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Glyphosate- and aminomethylphosphonic acid (AMPA)-induced mortality and residues in juvenile brown trout (*Salmo trutta* f. *fario*) exposed at different temperatures

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Abstract

Background Glyphosate is a broad-spectrum, non-selective systemic herbicide with a commonly assumed low potential for accumulation in biota. Nevertheless, glyphosate has been shown to bioaccumulate in the tissues of several organisms. To understand the bioconcentration dynamics of glyphosate in fish, brown trout (*Salmo trutta* forma *fario*) of different age were exposed to different concentrations of glyphosate, the formulation Roundup® LB Plus, and the major transformation product aminomethylphosphonic acid (AMPA) for two, three, or four weeks at different temperatures in the laboratory. Mortality rates were determined, and tissue samples were collected at the end of the experiment to ascertain concentrations of glyphosate and AMPA residues by liquid chromatography coupled to mass spectrometry (LC–MS/MS).

Results Brown trout mortality during exposure to glyphosate or AMPA was considerably higher at 15 °C than at 7 °C. Also, a significant increase in glyphosate concentrations in samples containing muscle, head, backbone, and caudal fin tissue with increasing exposure concentrations and temperatures was observed. Six-month-old fish contained more glyphosate per kg wet weight after exposure than ten-month-old fish. The bioconcentration factors (BCFs) for glyphosate and AMPA were much higher at 15 °C than at 7 °C, but in both cases decreased with higher glyphosate concentrations. The BCF for glyphosate formulated in Roundup® was higher than the one for the parent compound. Approximately 30–42% of the organ-absorbed glyphosate and AMPA remained in the tissues even when the fish were kept in clean water lacking the test substances for three weeks after termination of exposure.

Conclusion Our study demonstrated that there is an interaction between glyphosate and ambient temperature in terms of toxicity. Further it was shown that increasing concentrations of glyphosate and AMPA in the surrounding media lead to significantly increased concentrations of these substances in brown trout tissues, although neither bioconcentration nor bioaccumulation of glyphosate in animal tissues is expected due to the high water solubility of this chemical. As a consequence, the uptake of glyphosate by humans through the consumption of contaminated edible fish is very likely.

Keywords Acute toxicity, Bioconcentration, Glyphosate uptake, Herbicide, Maximum residue limits, Temperature

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Background

The herbicidal properties of glyphosate (N-(phosphonomethyl)-glycine, CAS: 1071-83-6) were first discovered in 1974 by J. E. Franz while he was working for Monsanto Company. As a broad-spectrum non-selective systemic herbicide which can be used for total control of weed

plants [1], glyphosate quickly gained high importance in worldwide agriculture [2, 3] with dramatically increasing sales figures during the last decades [4, 5]. The intended mode of action of glyphosate is related to its influence on the shikimate pathway in plants via inhibition of the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), resulting in a cessation of aromatic amino acid synthesis [6–8]. Since aromatic amino acids such as phenylalanine, tyrosine, and tryptophan are essential for cellular metabolism, their deficiency results in a perturbation of the metabolic homeostasis in plants [9], leading to growth arrest and ultimately to plant death. In addition to this disruption of the shikimate pathway, glyphosate has been shown to affect plant physiology by inhibiting photosynthesis, increasing oxidative stress, and modulating diseases, e.g., root infections [10]. Furthermore, numerous microorganisms are known to synthesize aromatic amino acids via the shikimate pathway, which makes them additional target organisms for glyphosate, e.g., in soils, river sediments, or in the microbiome of animals [11–14]. Considering a worldwide application of more than 750 000 tons of glyphosate per year, this chemical and/or its metabolites can now be detected in all ecosystem compartments worldwide [15–17]. Emerging evidence has shown that glyphosate and its commercial formulations have negative impact on fish [18, 19], including organ damage [20–22], teratogenic and genotoxic effects [23, 24], behavioral abnormalities [25], and disorders in reproduction [26–28].

