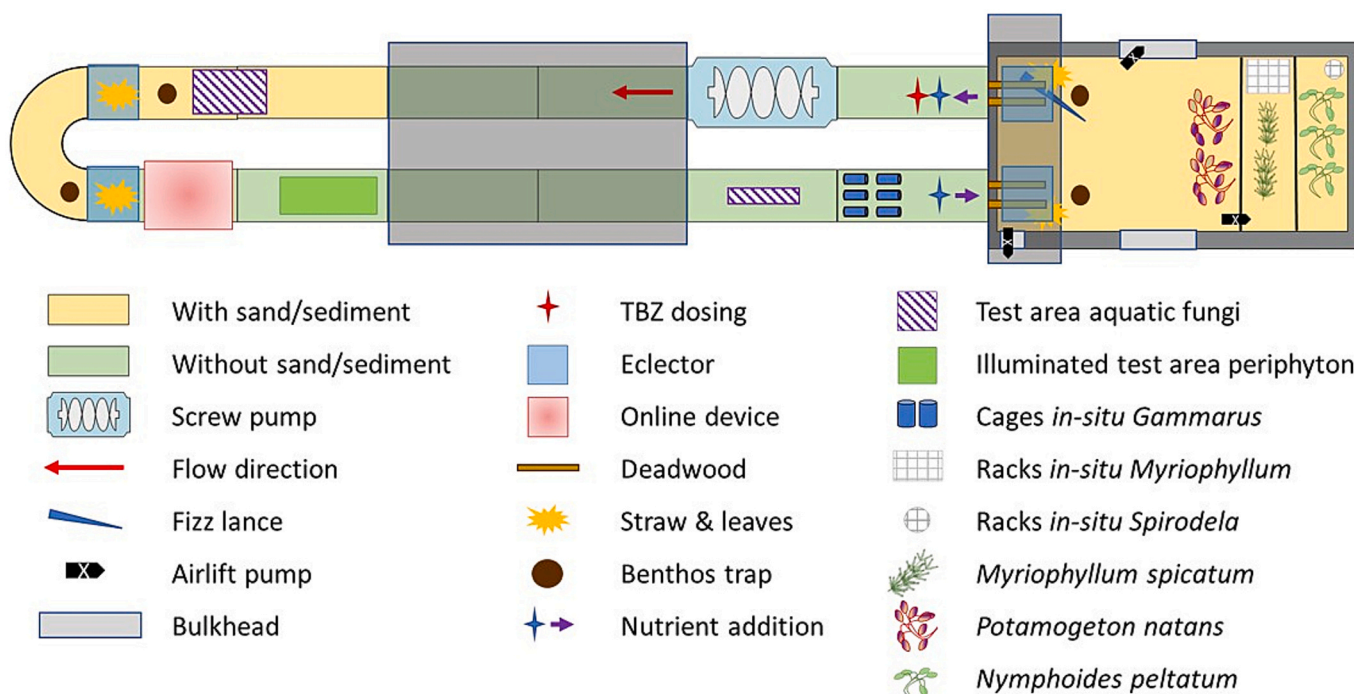


Fig. 1. Setup of stream-pond mesocosms stocked with sediment, macrophytes, macro-invertebrates, periphyton, plankton and microbes, three months prior to TBZ application. Special sampling racks and traps were used to assess the effects of the fungicide on biota in different habitats.

traps were installed (see Fig. 1 for positions). For further details on the biological set-up of the systems, see also Mohr et al. (2025).

2.1.2. Application procedure

The volumes and concentrations of the tebuconazole stock solutions, based on a tebuconazole content of 250 g/L in Folicur® (Bayer product data), were diluted with ultra-pure water (~4 µS; Merk Millipore Advantage A10, Germany) and prepared as follows: 10 L with 15.4 mg/L tebuconazole for the 5000 µg/L treatment, 10 and 5 L with 3.08 mg/L tebuconazole for the 1000 and 500 µg/L treatment, respectively, 10 and 5 L with 308 mg/L tebuconazole for the 100 and 50 µg/L treatment, respectively, and 5 L with 30.8 mg/L tebuconazole for the 5 µg/L treatment. Dosing of each stream-pond system was carried out using valve-less rotary piston pumps (Ismatec, Switzerland). The solutions were dispensed via 2 stainless steel/Teflon pipes (diameter 4 mm, spaced 30 cm apart) in the flow direction at the inlet of the channel area and at a water depth of 20 cm (see Fig. 1). The delivery rate was adapted to the dosing volume, and corresponded to two total water volume turnovers (110 and 220 mL/min for 5 and 10 L dosing solutions, respectively). The control systems were dosed with ultra-pure water only. Subsequently, all systems were aerated for 1 h with bubbling lances to promote homogeneous distribution of the dosing solution in each system and to counteract possible immediate oxygen-consuming processes as a result of the substance application.

