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Ruth Müller [a]; Rüdiger Berghahn [b]; Sabine Hilt [c]
[a] Freie Universität Berlin, Berlin, Germany [b] Federal Environment Agency, Berlin, Germany [c] Leibniz-Institute for Freshwater Ecology and Inland Fisheries, Berlin, Germany

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Herbicide effects of metazachlor on duckweed (*Lemna minor* and *Spirodela polyrhiza*) in test systems with different trophic status and complexity

RUTH MÜLLER[1], RÜDIGER BERGHAHN[2] and SABINE HILT[3]

[1]*Freie Universität Berlin, Berlin, Germany*
[2]*Federal Environment Agency, Field Station Marienfelde, Berlin, Germany*
[3]*Leibniz-Institute for Freshwater Ecology and Inland Fisheries, Berlin, Germany*

Growth of common duckweed *Lemna minor* under optimal standard test conditions was compared to growth of *L. minor* exposed to nutrient-poor water in both a modified standardised test and in oligo- to mesotrophic indoor pond mesocosms in order to test the impact of trophic conditions and test system complexity on the effect of the herbicide metazachlor (2-chloro-*N*-(pyrazol-1-ylmethyl)acet-2′,6′-xylidide) on aquatic macrophytes. In the mesocosms *L. minor* was replaced by greater duckweed *Spirodela polyrhiza* after 3 weeks due to high mortality even in the controls. The pond systems contained other macrophytes and biota as well as sediment and were thus more complex than standard test systems. For *L. minor* front area, the ErC_{50} (50% effective concentration related to growth rate) was 2.8 $\mu g\ L^{-1}$ metazachlor in the standardised and 4.7 $\mu g\ L^{-1}$ in the modified laboratory test after 7 days (4.9 $\mu g\ L^{-1}$ and 52.9 $\mu g\ L^{-1}$ metazachlor when using front number). In the oligo- to mesotrophic pond mesocosms, similar sensitivities to metazachlor (ErC_{50} 4.5–6.4 $\mu g\ L^{-1}$) were noted for *S. polyrhiza* after 21 and 28 days of exposure. In comparison with dicotyledonous macrophytes, duckweed species are more sensitive for irreversible enzyme inhibitors of growth such as metazachlor independent of trophic status and complexity of the test system.

Keywords: Growth rate; herbicide; nutrients; macrophytes; mesocosm; ponds.

Introduction

The importance of macrophytes as functional and structural elements of aquatic communities, as a food source, habitat and refuge for aquatic organisms and as a major force in chemical cycles has been recognized for a long time.[1,2] At the lower tier of aquatic toxicity testing in the EU risk assessment, the only—but well—established standardised laboratory macrophyte test available uses the free-floating, rooted, monocotyledonous genus *Lemna*.[3] This single-species test provides optimal growth conditions (optimal nutrient, radiation, and temperature conditions), since the growth inhibition test is based on maximum growth in the controls.

However, toxic effects may be even higher under oligo- to mesotrophic conditions and should be considered in risk assessment to properly cover the full range of macrophyte biocoenoses. Furthermore, the system complexity may influence herbicide effects. Mesocosms studies covering manifold abiotic and biotic interactions and thereby simulating more realistic field conditions are thus needed for comparison with laboratory tests.[4] In this study, the effect of herbicide metazachlor on the growth of common duckweed *L. minor* in standardised laboratory test[3] was compared to (i.) a modified standardised test with mesotrophic conditions and (ii.) to a complex mesotrophic pond mesocosm experiment. (iii.) Additionally, growth of greater duckweed *Spirodela polyrhiza* (L.) Schleid. was examined in pond mesocosms.

The model substance metazachlor (2-chloro-*N*-(pyrazol-1-ylmethyl)acet-2′,6′-xylidide, BASF, Germany) is an α-chloroacetamide derivate and commonly used in Europe[5] to protect rape and other species of the Brassicaceae family.[6] It is applied as pre-emergence herbicide in autumn as well as in spring and early summer. During these periods, it may enter the aquatic environment in relevant amounts by run-off or spray-drift. Metazachlor has been detected in natural waters in concentrations up to 100 $\mu g\ L^{-1}$[7] and may therefore potentially harm non-target macrophytes, in

Address correspondence to Ruth Müller, LOEWE Biodiversity and Climate Research Centre, Siesmayerstrasse 70, Frankfurt/Main, 60054; E-mail: ruthmueller@bio.uni-frankfurt.de
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particular in stagnant surface waters adjacent to the area of application.