Particular attention must be paid to the interactions between temperature and pollutant inputs to water bodies, especially in the context of climate change with rising air and water temperatures, increasing heavy rainfall events leading to increased pollutant drift from the surface, and increasing periods of drought leading to increased pollutant concentrations due to lower dilution ratios [29–33]. Regardless, pesticides are used throughout the year for different purposes and therefore at different ambient temperatures. Diffuse inputs of these substances into water bodies via the air or after rainfall also occur at different temperatures [34]. The corresponding water temperatures influence the material properties of the introduced substances, such as their solubility or volatility, their biotic and abiotic degradation in the water, and the physiological state and metabolism of poikilothermic aquatic organisms, which are continuously exposed to substances dissolved in the water or bound to particles throughout their lives [35, 36]. Water temperature therefore influences the uptake, biotransformation, and possible detoxification as well as the possible excretion or storage of substances in the organisms [37].

Based on its low octanol/water partition coefficient (Log K_{ow}: − 3.4), a low accumulation potential of glyphosate in biota can be expected [38]. Nevertheless, the herbicide has been shown to bioaccumulate in the tissues of several organisms, after exposure through food, water, or air. For example, in *Lumbriculus variegatus*, the total amount of glyphosate that accumulated in the tissue increased to approximately 8 µg/g fresh weight with the concentration of glyphosate and Roundup® in the surrounding media (0.05–5 mg/l) [39]. Numerous studies have been conducted to measure glyphosate concentrations in the tissues of farm animals that serve as food sources. Up to now, glyphosate was not detected in meat, milk, and eggs, but in some sensitive organs [40]. In cows, the kidney and lung are the most susceptible organs with a glyphosate concentration of about 60–80 ng/g [41], while in chickens, the glyphosate concentration in the intestine was the highest at about 100 ng/g [42]. An average of 2 mg/kg glyphosate was detected in the livers of malformed piglets [43], and 5–16 mg/kg was found in the livers of pigs receiving glyphosate-contaminated food [44]. This phenomenon cannot be explained in general by passive diffusion of the compound and may be rather due to the active uptake of glyphosate into cells by amino acid transporters with a high affinity for glyphosate, which have been identified in mammalian cells [45].

Glyphosate-based herbicides (GBHs) like Roundup® always contain co-formulants additional to the active ingredient, which function as surfactants, diluents, or stabilizers, e.g., polyethoxylated tallow amine (POEA) [46], and improve the uptake of glyphosate into cells of target organisms [46, 47]. Against this background, it was of interest whether residues in biota exposed to pure glyphosate were lower than those in biota exposed to a commercial herbicide containing equimolar concentrations of the active ingredient. This is the more important because GBHs are often reported to be more toxic to non-target organisms than the herbicide itself [48–51].

Not only glyphosate but also its major metabolite AMPA has been shown to persist in the environment [52, 53]. It is assumed that the affinity of both glyphosate and AMPA to solid material, e.g., in soil, does not differ to a great extent [54], resulting in half-lives of 2–240 days, depending on the intrinsic chemical and physical properties of the soil particles or sediments that affect degradation [38, 55–57]. In general, the half-life of AMPA is somewhat greater than that of glyphosate, suggesting a slower degradation rate and making AMPA more persistent in the environment [57–60]. To date, data on AMPA accumulation in biota are scarce and, when available, mostly related to plants [61]. Recently, AMPA has been shown to bioaccumulate in the liver and muscle of broiler chickens [62] and in the armored catfish *Hoplosternum littorale* collected from a rice field [63], but further data

on fish, especially related to possible adverse effects, are lacking.

Even though glyphosate residues can be expected in fish for human consumption, no threshold values for maximum residue levels have been determined for food fish so far, neither in German national legislation nor in European Union regulations. The present paper provides data on the bioconcentration of glyphosate and its main metabolite AMPA in brown trout, a commercially important fish species with high ecological relevance in freshwater ecosystems [64, 65] as a basis for a future derivation of such values.

Experiments required for the risk assessment of substances are usually conducted at standard temperatures and, thus, influences of temperature on the toxicity of chemicals are therefore often ignored. To address this shortcoming, in the present study mortality and bioconcentration of glyphosate and AMPA were measured in brown trout after exposure to different concentrations of glyphosate, AMPA, and Roundup®—at concentrations equimolar to the highest glyphosate test concentration—for two, three or four weeks at 7 °C or 15 °C. The selected test concentrations were based on the 2016 template for the annual average environmental quality standard published by the EU, which has defined a concentration of 56 µg/l glyphosate as a regulatory acceptable concentration (RAC) [66], and therefore 1, 10, and 100 times this concentration were chosen for the experiments. In the meantime, a RAC of 100 µg/l [67] and Environmental Quality Standards (EQS) were determined for surface waters including an AA-EQS (average allowed concentration) of 86.7 µg/l for freshwater not used for the abstraction and preparation of drinking water, and an AA-EQS of 0.1 µg/l for freshwater used for drinking water purposes [68].