2.2. Sampling and analysis of tebuconazole in water and sediment

2.2.1. Sampling procedures

During the 1-year mesocosm study, physico-chemical parameters, i. e. oxygen content, conductivity, pH and temperature were recorded continuously (hourly measurements) via online measurement technology and verified weekly using portable measuring probes. To check for homogeneous distribution and whether the desired test concentration was reached, tebuconazole concentrations were analyzed in water samples taken from the channel bend and from the pond area of each system, 1 and 4 h post application. The tebuconazole concentration in water of all systems was then determined after 1, 3 and 7 days, followed by weekly analysis until week six and then monthly determination until the end of the experiment. Water samples were taken from the channel bend as an integral sample at 20 cm water depth using a 1 L brown glass bottle (Duran®) and dividing the volume into three sub-samples that were frozen until further analysis. For selected stream-pond mesocosms (50, 100 and 500 µg/L), the tebuconazole sediment concentration was determined 1- and 3-days post application, as well as after 1, 2, 4, 6, 14, 22, 30, 42 and 52 weeks. For this purpose, three sediment cores per mesocosm and sampling date were taken from the main pond section with a polymethylmethacrylate sampling corer ($d = 6.7$ cm) at identical positions in each pond, but different positions at each sampling date. The top 7.5 cm of the three cores were cut into three layers (0–2.5, 2.5–5, and 5–7.5 cm depth), and the 3 samples per layer and pond were pooled and stored frozen until further processing. To rule out contamination of the systems with tebuconazole prior to the start of the experiment, water and sediment samples were analyzed for tebuconazole residues seven and five days respectively, prior to dosing.

2.2.2. Analysis of TBZ in water and sediment samples

Water samples were enriched and purified using SPE (Strata XL, 200 mg, 6 mL, Phenomenex, Aschaffenburg, Germany) on a 24-port vacuum manifold (Chromabond®, Macherey-Nagel, Düren, Germany), including the following steps: equilibration (2×2 mL methanol and 2×2 mL ultrapure water), sample loading (5 mL), interference elution (2×1 mL methanol/ultrapure water, 20/80% v/v), drying (1 h, 2 bar, N₂) and tebuconazole elution (2×2 mL ethyl acetate), adjusted to 5 mL in graduated centrifuge tubes. For water samples with nominal concentrations ≤ 5 µg/L, the ethyl acetate extract was evaporated to dryness (40 °C, N₂) and reconstituted in 60 µL ethyl acetate prior to GC–MS/MS

analysis. For the analysis of tebuconazole in sediment, the extraction followed the citrate-buffered version of QuEChERS as introduced by Anastassiades et al., 2003 (for a detailed description see the SI Part 2). All aliquots of the water and sediment extracts were spiked with internal standard tebuconazole-D6 and an analyte protectant mix, and analyzed for tebuconazole using a 7890B GC system coupled to a 7010B triple-quadrupole mass spectrometer with high efficiency ion source (all from Agilent, Waldbronn, Germany). Electron impact ionization in MRM mode (multiple reaction monitoring) was used for detection and quantification (tebuconazole: m/z 250 $\rightarrow$ 125 and m/z 252 $\rightarrow$ 127; tebuconazole-D6: m/z 258 $\rightarrow$ 127). For detailed GC and MS, settings see the SI Part 2. Both methods were validated in accordance with the residue analytical method guideline SANTE/2020/12830, Rev. 1 at concentrations of 0.5 and 500 µg/L in water, and 5 µg/kg and 500 µg/kg in sediment (for details see the SI Part 2). Limits of detection (LOD) and limits of quantification (LOQ) for tebuconazole in water were 0.15 µg/L and 0.5 µg/L, and in sediment 1.5 µg/kg and 5 µg/kg, respectively.

2.2.3. Analysis of transformation products

The water samples from the 50, 100 and 500 µg/L tebuconazole treatment were also analyzed for transformation products (TPs), after spiking with an internal standard mix solution containing 1,2,4-triazole-¹³C₂ and tebuconazole d₉, and filtering through syringe filters (Rotilabo PP41 RC 0.20 µm, Carl Roth, Karlsruhe, Germany). Tebuconazole, 1,2,4-triazole, tebuconazole-1-hydroxy, tebuconazole-o-hydroxy, tebuconazole-m-hydroxy, tebuconazole-lactone and tebuconazole-pentanoic acid were determined by LC/MS-MS using a LC20AD HPLC system (Shimadzu, Duisburg, Germany) coupled to a 4000 QTRAP triple-quadrupole mass spectrometer (AB Sciex, Darmstadt, Germany). Electrospray ionization in positive mode was used for detection and quantification of the compounds. Compound-specific MS settings and general interface parameters can be found in the SI Part 2. The method was validated in accordance with the Residue Analytical Method Guideline SANTE/2020/12830, Rev. 2 at 0.5 µg/L and 200 µg/L, resulting in LOD and LOQ for all specified compounds in water of 0.15 µg/L and 0.5 µg/L, respectively. In the case of 1,2,4-triazole, the second mass transition used for confirmation was a specific pseudo-transition (m/z 70 $\rightarrow$ 70), verified using matrix blanks and spiked samples, as permitted by the guideline.

2.3. Luminescent bacteria test

In a side experiment, a luminescent bacteria test using *Photobacterium leiognathi* was carried out across a concentration range of 0.24–117 mg/L tebuconazole (applied as Folicur®). The test solutions were prepared via an eight-step serial dilution from three stock solutions (3.13, 5.14 and 11.7 g/L). The test was conducted at room temperature in a Fluostar Omega plate reader (BMG LabTech) in 96-well plates (CELLSTAR® TC) for 30 min with cryopreserved bacteria (LGC, strain accession ATCC-33469) in 150 µL/well autoclaved test medium (100 mM KH₂PO₄, 450 mM NaCl, 50 mM MgSO₄, 10 mM KCl) at pH 4.0. As reference inhibitor for quality control of the test procedure 3,5-dichlorophenol was used. Dose-response curves, EC_x values and 95%-confidence limits (calculated according to Fieller's theorem) for the three pooled series were calculated using ToxRat Professional (Version 3.3 ToxRat Solutions GmbH).