Materials and methods

Laboratory experiments

Sterile culture of *L. minor* was purchased from Ökotox (Stuttgart, Germany) clone Stuttgart (ST). Prior to the metazachlor experiments, the appropriateness of *L. minor* culture was affirmed with a 3.5-dichlorphenol (DCF) reference test after Organisation for Economic Co-operation and Development (OECD) guideline 221[3] at a range of 1.2–2.4 mg L^{-1} DCF in water without solubiliser by stirring for 24 h. The reference test was valid, since more than 96 fronds occurred in the controls on day 7, the growth rates of controls were 0.31 d^{-1} (frond number) and 0.32 d^{-1} (frond area), and the inhibition of growth rate amounted to 3.8–62% (frond number) and 2.4 to 60% (frond area) at a range of 1.2–2.4 mg L^{-1} DCF. Afterwards, the growth of *L. minor* (initially 4 × four days old, three-frond colonies) was determined in a standardised static laboratory test according to the OECD guideline 221[3] at metazachlor concentrations of 0, 0.6, 1.8, 5.4, 16.2, 48.6 and 150 μg L^{-1} modified Steinberg medium[8] (n = 3, see Table 1).

In addition, *L. minor* was exposed to 0, 4, 8, 16, 32, and 64 μg L^{-1} metazachlor in a modified laboratory test (n = 3, see Table 1). The main differences to the OECD test[3] was the use of 0.45 μm filtered, nutrient-poor mesocosm water as medium instead of nutrient-enriched water (Steinberg medium, modified after[8]) and exposure to variable environmental conditions (temperature, light) in the mesocosm hall instead of environmentally controlled exposure in a test chamber. Similarly to the standard test, *L. minor* should exhibit exponential growth at the start of the experiment. Therefore, lab culture of *L. minor* was exposed to new environmental conditions in the mesocosm hall without pre-adaptation. For more details about the environmental conditions during laboratory experiments see Table 1.

Mesocosm experiment

Mesocosms

The mesocosm effect study was carried out in 8 indoor pond mesocosms ([9], www.umweltbundesamt.de/fsa). Each pond mesocosm (690 × 325 × 250 cm, length × width × height) had a littoral zone and contained 46 m^3 of sand with an upper layer of fine sediment as well as 14 m^3 of water taken from a well after reduction in Fe, Mn, and electrical conductivity to 450 μS cm^{-1} (see[9] for technical details). One year prior to the experiment, the ponds had been stocked with aquatic macrophytes (*Potamogeton natans* L., *Myriophyllum verticillatum* L., *Persicaria amphibia* (L.) Delarbre, *Chara vulgaris* (L.)), macroalgae (*Cladophora glomerata* (L.) Kütz.), plankton, periphyton, molluscs, and larvae of insects (Chironomidae and Chaoboridae).

Metazachlor application and water quality parameters

Metazachlor was added to five pond and stream mesocosms at nominal concentrations of 5, 20, 80, 200 and 500 μg L^{-1} on 2 June and homogenized by means of an electric outboard motor. Three ponds and streams served as controls. Routine sampling was carried out during the entire experiment on a fortnightly basis. Parameters measured included total organic carbon, dissolved organic carbon, total nitrogen, NH_4^+, NO_2^-, NO_3^-[10] total phosphorus, PO_4^{3-}[11], silicate,[12] macronutrients, heavy metals, metazachlor and its metabolites as well as temperature, pH, conductivity, oxygen, turbidity, photosynthetic active radiation (PAR, measured via LI-250 with cosine corrected underwater sensor, LI-COR Bioscience, Lincoln, USA). Mercury-vapour lamps provided 150–250 μmol m^{-2} s^{-1} PAR at the water surface. The dark/light regime was adjusted monthly to outdoor conditions during the mesocosm experiment. Details including metazachlor analysis and degradation in the mesocosms have been reported earlier.[13]