Methods

Test organisms

Approximately six- and ten-month-old brown trout (*Salmo trutta* f. *fario*) were obtained from a commercial fish farm (Forellenzucht Lohmühle, 72275 Alpirsbach-Ehlenbogen, Germany). This breeding facility is classified as category I, i.e., disease free, according to the EC Council Directive 2006/88 [69]. Prior to the experiments, fish were acclimated to laboratory conditions for one week in a climate chamber at 7 °C or 15 °C, respectively, with a 10/14 h light/dark cycle. Experiments were conducted at two temperatures in order to simulate fish exposure at different seasons of the year (early spring and summer in Central Europe), resulting in different water temperatures in the field, which may influence the uptake, biotransformation, and possible detoxification as well as the possible excretion or storage of substances in an organism. Prior to the experiments, the fish were maintained in a 250-l aquarium with constantly aerated filtered tap water (iron, particle, and activated carbon filters) equipped with an external filter (CristalProfi, JBL, Germany). To avoid direct light exposure, two-thirds of the side glass panes and the top of the tank were covered with 0.5 mm black PVC. Fish were observed daily and fed commercial trout feed (Inico Plus, BioMar, Brande, Denmark).

Exposure experiments

All experiments were conducted under the same conditions in the same climate chamber. Three times six 25-l aquaria, each containing 15 l of the respective test solution, were placed in this climate chamber resulting in a three-block design. Also in these aquaria, the lower two-thirds of the side glass panes and the aquaria lids were covered with black PVC to prevent direct lighting. Between 30 and 40 six-month-old individuals or 10 ten-month-old individuals were exposed in each aquarium to account for possible age-dependent difference in body size. Fish were daily fed a defined portion of appropriate commercial food (Inico Plus, BioMar, Brande, Denmark) corresponding to 0.5–1% of the initial body weight. The health condition of the fishes was checked twice a day and dead fish were immediately removed. Every two to three days, half of the respective test solutions, feces, and food residues were removed from each tank and replaced by fresh test solution. Water parameters (NH_4, temperature, oxygen content, pH, and conductivity) were controlled regularly, and although ammonium compounds were sometimes elevated despite even more frequent water changes, this had no effect on mortality or other measured variables. In addition, water samples were taken at the beginning and at the end of the exposure experiment to determine glyphosate and AMPA levels.

For the exposure experiments, glyphosate (N-(phosphonomethyl) glycine; Sigma-Aldrich, Merck KGaA, Darmstadt, Germany), Roundup® (Roundup® LB Plus, purchased at a local retail store), and AMPA (aminomethylphosphonic acid; Acros Organics BVBA, Geel, Belgium) were used. Stock solutions for each test medium were prepared with ultrapure water (glyphosate: 500 mg/l and 560 mg/l, respectively; Roundup®: 560 mg/l; AMPA: 366 mg/l) and diluted to the respective test concentrations. Table 1 provides information on the respective treatments/test concentrations and gives also further information about the test fish in the five experiments conducted. In a range-finding experiment, brown trout were exposed to 500 µg/l and 5000 µg/l glyphosate as well as Roundup® with 5000 µg/l glyphosate prior to the exposure experiments. In the following experiments 1 (E1) and 3 (E3), fish were exposed to an ascending series of glyphosate concentration, the highest

Table 1 Exposure experiments: brown trout at various ages with glyphosate, glyphosate in Roundup®, AMPA, and PEP