2.4. DOC in water/TOC in sediment

Dissolved organic carbon (DOC) in water samples was determined in duplicate, according to DIN EN 1484 as Non-Purgeable Organic Carbon (NPOC) using a Shimadzu TOC-L analyzer with autosampler ASI-L. As a deviation from DIN EN1484, filtration was carried out using 0.2 µm membrane filters.

The freeze-dried and ground sediment samples from the 500 µg/L pond were also tested for total organic carbon (TOC). All 3 layers (0–7.5

cm) were pooled and TOC measured with a VarioTOC (Elementar) according to DIN EN 15936 by dry combustion, using 10% hydrochloric acid. Each sample was measured in duplicate, using 100 mg per measurement.

2.5. Calculation of dissipation time

The time series of tebuconazole residue concentrations were normalized to 12 and 20 °C using time-step normalization (Eq. (1)) $f_{temp} = Q_{10}^{\frac{T_{act}-T_{ref}}{10}}$ with f_{temp} as correction factor for temperature, T_{act} as the measured and T_{ref} as the respective reference temperature and a Q_{10} of 2.58 (Focus, 2014) to allow for comparison of dissipation time in water (DT_{50} water) between persistence guidelines used for plant protection products (20 °C) and biocidal products (12 °C) (ECHA, 2014, 2016; Focus, 2014). Data analysis was conducted using the R package *mkim* (Version 1.2.9) in R (Version 4.4.1), applying the five most common degradation kinetic models; single first-order (SFO), double first-order in parallel (DFOP), first order multi-compartment (FOMC), hockey stick (HS) and single first-order reversible binding (SFORB) with different error models (two component (*tc*), constant variance (*cv*) and variance by variable (*obs*)) to 'tebuconazole in water' for all systems (Ranke and Meinecke, 2019).

As a significant advancement in degradation kinetic modeling, a newly developed non-linear hierarchical model (NLHM) was applied to our data sets (degradation kinetic as mentioned above, error models excluding 'obs' due to limitation of the *saemix* package used for the NLHM calculation) with additional consideration of the covariate 'concentration' for best fit (SI Part 1 Snippet 1, Ranke et al., 2021, Ranke, 2023).

Exemplarily, tebuconazole sediment concentrations (treatments with 50, 100 and 500 µg/L tebuconazole starting concentration) were considered for DT_{50} system and DT_{50} water-sediment modeling (SI Part 1 Snippet 2). For the calculation of the system half-life (DT_{50} sys), the same kinetics and error models as outlined above were applied to the sum of tebuconazole remaining in water and sediment. In the water-sediment half-life modeling, the two compartments were evaluated separately, either with or without formation fraction and either with or

without sediment sink. The tebuconazole content in water and sediment is expressed as a percentage of the total amount of tebuconazole initially added to each system.

3. Results

3.1. Tebuconazole dissipation

After one year, 11, 29, 30, 45, 48 and 77% tebuconazole, relative to the initial concentrations, were still present in the mesocosms treated with 5, 50, 100, 500, 1000 and 5000 µg/L, respectively (Fig. 2A). For the 50, 100 and 500 µg/L treatments, the content of tebuconazole in the upper (7.5 cm) layer of sediment after one year was approx. 3–4% of the initial concentration in water (Fig. 2B, SI Part 1 Fig. 1).

Since SFORB kinetic with *cv* as error model showed the most reliable results for the initial data set based on AIC and χ^2 error levels, it was applied to the individual data sets and the temperature normalized data. Two DT_{50} values can be derived from the biphasic kinetic SFORB: one from phase 1 (TBZ_parent_b1, DT_{50} (fast)), with faster disappearance of the substance, as a value for short-term release or displacement considerations, and one from phase 2 (TBZ_parent_b2, DT_{50} (slow)), which reflects the slower, dominant dissipation over a longer period of time. In the following, the DT_{50} value of the second phase is considered, as it is used as a conservative reference value for long-term effects in regulatory contexts (Focus, 2014, SI Part 1 Table 1). The calculated DT_{50} (slow) values derived from TBZ_parent_b2 for non-temperature normalized data (mean 14.2 °C, min/max = 7.2/22.6 °C) ranged from 176 days in the 5 µg/L tebuconazole treatment to 1079 days in the 5000 µg/L treatment (Table 1). The DT_{50} (slow) calculation after time-step normalization for lower (12 °C) and higher (20 °C) standard temperatures resulted in longer or shorter degradation times, respectively (Table 1). When the entire data set was considered with the substance concentration as a co-variate, a DT_{50} (slow) value of 409 days (as the fiftieth percentile from the NLHM) was obtained for our specific test conditions, 470 days at 12 °C and 216 days at 20 °C. When the NLHM was applied to the concentrations ranging from 5 to 500 µg/L tebuconazole, this resulted in a DT_{50} (slow) of 322 days (Table 1).