Exposure

One hundred fronds of *L. minor* from a sterile lab culture (Ökotox, Stuttgart, Germany) were inoculated in silicon tube rings (177 cm^2 enclosed area, n = 7) that were exposed in the mesocosms. They were replaced 3 weeks after metazachlor application of pond mesocosms by *S. polyrhiza* since *L. minor* displayed high mortality even in the control ponds (due to chlorosis). The substitute from an aquarium culture was proliferated at 30–65 μmol m^{-2} s^{-1} PAR in the mesocosm hall in a polyethylene box containing 9 L unfiltered water from nearby stream mesocosms (for water parameters see[13]) and 1 L Steinberg medium (modified after[8]). It was exposed at 150 to 250 μmol m^{-2} s^{-1} PAR for 28 days to metazachlor concentrations which had decreased in the ponds due to degradation to 4.1, 17.4, 70.9, 187.7 and 454 μg L^{-1} (Table 1).

Data analysis

The fronds of *L. minor* were equidistantly photographed (Camera Cosmic, Pentax, Hamburg, Germany) and the area and number of fronds analysed with the program Medealab (Erlangen, Germany). Frond growth of *L. minor* and *S. polyrhiza* exposed in the ponds was documented using digital top view photography equidistantly taken once a week (cp.[14,15]). Photos were processed with Adobe Photoshop® 3.0 (Adobe System Incorporated, San Jose, USA) to erase superfluous background information. The endpoint area of fronds was measured with analySIS® 3.1 (Soft Imaging System GmbH, Münster, Germany) as percent pixel of the entire image. The calculation of the parameter number of fronds was not useful in case of *in situ* exposed duckweeds owing to light reflections at the water

Table 1. Growth experiments with the duckweeds *Lemna minor* (*L. min.*) and *Spirodela polyrhiza* (*S. pol.*) under different environmental conditions and test systems are summarized. Growth rates and effective metazachlor concentrations are given for each experiment.

	L. min.[a] *(standard)*	*L. min.*[b] *(modified)*	*S. pol.*[c] *(pond)*	*S. pol.*[c] *(pond)*	*S. pol.*[c] *(pond)*	*S. pol.*[c] *(pond)*
Medium	modified Steinberg enriched water	mesocosm water (0.45 μm filtered)	mesocosm water	mesocosm water	mesocosm water	mesocosm water
Exposure time [d]	7	7	7	14	21	28
PAR [μmol m^{-2} s^{-1}]	85–125	30–65	150–250	150–250	150–250	150–250
Light : dark cycle [h d^{-1}]	24 : 0	13 : 11	16 : 8	16 : 8	16 : 8	16 : 8
Temperature [°C]	24 ± 2	16 ± 2	19	19	21	23
pH	5.5	8.2	7.9–10	7.9–10	7.9–10	7.9–10
PO_4-P [mg L^{-1}]	44.71	0.05	0.002–0.003		0.00–0.014	
(NO_3+NO_2)-N [mg L^{-1}]	83.49	0.0–0.55	0.471–0.882		0.003–0.322	
Range of tested concentrations [μg L^{-1} metazachlor]	0.6–150	4.0–64	4.1–454	4.1–454	4.1–454	4.1–454
Growth rate of controls [increase in frond area d^{-1}]	0.30 ± 0.00	0.08 ± 0.00	0.05 ± 0.02	0.03 ± 0.01	0.05 ± 0.01	0.03 ± 0.01
Growth rate of controls [increase in frond number d^{-1}]	0.31 ± 0.01	0.10 ± 0.01	—	—	—	—
ErC_{10} (frond area) [μg L^{-1} metazachlor] (C.I. 95%)	0.6 (0.47–0.79)*	1.1 (0.53–1.63)*	—	—	—	—
	0.8 (0.63–1.13)**		n.d.	n.d.	0.4 (0.01–18.49)**	1.9 (0.81–4.44)**
ErC_{10} (frond number) [μg L^{-1} metazachlor] (C.I. 95%)	0.9 (0.34–1.43)*	1.5 (n.d.)*	—	—	—	—
	0.6 (0.18–2.13)**	—	—	—	—	—
ErC_{50} (frond area) [μg L^{-1} metazachlor] (C.I. 95%)	2.8 (2.45–3.14)*	4.7 (3.74–5.51)*	—	—	—	—
	2.9 (2.58–3.36)**	—	n.d.	n.d.	6.4 (1.34–30.27)**	4.5 (3.48–5.88)**
ErC_{50} (frond number) [μg L^{-1} metazachlor] (C.I. 95%)	4.9 (3.49–6.78)*	52.9 (n.d.)*	—	—	—	—
	3.8 (2.13–6.63)**	—	—	—	—	—