Experiment number:	Range-finding	E1	E2	E3	E4
Time point	September 2020	November 2021	July 2022	June 2023	July 2023
Total number of exposed individuals	120	180	540	664	359
Age of exposed fish	10 months	10 months	6 months	6 months	7 months
Average weight in g ± SD	6.5 ± 1.9	6.7 ± 2.0	0.8 ± 0.2	1.2 ± 0.4	1.4 ± 0.4
Average size in cm ± SD	8.0 ± 1.0	8.4 ± 0.9	4.1 ± 0.5	5.1 ± 0.6	5.4 ± 0.5
Duration of exposure	4 weeks	3 weeks	3 weeks	2 weeks	3 weeks
Temperature	7 °C	7 °C	7 °C	15 °C	15 °C
Treatments	Control; glyphosate: 500 µg/l, 5000 µg/l; glyphosate in Roundup®: 5000 µg/l	Control; glyphosate: 56 µg/l, 560 µg/l, 5600 µg/l; AMPA: 3666 µg/l; glyphosate in Roundup®: 5600 µg/l	Control; control + PEP; glyphosate: 5600 µg/l; 5600 µg/l + PEP: AMPA: 3666 µg/l; 3666 µg/l + PEP	Control; glyphosate: 56 µg/l, 560 µg/l, 5600 µg/l; AMPA: 3666 µg/l; glyphosate in Roundup®: 5600 µg/l	Pure water for "recovery"

concentration of glyphosate in Roundup®, and an equimolar amount of the major metabolite AMPA. The two experiments differed in the age of the fish (approximately 10 months and 6 months, respectively), the temperature of exposure (7 °C and 15 °C, respectively), and the duration of the experiment, as an increased total mortality of more than 10% at 15 °C necessitated termination of the exposure in this experiment (E3) after two weeks due to animal welfare reasons. Half of the survivors were sampled, and the other half was allowed to recover in pure filtered tap water for three weeks (experiment 4; E4). Again, water exchange was conducted every two to three days to remove feces and food residues. In the second experiment (E2), fish were exposed not only to 5600 µg/l glyphosate and 3666 µg/l AMPA but also to phosphoenolpyruvate (PEP; Biosynth Carbosynth, Bratislava, Slovakia), which is the glyphosate antagonist for the binding site on the EPSPS enzyme in the shikimate pathway in order to prove whether the addition of PEP may interfere with uptake and toxicity of glyphosate. PEP was added to the aquaria at a concentration equimolar to 5600 µg/l glyphosate. After exposure, fish were anesthetized and killed by 1 g/l tricaine methanesulfonate (MS-222; Pharmaq, Overhalla, Norway), buffered to a neutral pH level with $NaHCO_3$, followed by a cut of the cervical spine. Tissue samples were taken for the assessment of several endpoints of toxicity (data not included in this manuscript) and the determination of glyphosate and AMPA residue concentrations (data presented here).

Chemical analysis

At the beginning and at the end of the experiment, 45 ml of test solution was sampled from each aquarium and frozen at − 20 °C until further processing. After dissection of fish and removal of viscera for other analyses, the remaining fish tissues (mainly muscle tissue, head, backbone, and caudal fin besides fillets from the range-finding experiment) had to be pooled (up to 40 individuals per sample) in order to obtain the minimum tissue weight required for chemical analyses. In 2020 (time of the range-finding experiment), the required minimum weight per sample was lower than in subsequent years, so the fillets alone could be chemical analyzed as a pool (approximately 15 g of tissue). Later, the total weight of the fillets was not sufficient for analysis and samples containing muscle, head, backbone, and caudal fin tissue were pooled to reach the required amount of tissue. Tissue samples were stored at − 80 °C until further processing. Details on sample sizes and characteristics, etc., are provided in Additional file 1: Table S1.

The actual concentrations of glyphosate and AMPA in water samples from the test aquaria and in the exposed fish were determined by Eurofins (Eurofins Sofia GmbH, Berlin, Germany) according to their routine lab procedure to determine the concentration of glyphosate in food and liquids: After addition of 0.1 N HCl, shaking, and centrifugation, the tissue extract was taken and neutralized with 0.1 N KOH. After another centrifugation step, an internal, isotope-labeled standard was added and everything was derivatized with fluorenylmethyloxycarbonyl chloride (Fmoc-Cl), before HCl was added. A minimum of 20 g of tissue was required and the material matrix used was specific for plant material, food, and feed. For water analysis, an internal, isotope-labeled standard and HCl were added to 500 µl of sample. After derivatization with Fmoc-Cl, quantification in both cases was performed by liquid chromatography coupled to mass spectrometry (LC–MS/MS). The limit of quantification was 0.01 mg/kg in tissue and 0.05 µg/l in water, respectively. The results for glyphosate and AMPA

concentrations were reported together with a generally anticipated and accepted measurement uncertainty of 50%, which is common in pesticide residue analysis (according to SANTE/11813/2017 [70]).