Sorption to the sediment was evaluated in the mesocosms treated

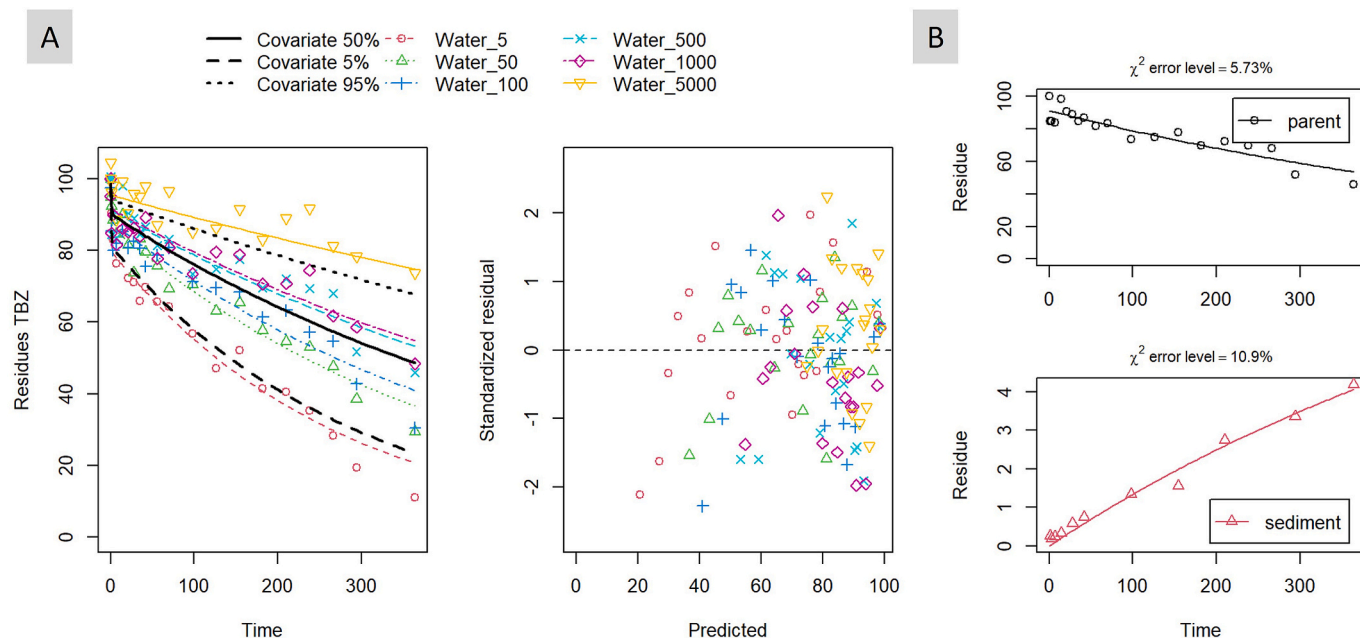


Fig. 2. (A) TBZ residues in water for each mesocosm, SFORB kinetic (best fit) with 'TBZ concentration' as co-variate (graphical output from NLHM) and (B) exemplarily for 500 µg/L TBZ Water-Sediment-Model providing DT_{50} values for 'parent in water' and for 'parent in sediment' (best fit SFO-SFO without formation fraction).

Table 1

Dissipation time (DT₅₀) and associated Chi² error for tebuconazole 'water_parent_b2' (slow phase), derived from the kinetic model with best fit (SFORB), and the error model 'constant variance' (cv) for each concentration and based on non-linear hierarchical modeling (in bold), considering the co-variate 'concentration' including all data sets or excluding the two higher concentrations (DT₅₀ in brackets) at our specific test conditions (real temp), and normalized to 12 and 20 °C, respectively. The DT₅₀ tebuconazole system and DT₅₀ tebuconazole water/sediment calculation includes the tebuconazole 'sediment_parent' fraction, with SFO and SFO-SFO (both with sink) without formation fraction, respectively, being the kinetic models with best fit. n.p. = not predictable.

TBZ [μg/L]	DT ₅₀ water_parent_b2 (SFORB cv) & Chi ² error			DT ₅₀ system (SFO*) & Chi ² error		DT ₅₀ water/sediment (SFO-SFO without formation fraction) & Chi ² error (all data/water/sediment)	
	12 °C	real temp	20 °C	real temp		real temp	
5	189	4.88	176	5.70	89	4.88	
50	304	3.33	284	4.28	142	3.33	282
100	394	4.18	356	4.96	185	4.18	368
500	542	4.78	487	5.27	254	4.78	497
1000	629	3.73	568	4.38	295	3.73	
5000	1342	3.74	1079	4.27	629	3.74	
5–5000 (5–500)	470	409 (322)	216				

* DT₅₀ identical results for SFO and SFORB, except for 5 μg/L tebuconazole with a DT₅₀ of 242 days and Chi² error 4.95 if modeled with SFORB.

with 50, 100 and 500 μg/L tebuconazole. The results showed a low and steady displacement of tebuconazole into the sediment (<5% of the initial concentration after 364 days, SI Part 1 Fig. 1). The DT₅₀ for the whole system was 50 to 100 days longer than for water only (Table 1). Moreover, in the 100 and 500 μg/L tebuconazole treatments, the DT₅₀ sediment values were much higher or even unpredictable, compared to water only.