[a]standardised *L. minor* test (after Organisation for Economic Co-operation and Development [OECD] 2002). [b]*L. minor* was tested in 120 mL glasses as in *a*, but under modified environmental conditions in the mesocosm hall. [c]*In situ* exposed *L. minor* in the pond mesocosms was threatened with extinction after the second day of exposure and therefore substituted by *S. polyrhiza*. n.d.= not detectable, C.I. = confidence intervals *probit analysis with ToxRat software, **log logistic analysis with Prism4 software.

surface. Relative growth rates per day (R) were calculated for each experiment employing the Equation 1.

$$R = (\ln x_{t2} - \ln x_{t1})/(t_2 - t_1) \quad (1)$$

In Equation 1, x_{t1} denotes the frond area on day t_1 and x_{t2} the corresponding parameter on day t_2. Mean ± standard deviation (SD) was used according to the standardised laboratory test with duckweeds.[16]

On the basis of nominal concentrations, ErC_{10} and ErC_{50}(10% and 50% effective concentration related to growth rate) for the endpoint area of fronds and number of fronds of *L. minor* were calculated with probit analysis employing the software ToxRat Pro XT 2.09 (ToxRat Solutions GmBH, Alsdorf, Germany). Due to the longer exposure of *S. polyrhiza* of 21 and 28 instead of 7 days, the software ToxRat Pro XT 2.09 was not applicable. ErC_{10} and ErC_{50} for *S. polyrhiza* were calculated employing the logistic concentration response model Equation 2.

$$Y = \text{Bottom} + \frac{(\text{Top} - \text{Bottom})}{1 + 10^{(\text{LogErC}_{10/50} - X)\text{Hillslope}}} \quad (2)$$

In Equation 2, Y denotes the response, Bottom denotes the lower plateau of effect (0%) and Top the upper plateau of effect (100%). ErC_{10} is the effect concentration of 10% inhibition of growth, ErC_{50} is the effect concentration of 50% inhibition of growth, X the logarithm of the calculated nominal concentration 21 days after metazachlor application (verified by measurements) and Hillslope the form factor of slope (GraphPad Prism version 4.00 for Windows, GraphPad Software, San Diego California USA) including an add-on module (ErC_{10}, ErC_{50}) designed by the working group of Prof. Oehlmann, University Frankfurt, Germany. Degradation of metazachlor, which followed 1st order kinetics,[13] had to be considered and consequently ErC_x for *S. polyrhiza* had to be based on time weighted average (TWA) of the actual start concentrations employing the Equation 3.

$$TWA = \frac{1}{t - t_0}\int_{to}^{t} C(t)dt = \frac{1}{t - t_0}\left[-\frac{C_0}{k}\,e^{-kt}\right]_{to}^{t} \quad (3)$$

In Equation 3, t is the actual time (d), $t_\circ$ the start time (start at application, d), k the rate constant (d^{-1}), and C_0 the theoretic start concentration ($\mu g\ L^{-1}$). For comparison between the test results, the same logistic concentration-response model was used for the standardised *Lemna* test.

Results

In overall comparison, the lowest ErC_{50} of metazachlor was calculated in the standardised *L. minor* test after 7 days for the endpoint area of fronds (2.8 $\mu g\ L^{-1}$ metazachlor, Table 1, Fig. 1). Relative growth rates in the controls of the standardised *L. minor* test fulfilled the test criteria (Table 1). Negative growth rates due to necrosis were observed at metazachlor concentrations > 48.6 $\mu g\ L^{-1}$ (Fig. 2). Frond

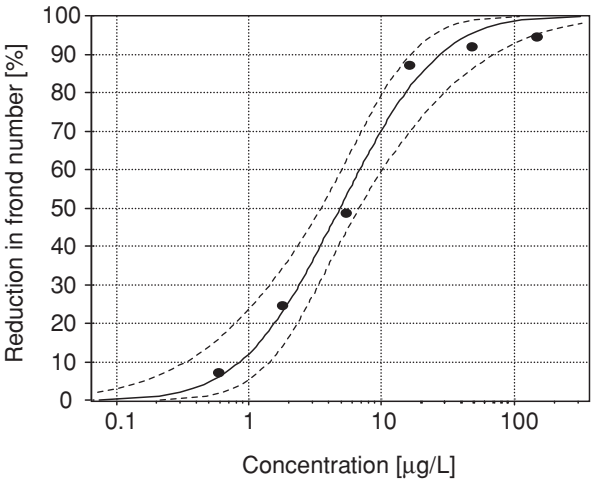

Fig. 1. Frond number of *L. minor* after 7 days exposure to different nominal start concentrations of metazachlor examined in the standardised test. Dotted lines indicate the 95% confidence interval.