Finally, the bioconcentration factor (BCF) of the investigated compounds was determined by means of division of the concentration of glyphosate or AMPA in biota (in mg/kg) by the concentration of glyphosate or AMPA in water (in mg/l). Due to the small size of the individual fish, samples had to be pooled for analysis.

Statistics

In order to quantify the explanatory potential of the parameters 'exposure concentration,' 'temperature,' 'age of fish,' and 'duration of exposure' for the variation of the internal concentrations of glyphosate and AMPA as well as the mortality of the fish, we carried out multiple regression modeling using SAS JMP 16.2.0 for the data resulting from the experiments with continuous exposure (namely E1, E2, E3). To model the effects of glyphosate, all approaches with the pure substance, with Roundup® and the controls were analyzed ($n=14$ mean values). With regard to AMPA, the modeling was carried out for the approaches with the pure substance and with the controls ($n=8$ mean values). As the high mortality observed in E3 resulted in an unplanned, earlier termination of exposure (two instead of three weeks), we excluded the parameter 'duration of exposure' from the models generated to explain mortality.

Results

The mortality of the brown trout varied greatly between the experiments (Fig. 1). In E1, where the fish were the oldest at 10 months, no animal died during the exposure period at 7 °C. In the younger animals, mortality at 7 °C water temperature (E2) averaged 5.2% and increased dramatically at 15 °C exposure (E3), forcing the experiment to be terminated earlier. In the controls of experiments 2 and 3, mortality rates were comparable at the different temperatures. AMPA, equimolar to the highest concentration of glyphosate, resulted in a mortality rate similar to that of glyphosate alone, while Roundup® had the highest averaged mortality rate. In the recovery experiment with clean water (E4), only one animal died in each of the treatment groups previously exposed to the highest concentrations of glyphosate, AMPA, and Roundup® (E3), although the temperature was the same (15 °C). The effect of glyphosate on mortality was clearly greater at 15 °C than at 7 °C.