3.2. Formation of tebuconazole transformation products

In the present study, nine TPs (SI Part 1 Table 2, TP 1–9) were found in water samples of the 50, 100 and 500 μg/L tebuconazole treatments, with a proportion of less than 5% during the entire test period. Of the six TPs for which analytical standards were available (Fig. 3A, TP 1–6), 1,2,4-triazole (TP 1), tebuconazole-lactone (TP 2) and tebuconazole-1-hydroxy (TP 5) could be quantified in the water samples (Fig. 3B, Fig. 4). The metabolite tebuconazole-1-hydroxy (TP 5) was first detected

after one week and remained relatively constant thereafter (Fig. 4). After one month, the concentration of tebuconazole-lactone (TP 2) began to increase continuously, while the late-stage degradation product 1,2,4-triazole (TP 1) was first detected after five months. In the treatment with 500 μg/L tebuconazole, tebuconazole-pentanoic acid (TP 3) and tebuconazole-m-hydroxy (TP 4) were detected after nine and twelve months, respectively, but in minor quantities (2.7–3.5 and 1.5 nmol, data not shown). During chromatographic separation, three further TPs were found that did not match the analytical standards previously included into the method (Fig. 3B, TP 7–9). TP 8 was putatively identified as tebuconazole-ketone, while the structures of TP 7 and TP 9 remained unclear.

3.3. Testing for experimental artefacts - bacterial toxicity, dissolved and total organic carbon

Probit analysis of bacterial luminescence in *P. leiognathi* exposed to

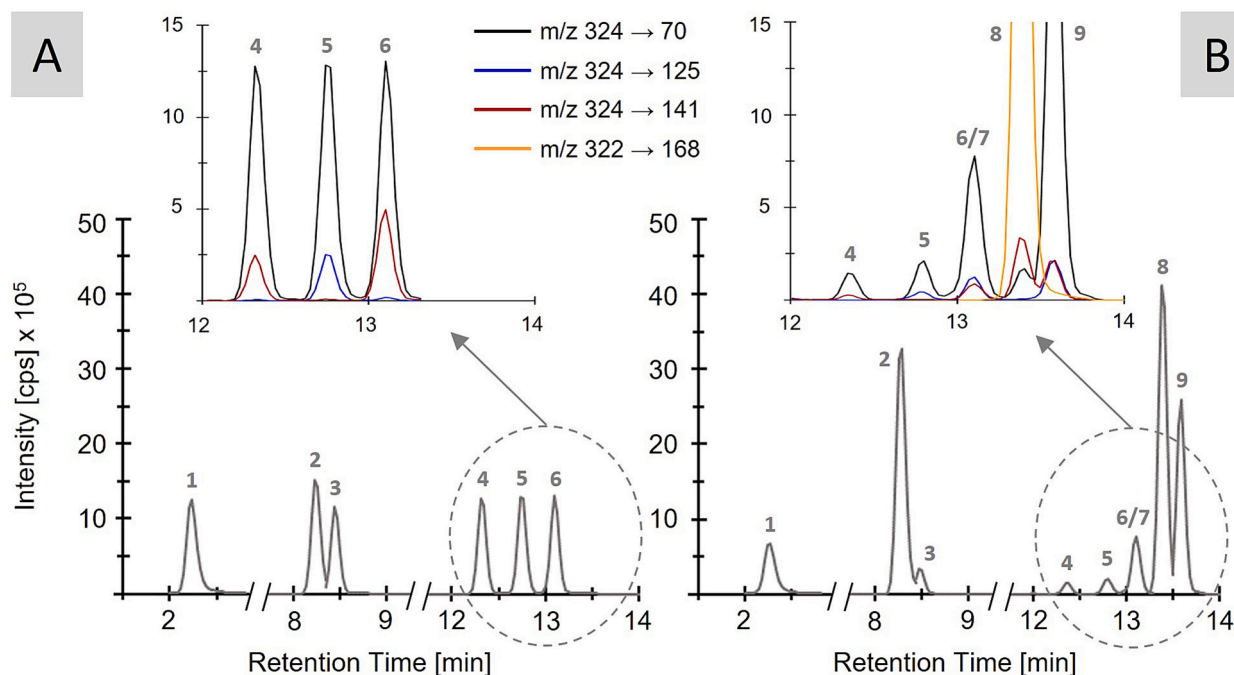


Fig. 3. Chromatographic separation of a calibration standard (A) and a water sample (B) from the 500 μg/L tebuconazole treatment after 364 days. Underlying MRM traces for identification of unknown transformation products, are highlighted. Peak numbers: 1) 1,2,4-triazole, 2) tebuconazole-lactone, 3) tebuconazole-pentanoic acid, 4) tebuconazole-m-hydroxy, 5) tebuconazole-1-hydroxy, 6) tebuconazole-o-hydroxy, 7) tebuconazole-X₁-hydroxy, 8) tebuconazole-5-keto, and 9) tebuconazole-X₂-hydroxy.