area was the more sensitive endpoint (Table 1), while results with the two calculation models employed for the ErC in the standardised *Lemna* test were almost identical. The preferential biomass parameter number of fronds of the OECD *Lemna* test guideline[3] led to higher ErC_{50} in the *L. minor* standardised as well as in the modified laboratory test, which was conducted in the mesocosm hall (Table 1). The modified *Lemna* test was considered not valid by the software due to low growth rates (Table 1, Fig. 3). Nevertheless, the $ErC_{50}$and ErC_{10} were in the same order of magnitude in the 3 setups apart from the ErC_{50} for the number of fronds in the modified *Lemna* test (Table 1).

In contrast to the negative growth of *L. minor* in the control ponds, *in situ* exposed *S. polyrhiza* exhibited exponential growth in the controls ($y = 1.40e^{0.37x}$, $R^2 = 0.84$, Fig. 4). At low nutrient concentrations (Table 1) growth rates of controls were between 0.03 ± 0.01 and 0.05 ± 0.02 d^{-1} (frond area). After 21 days, exponential growth could not be observed in the controls (Fig. 4). Metazachlor effects

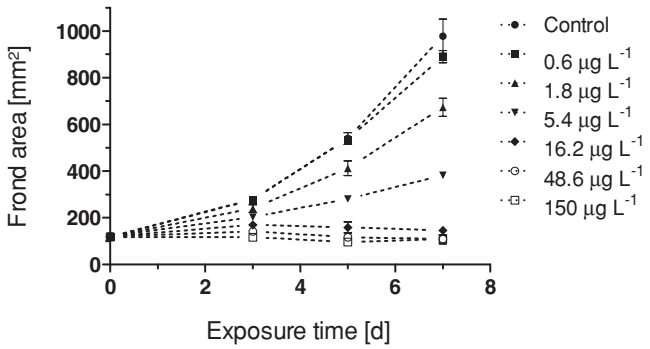

Fig. 2. Frond area of *L. minor* during the standardised test at different nominal start concentrations of metazachlor.

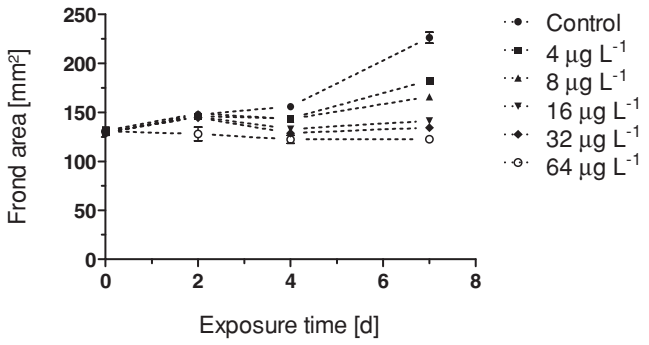

Fig. 3. Frond area of *L. minor* during the modified test in the mesocosm hall at different nominal start concentrations of metazachlor.

on *S. polyrhiza* became stronger with increasing exposure time and at higher growth rates (Table 1, Fig. 4). Growth was significantly inhibited in pond mesocosms treated with 4.1, 70.9 and 454 μg L^{-1} metazachlor after 7 days of exposure and in all treatments after 14 days of exposure (Fig. 4). However, the concentration-effect-relationship on day 7 and 14 was not monotonous and effect concentrations could not be determined. After 21 days exposure, the log logistic analysis of effective concentrations revealed a higher ErC_{50} based on TWA of 6.4 μg L^{-1} (95% C.I. 1.34–30.27 μg L^{-1}, $R^2 = 0.99$) than after 28 days. In contrast, a little higher ErC_{10} of 1.9 μg L^{-1} with a lower confidence interval (95% C.I. 0.81–4.44 μg L^{-1}) was found after 28 days exposure (cp. also Table 1).