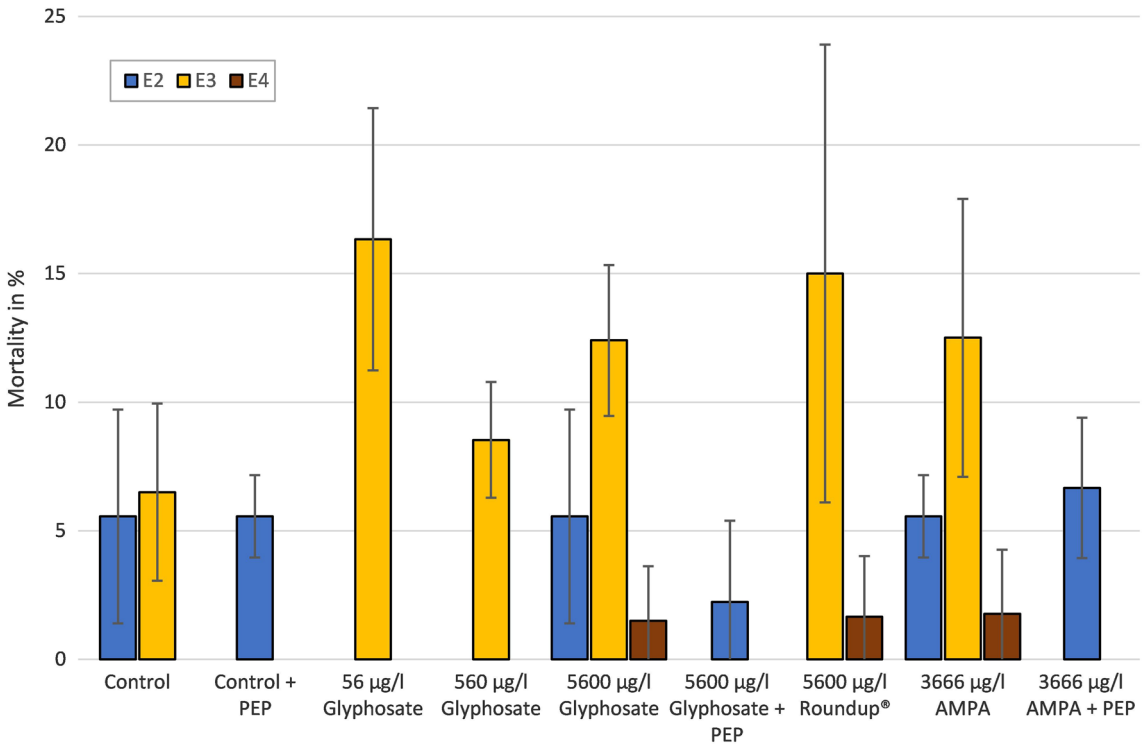

Fig. 1 Mean mortality in percent ± standard deviation of brown trout in experiment 2 (E2, exposure at 7 °C), experiment 3 (E3, exposure at 15 °C), and experiment 4 (E4, recovery at 15 °C). Data of experiment 1 (E1, exposure at 7 °C) are not shown as mortality was always 0. Data are calculated as the means of three respective replicates of each treatment

Multiple regression modeling revealed the variation in mortality in the glyphosate-exposed animals ($n=14$) to be explained to 81.97% ($p=0.0005$) by the parameters 'temperature' ($p=0.0035$), 'age of fish' ($p=0.0310$), 'glyphosate concentration' (insignificant contribution to the model, n.s.), and the intercept (n.s.). 'Temperature' alone explained 70.07% ($p=0.0002$) of the variation in mortality; 'age' alone explained 56.03% ($p=0.0021$). With increasing temperature, the mortality increased—this was already the case for 56 µg/l glyphosate, an environmentally relevant concentration. With increasing age of the fish, mortality decreased. The variation in AMPA-induced mortality ($n=8$) could be explained by 88.97% ($p=0.0230$) by the parameters 'age of fish' ($p=0.0192$), 'temperature' (n.s.), 'concentration' (n.s.), and the intercept ($p=0.0491$). 'Age of fish' alone explained 66.70% ($p=0.0130$) of the variation in mortality with a significant intercept ($p=0.0029$). The highest AMPA-induced mortality was associated with the highest temperature (15 °C), but due to this single data point, the contribution of the parameter 'temperature' to the model remained insignificant. As in the glyphosate experiments, also AMPA-induced mortality decreased with increasing age of fish.

The real water concentrations of glyphosate and AMPA were determined in the range-finding experiment at the end of the experiment (Additional file 1: Table S2). In the exposure experiments 1 to 3, samples were taken before and after the exposure experiments. Real concentrations were very close to the respective nominal concentrations in the different treatments (Table 2). In the control group, very low amounts of glyphosate and/ or AMPA were measured in some cases, which is most likely due to accidentally carryover during water exchange. The low levels of AMPA measured in the glyphosate and Roundup® treatment groups may indicate degradation processes of glyphosate. In the second experiment, a greater amount of glyphosate was found in the treatment with AMPA and PEP—as PEP is structurally very similar to glyphosate, re-metabolization may have occurred, but this requires further investigation. PEP did not interfere with the uptake of glyphosate.

Table 2 Real concentration of glyphosate and AMPA in water in the exposure experiments 1–3

Treatment group	Nominal concentration in µg/l	Real concentration at the beginning in µg/l		Real concentration at the end in µg/l	
		Glyphosate	AMPA	Glyphosate	AMPA
First experiment					
Control	0	0.12 ± 0.06[a,b]	< DL	0.1 ± 0.05[a,b]	< DL
56 µg/l glyphosate	56	88 ± 44[a]	< DL	56 ± 28[a]	0.18 ± 0.09[a]
560 µg/l glyphosate	560	550 ± 270[a]	0.26 ± 0.13[a]	610 ± 300[a]	0.80 ± 0.40[a]
5600 µg/l glyphosate	5600	6000 ± 3000[a]	1.6 ± 0.8[a]	6200 ± 3100[a]	21 ± 10[a]
3666 µg/l AMPA	3666	23 ± 11[a]	4300 ± 2100[a]	19 ± 9.50[a]	4400 ± 2200[a]
5600 µg/l glyphosate in Roundup®	5600	6400 ± 3200[a]	28 ± 14[a]	4100 ± 2000[a]	27 ± 13[a]
Second experiment					
Control	0	< DL	< DL	< DL	0.1 ± 0.05[a,b]
Control + PEP	0	< DL	< DL	0.15 ± 0.075[a,b]	0.31 ± 0.16[a,b]
5600 µg/l glyphosate	5600	4700 ± 2400[a]	< DL	5400 ± 2700[a]	< DL
5600 µg/l glyphosate + PEP	5600	4700 ± 2400[a]	< DL	5600 ± 2800[a]	< DL
3666 µg/l AMPA	3666	57 ± 29[a]	3600 ± 1800[a]	86 ± 43[a]	3800 ± 1900[a]
3666 µg/l AMPA + PEP	3666	< DL	3800 ± 1900[a]	1200 ± 620[a]	3700 ± 1900[a]
Third experiment					
Control	0	< DL	< DL	< DL	< DL
56 µg/l glyphosate	56	57 ± 29[a]	< DL	50 ± 25[a]	< DL
560 µg/l glyphosate	560	570 ± 290[a]	< DL	470 ± 240[a]	< DL
5600 µg/l glyphosate	5600	5500 ± 2800[a]	< DL	5400 ± 2700[a]	< DL
3666 µg/l AMPA	3666	< DL	3800 ± 1900[a]	< DL	3900 ± 2000[a]
5600 µg/l glyphosate in Roundup®	5600	5700 ± 2900[a]	< DL	5100 ± 2600[a]	< DL