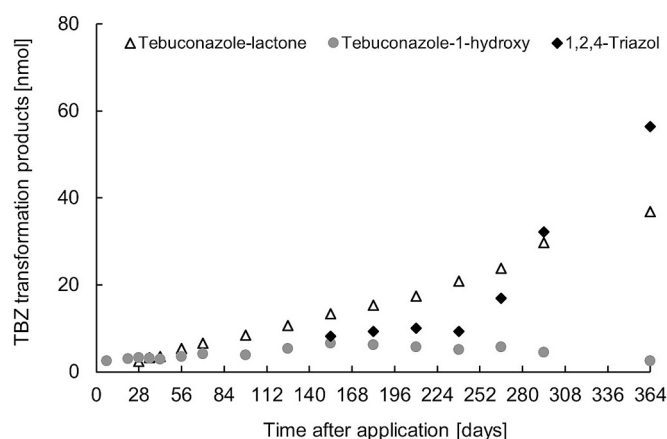


Fig. 4. Formation of main transformation products [nmol] tebuconazole-lactone, tebuconazole-1-hydroxy and 1,2,4-triazole over the experimental trial period [in days], shown exemplarily for the 500 $\mu\text{g/L}$ tebuconazole treatment.

tebuconazole concentrations ranging from 240 to 11,700 $\mu\text{g/L}$ revealed an EC_{10} value of 650 (540–760), an EC_{20} of 1180 (1030–1330) and an EC_{50} of 3720 (3440–4020) $\mu\text{g/L}$, demonstrating a clear concentration-dependent response (SI Part 1 Fig. 2).

The addition of tebuconazole and dimethyldecanamide provided a potential carbon source, contributing 6.7 mg/L and 1.4 mg/L dissolved organic carbon in water for the 5000 and 1000 $\mu\text{g/L}$ tebuconazole systems, respectively. In the treatments with the four lower tebuconazole concentrations, the addition of tebuconazole and dimethyldecanamide did not result in a significant increase in the carbon content compared to the natural carbon availability in the systems (SI Part 1 Fig. 3). The total organic carbon content in sediment was $0.29 \pm 0.04\%$ and displayed no significant change during the experiment (SI Part 1 Table 3), measured for the 500 $\mu\text{g/L}$ treatment. The resulting calculated log Koc for the 500 $\mu\text{g/L}$ treatment increased from 1.6 at the beginning to 2.7 at the end of the experiment (SI Part 1 Table 3).

4. Discussion

4.1. Tebuconazole dissipation

The design of the indoor mesocosm study aimed to mimic field conditions by ensuring that the two lowest test concentrations were environmentally relevant, applying a temperature regime that covered a one-year cycle at mid-latitudes, establishing near-natural water and sediment characteristics, and including a biological community representing nearly all trophic levels. The simulated conditions with regard to the residence time and complete circulation of the water body were comparable to Lake Type 11 'Polymictic Lowland Lake', considered to be unstratified, with a relatively large catchment area and a water residence time of >30 days (Riedmüller et al., 2022). For the present study, the recommendations for higher-tier studies in aquatic systems, such as sufficient sampling occasions, several test concentrations following a concentration response approach, a homogenous test concentration at the start of the experiment and the sampling of different compartments were met (Focus, 2014). Due to the hydrophobic properties of tebuconazole, its conventional application in the field is only possible in combination with a solubilizer; in the case of the tested formulation Folicur®, this solubilizer is dimethyldecanamide.

The fungicide tebuconazole is particularly interesting with regard to the concentration dependence of its dissipation time, as it is considered to be photolytically and hydrolytically stable (pH 5–9 at 25 °C) (EFSA, 2014). Since biological transformation is reported to be the main degradation pathway, the substance's toxicological inhibition of this

process at higher concentrations is expected to result in an extended half-life. In the authorization dossiers, whether as PPP or biocidal product, disparate information is given regarding the dissipation time of tebuconazole in soil, water and water-sediment systems, ranging from short-lived to highly persistent, depending on soil or sediment type and geographical location. For degradation in water-sediment systems in particular, DT_{50} values ranging from approximately 50 days (outdoor microcosm study) to over one year (laboratory water-sediment study) can be found (EFSA, 2014). One crucial problem is that the DT_{50} value is not a well-defined term. Different types of DT_{50} values (e.g. "total system", "water") are obtained in water-sediment studies. DT_{50} in water is based on dissipation when it is a water-sediment test. It can be the time at which residues first reach 50%, or it can be understood as a half-life intended to describe the entire course of the curve (only applicable for SFO), or it can be a half-life that describes only the slower degradation in the later part of the degradation curve. This raises the question of which values are truly reliable in terms of their transferability to the real environment and how many data sets should be considered in total.

Our study also revealed significant discrepancies in half-life, depending on the initial concentration applied (176 days at 5 $\mu\text{g/L}$, 1079 days at 5000 $\mu\text{g/L}$). This prompted us to evaluate our entire data set using the newly developed modeling tool by Ranke (2023). The NLHM fitting method calculates the half-life, using the 'stochastic approximation maximization algorithm' (SAEM, Kuhn and Lavielle, 2005) which incorporates all measurement data from the individual data sets, and includes a calculation of the influence of co-variables, in our case the substance concentration. By testing different co-variables (e.g. pH, temperature, etc.), it is possible to assess their impact on the kinetic parameters and indirectly, on the half-life. Applying the NLHM to all treatments of our data set including the co-variate 'concentration' the SFORB kinetic with error model cv was determined as most suitable, due to best AIC and Chi^2 error values. An average water DT_{50} (slow) value of 409 days was obtained from second phase of the biphasic kinetic SFORB model, reflecting the slower, dominant dissipation over time, for our specific test conditions (temperature profile of the year). The co-variate 'log (concentration)' displayed a clear influence on the parameter log (k_{TBZ}), which is the logarithm of the dissipation rate of 'tebuconazole free'.