Discussion

Metazachlor toxicity data for *L. minor* could only be generated in the standardised and the modified laboratory test. Growth of *L. minor* in the oligo- to mesotrophic mesocosms was negative even in the controls. The most critical factor for the survival of *L. minor* in mesocosms might have been the transfer from optimum, high-growth conditions to extremely poor growth conditions without adaptation time. This assumption is supported by the fact that *S. polyrhiza* which had been pre-exposed to lower nutrient conditions performed very well in the mesocosms (cp.[17]). On the other hand, Coors et al.[15] used both, high-nutrient and poor-nutrient adapted *L. minor* strains successfully in outdoor mesocosm experiments. The high-nutrient laboratory strain grew better, although it exhibited a different response pattern to the tested herbicide than the nutrient-poor adapted strain.

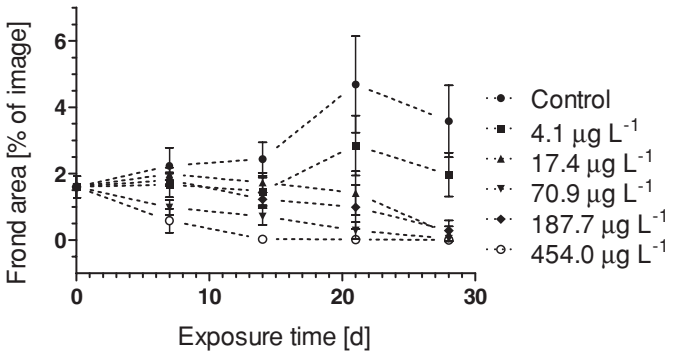

Fig. 4. Frond area of *S. polyrhiza* grown in pond mesocosms at different nominal start concentrations of metazachlor depending on exposure time.

Successful use of laboratory strains of *L. minor* in mesocosm studies might be strongly related to the phosphate level, which was significantly higher in the mesocosms of Coors et al.[15] than in the present study. Phosphate levels in the mesocosms (Table 1) were repeatedly below the minimum requirements of *L. minor* (minimal 0.0034 mg P L^{-1}).[18] This was in particular true for the control ponds and the ponds treated with low metazachlor concentrations since any nutrient input (18 June: NO_3–N: 1.0 mg L^{-1}, 1 July PO–P: 0.04 mg L^{-1}, and 11 July: PO_4–P: 0.04 mg L^{-1}) was taken up much faster by the less chemically impacted, greater standing stock of macrophytes. Nevertheless, mesocosms should be operated at mesotrophic to oligotrophic conditions for better experimental control.[13] In the course of long-term experiments, mesocosm investigators have to cope with the problem that the systems have to be fertilized and that each and every pulse of allochthonous nutrient inputs is immediately absorbed by the flora[13] as is the case under field conditions. This means that both median nutrient concentration and nutrient minima may control macrophyte growth.

Growth of the two test species *L. minor* and *S. polyrhiza* was strongly inhibited after single dosing of metazachlor (Figs. 1, 2, 3 and 4) at concentrations that are ecologically relevant for surface waters.[7,19–21] The ErC_{50}values of duckweed (area of fronds: 2.8–6.4 μg L^{-1}, number of fronds: 3.8–52.9 μg L^{-1}, cp. Table 1) obtained under different environmental conditions are close to the $ErC_{50}$of the duckweed *Lemna gibba* in the standardised test (2.3 μg L^{-1}[5]) and filamentous green algae in the oligo- to mesotrophic stream mesocoms (3 μg L^{-1}[13]). They are below the EC_{50} of rooted submersed dicotyledons (*Myriophyllum verticillatum*) and floating-leaved moncotyledons (*Potamogeton natans*) exposed in pond and stream mesocosms (11–38 μg L^{-1}[13]) and well below the effective concentration range for algae in standardised tests with metazachlor (31 to 9600 μg L^{-1}[6,22]).