At the two highest tested concentrations, bacterial toxicity was indicated by the bioluminescence test (see also section 3.3). However, bacterial growth might have been promoted, as the addition of tebuconazole and dimethyldecanamide provided a potential carbon source, with 6.7 mg/L and 1.4 mg/L DOC for the 5000 and 1000 $\mu\text{g/L}$ tebuconazole treatments, respectively (SI Fig. 3). Considering the absence of detectable bacterial toxicity at the treatments with the four lower concentrations, the NLHM was re-applied to the concentrations ranging from 5 to 500 $\mu\text{g/L}$ tebuconazole, which resulted in a water DT_{50} (slow) of 322 days.

Based on the published log K_{oc} (2.67–3.1) and log K_{ow} (3.7) values for tebuconazole, and in accordance with the Technical Guidance for Deriving Environmental Quality Standards of the European Commission (EC, 2018; Casado-Martínez et al., 2020), an additional effect assessment for sediment is required. Sorption to the sediment was evaluated in the mesocosms treated with 50, 100, and 500 $\mu\text{g/L}$ tebuconazole. The results showed a low and steady displacement of tebuconazole into the sediment (<5% of the initial amount after 364 days), with log K_{oc} values starting at 1.6 during the first weeks after application and increasing to 2.7 after one year in the 500 $\mu\text{g/L}$ treatment (SI Part 1 Table 3). The slow displacement into the sediment can be attributed to the relatively low total organic matter content of the sandy sediment used in the mesocosms (TOC 0.2–0.3%, see SI Part 1 Table 3). In terms of transferability to natural waters with low TOC content in the sediment, it can be assumed that reaching an equilibrium in the translocation processes may require a longer retention time of the compound in the system, potentially up to one year.

When comparing the DT_{50} values of tebuconazole with those of the

more than 20 azole fungicides currently on the market - which have been detected in surface waters worldwide at concentrations in the $\mu\text{g/L}$ range (Farah et al., 2024; Zubrod et al., 2019), it is striking that, tebuconazole exhibits the longest half-life, particularly in water. Along with epoxiconazole, paclobutrazol, triadimenol, and triticonazole, it is classified as very persistent (Creusot et al., 2020). In water-sediment systems, however, DT_{50} values for all azole fungicides are even higher, indicating that more than half of the active ingredients used must be classified as very persistent (Creusot et al., 2020). In-depth research into potential removal techniques - particularly for triazoles - in the context of wastewater treatment (Bian et al., 2025) is therefore commendable. The entry of azole fungicides via agriculture can primarily be counteracted by reducing their use, either through the application of active substances with greater efficacy at lower doses, or through the adoption of more environmentally friendly alternatives that ensure effective control of fungal diseases while meeting the relevant authorization requirements (Jørgensen and Heick, 2021).

4.2. Formation of tebuconazole transformation products (TPs)

In the context of the European risk assessment process, TPs have to be taken into account when modeling degradation kinetics, once their proportion exceeds 5% of the parent compound in the target compartment (on a molar basis relative to the parent compound at the start of the study) (Focus, 2014). Since the formation of quantifiable TPs in our study consistently remained below this 5% threshold, no further modeling of TP degradation was carried out. However, the detection of overall nine TPs provided valuable insights into the possible transformation steps of tebuconazole over time.

For the TPs for which no analytical standards were available (Fig. 3, 7–9), further attempts were made to assign potential structures using a water sample treated with 500 $\mu\text{g/L}$ tebuconazole (sampling date: 364 days). For this, a precursor ion scan from m/z 320–340 with m/z 70 as the product ion was used. As a result, the $^{35}\text{Cl}/^{37}\text{Cl}$ isotope ratio of TP 9 was highly consistent with that of tebuconazole-1-hydroxy (TP5), tebuconazole-m-hydroxy (TP4) and tebuconazole-o-hydroxy (TP 6) reference standards indicating one chlorine atom in unknown TP 9. In contrast, the conformance was notably poorer for TP 7, suggesting a possible coelution of at least two substances (see SI Part 1 Figs. 4 and 5). Molecular ions of m/z 324 $[\text{M} + \text{H}]^+$ and m/z 326 $[\text{M} + 2 + \text{H}]^+$ for the unknown TPs indicate the presence of a hydroxyl group when compared to tebuconazole (m/z 306 $[\text{M} + \text{H}]^+$ and m/z 308 $[\text{M} + 2 + \text{H}]^+$ Δ 18 amu). Evidence for a further TP, tebuconazole-ketone (TP 8), was also obtained from the precursor ion scan. Based on molecular ion peaks at m/z 322 $[\text{M} + \text{H}]^+$ and m/z 324 $[\text{M} + 2 + \text{H}]^+$ a keto group is proposed due to a mass difference of 2 amu when compared to TPs 5, 7 and 9. In addition, the $^{35}\text{Cl}/^{37}\text{Cl}$ isotope ratio of TP 8 matched the $^{35}\text{Cl}/^{37}\text{Cl}$ isotope ratio of tebuconazole-1-hydroxy (TP 5) in the same water sample (see the SI Part 1 Fig. 5, A and C), demonstrating the presence of one chlorine atom in the molecule. The identification of tebuconazole-ketone based on MRM and EPI (enhanced product ion scan) experiments was further supported by the specific mass fragments and transitions reported by Fu et al., 2025, yielding comparable EPI spectra, with m/z 168 being the most intense peak and m/z 322 $\rightarrow$ 168 the most sensitive mass transition (see Fig. 3B).