The lower sensitivity of the endpoint number of fronds may be related to the lack of information about small and necrotic fronds that were regularly observed in duckweed after exposure to concentrations above 1.8 μg L^{-1} metazachlor. The parameter number of fronds was also less sensitive for the detection of metazachlor effects in *Potamogeton natans* with floating leaves exposed in the same pond mesocosms.[14] Metazachlor-induced smaller fronds are assumed to result from the irreversible inhibition of

very long chain fatty acids (VLCFA) synthesis since the enzyme VLCFA elongase is the target site of metazachlor action.[23,24] VLCFA inhibition should make it difficult for duckweed to construct cell membranes in new arising fronds.[13]

With reference to the metazachlor-sensitive endpoint area of fronds, the *in situ* exposed *S. polyrhiza* under nutrient-poor conditions in pond mesocosms (Fig. 4, Table 1) was as sensitive to metazachlor as *L. minor* examined in the standardised test (Fig. 2, Table 1), although metazachlor concentrations in the ponds decreased continuously (DT_{50} of 37–48 days[13]). Similarly, the investigation of *M. verticillatum* in a parallel running metazachlor experiment in lotic mesocosms revealed significant concentration- and time-dependent effects of metazachlor on plant length (unpublished data). On day 16, 31, and 43 the three highest metazachlor treatments (nominal 80, 200 and 500 $\mu g\ L^{-1}$) were significantly different from the controls. On day 59 the next lower concentration (nominal 20 $\mu g\ L^{-1}$) joined this group and on day 73 all metazachlor treatments were significantly different from the controls.

Delayed metazachlor effects on macrophytes (cp. Figs. 2, 3 and 4) may be attributed to low nutrient concentrations and thus to slow growth rates as earlier assumed by Mohr et al.[13] Due to the covalent binding of the herbicide to the active enzyme[25] the enzymatic reaction can be expected to be not only dependent on herbicide concentration as known for reversible inhibitors[26] but also on the growth rate of macrophytes (cp.[23]). In fact, the lower growth rate of *L. minor* in the modified nutrient-poor test system indicated slightly higher ErC than the standardised test at nutrient-rich conditions (Table 1, Figs. 2 and 3). Furthermore, the fast-growing duckweed species were generally more rapidly and more strongly affected by metazachlor than slower-growing submerged and rooted species such as *M. verticillatum* in parallel running stream mesocosms (unpublished data) and the macrophyte standing stock in the same experimental pond mesocosms.[13] The rooted floating-leaved *P. natans* and the rooted submersed *M. verticillatum* exposed for 140 days in pond mesocosms were more tolerant to metazachlor with an EC_{50} (total WW and ash-free DW, TWA) between 38 and 37 $\mu g\ L^{-1}$ and between 22 and 21 $\mu g\ L^{-1}$[13] than the fast-growing duckweed *S. polyrhiza* (Table 1).

Similar observations were made with the metazachlor-related acetoanilides metolachlor and S-metolachlor which replaced the less effective metolachlor in 1999.[27] The EC_{50} of metolachlor is 48 $\mu g\ L^{-1}$ and 343 $\mu g\ L^{-1}$ for the fast-growing duckweed species *L. gibba* and *L. minor*[28,29], but more than 3,000 $\mu g\ L^{-1}$ for the submersed, slow-growing *Myriophyllum heterophyllum*.[30] The successor S-metolachlor inhibited the growth of *M. spicatum* in a microbioreactor to 30% at a concentration of 1,000 $\mu g\ L^{-1}$.[27] Stronger effects were induced by the acetoanilide dimethachlor in a 84 d outdoor microcosm study: After 56 days, the biomass of *Myriophyllum* sp. (DW) and the growth of *Chara* sp. showed significant adverse effects at the second lowest concentration of 26.8 $\mu g\ L^{-1}$ dimethachlor.[27]

For other chemicals, different modes of action may result in differences in the toxic potential for dicotyledonous, monocotyledonous plants and algae.[31,32] Moreover, it has to be considered whether the chemical is taken up from the sediment and the water by roots or by other parts of the plant.[4,33] Unfortunately, only few toxicity data for macrophyte genera other than *Lemna* are available from higher tier mesocosm studies[13,15,27,32,34,35] and there is still a considerable information gap on macrophyte toxicity of chemicals. Several test methods employing the macrophyte species *Myriophyllum* sp. or other species were developed by now but there is still no standardised procedure.[32,34,36]

Our study shows that tests with fast-growing macrophytes such as duckweed are most appropriate for acetoanilide risk assessment at ecologically relevant concentrations regardless the complexity and nutritional status of the test system with *S. polyrhiza* being a suitable substitute for *L. minor* under oligo- to mesotrophic conditions.

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