Furthermore, by testing an orthogonal stationary phase (Kinetex® Biphenyl, 150 $\times$ 3 mm, 2.6 μm , Phenomenex, Aschaffenburg, Germany) under the same gradient conditions in MRM mode as described in the SI Part 2 (analytical method details), the separation of TP 6 and TP 7 was achieved. TP 8 eluted after tebuconazole rather than before (see SI Part 1 Fig. 6). TP 6 was identified as tebuconazole-o-hydroxy matching the characteristic mass transitions of m/z 324 $\rightarrow$ 70 and m/z 324 $\rightarrow$ 141 (with absence of m/z 324 $\rightarrow$ 125), as well as the retention time and ion ratios compared to the reference standard (see SI Part 1 Table 2). Fragments of m/z 70, 125, and 141 from precursor 324 were still observed in TP 7, which may indicate the presence of tebuconazole-5-

hydroxy (Fu et al., 2025).

The occurrence of 1,2,4-triazole, tebuconazole-lactone, and tebuconazole-pentanoic acid is in line with their presence as main TPs in soil and surface water (Azhari et al., 2018; EFSA, 2014). Furthermore, a large number of hydroxylated TPs were found in distinct environmental matrices and crops by Dong (2024), consistent with our observations and supporting the involvement of biological transformation processes. For some of these TPs, Ecological Structure-Activity Relationship (ECOSAR) predictions by de Oliveira and Sant'Ana (2023) suggest an equal or slightly lower toxicity compared to tebuconazole (see also Dong, 2024).

4.3. Bacterial toxicity

In the present study, bacterial luminescence exhibited a clear concentration-dependent response. The marine, luminescent bacterium *P. leiognathid* is only partially representative of the microbial communities involved in freshwater degradation processes, but due to the high sensitivity of the test, it is considered a useful indicator of incipient toxicity. Even at concentrations not leading to direct bacterial or microbial toxicity, shifts in the composition of the entire saprobic community can still occur (Baćmaga et al., 2021; Han et al., 2021; Staley et al., 2015). Tebuconazole is marketed as specifically targeting fungi by inhibiting the cytochrome P450 enzyme 14 α -demethylase, which disrupts ergosterol synthesis, an essential component of fungal cell membranes. However, several studies have shown that it also interferes with the biosynthesis of sterols in animals and plants (Dong, 2024), as these metabolic pathways are (among others) also regulated by 14 α -demethylases. For bacterial communities in soil, it has been shown that tebuconazole exposure alters species composition, biochemical activity, and functional profiles, due to a decline of e.g. actinobacteria or an increased abundance of e.g. potentially tebuconazole-degrading bacteria (Baćmaga et al., 2021; Han et al., 2021). Further studies, such as that by Artigas et al. (2014), suggest that microbial freshwater communities may be affected by tebuconazole concentrations as low as 20 $\mu\text{g/L}$, including potential growth-stimulating effects.

In general, the biodegradation of fungicides by microbes is an important sink for these compounds, which relies not only on bacterial processes, but also on the broad enzymatic capabilities of fungi (Obanda and Shupe, 2009; Sehnem et al., 2010; Meng et al., 2023). Especially for freshwater fungi, such metabolic pathways are discussed as detoxification mechanisms (Artigas et al., 2017), contributing to the self-cleaning of water bodies (Krauss et al., 2011; Seena et al., 2023). Conversely, exposure of non-target organisms can lead to the development of e.g. drug resistances (Rhodes et al., 2022; Akosah et al., 2023; Gao et al., 2024), leading to unintended side effects in other sectors. Therefore, the need for further investigation into microbial pesticide degradation and possible consequences of pesticide exposure, including the protection of the affected communities, cannot be emphasized enough.

5. Conclusion

The use of the NLHM tool proved valuable for this study, as it demonstrated a clear concentration dependency in the dissipation time for the tested tebuconazole. The tool has the advantage of identifying influencing co-variables and integrating them when deriving kinetic parameters from diverse data sets. It is particularly useful when considering multiple co-variables as possible influencing factors, as well as in calculating their relative strength or influence. In the case of substances that are primarily degraded microbially but also have a toxic effect on the microbiome, a concentration-dependent dissipation rate, as observed in our study, can be expected and can be accounted for using the new non-linear hierarchical model.

The possibility of near-natural simulation in mesocosms to study the dissipation behavior of substances potentially toxic to aquatic ecosystems offers clear advantages and serves as a valuable supplement to

standard laboratory tests for substance evaluation and approval. Mesocosm studies also provide important insights into transformation processes and metabolite stability. Therefore, we propose expanding the research on this topic with similar studies, allowing for continuous evaluation and improvement of risk assessment.

CRediT authorship contribution statement

Valeska Contardo-Jara: Writing – original draft, Visualization, Supervision, Project administration, Methodology, Investigation, Conceptualization. **Stefan Bader:** Writing – original draft, Visualization, Validation, Methodology, Formal analysis, Data curation. **Björn Kusebauch:** Writing – review & editing, Validation, Methodology, Investigation, Conceptualization. **Johannes Ranke:** Writing – review & editing, Validation, Software. **Silvia Mohr:** Writing – review & editing, Project administration, Conceptualization. **Ulrike Scholz:** Writing – original draft, Investigation. **Steffen Carl:** Writing – review & editing, Project administration, Conceptualization. **Stefan Meinecke:** Writing – review & editing, Validation, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2026.182286>.

Data availability

Data will be made available on request.

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