Information on the nominal and real concentration of glyphosate and AMPA in water at the beginning and the end of the exposure experiments 1–3. The results for glyphosate and AMPA concentrations are reported as concentrations in µg/l along with a generally anticipated and accepted measurement uncertainty of 50%

[a] Which is common in pesticide residue analysis (according to SANTE/11813/2017 [70])

[b] Glyphosate/AMPA measured in the control group, most likely because a small amount of glyphosate accidentally entered the control aquaria during water exchange. < *DL* below detection limit (= 0.05 µg/l)

In all experiments, uptake of glyphosate was detected in the tissues of brown trout (Fig. 2). In the range-finding experiment, glyphosate levels were analyzed in the fillets of the fish only, and the detected concentrations of glyphosate were much lower than in the samples in the following experiments containing muscles, head, backbone, and caudal fin tissues. In the latter, concentrations of glyphosate residues increased with increasing exposure concentrations. Measurements in E1 and E2 originally consisted of multiple pools, but due to miscommunication the samples from each treatment in E1 were pooled again for analysis. Instead, in E2 each measurement consisted of two different pools so that a standard deviation could be calculated. In control fish, neither glyphosate nor AMPA was detected in all experiments (Additional file 1: Table S1). Ten-month-old fish in E1 contained less glyphosate and AMPA than the fish in E2 and E3, which were about four months younger. In the latter experiments, tissue from more individuals had to be pooled to achieve the minimum tissue weight required for chemical analysis. Although the age of the fish in E2 and E3 was comparable, the individuals exposed at 15 °C showed higher glyphosate and AMPA uptake than the fish exposed at 7 °C, even though the exposure time in E3 was one week shorter. Roundup® LB Plus resulted in the highest glyphosate uptake in fish in both experiments E1 and E3.

Varying internal concentrations of glyphosate in fish tissues ($n=14$) could be explained to 96.11% ($p<0.0001$) by the parameters 'external glyphosate concentration' ($p<0.0001$), 'age of fish' (n.s.), 'temperature' (n.s.), 'time of exposure' (n.s.), and the intercept (n.s.). The glyphosate concentration in the water of the aquaria alone explained 93.14% ($p<0.0001$) of the variation in tissue concentrations of glyphosate. When the parameters 'external glyphosate concentration' and 'temperature' were crossed and included in a multiple regression model together with 'external glyphosate concentration' alone ($p<0.0001$), 'temperature' alone ($p=0.0270$), and the intercept ($p=0.0466$), the crossed parameter 'external glyphosate concentration × temperature' contributed significantly to the model ($p=0.0247$) which in total explained 97.07% ($p<0.0001$) of the variation in internal glyphosate concentration in fish. With increasing temperature, glyphosate concentration in the water and 'temperature x water concentration' the internal glyphosate concentrations in fish tissue increased. A total of 94.21% ($p=0.0062$) of the variation in internal AMPA concentrations in AMPA-exposed fish ($n=8$) was explained by a model comprising the parameters 'external AMPA concentration' ($p=0.0014$), 'temperature' (n.s.), 'exposure

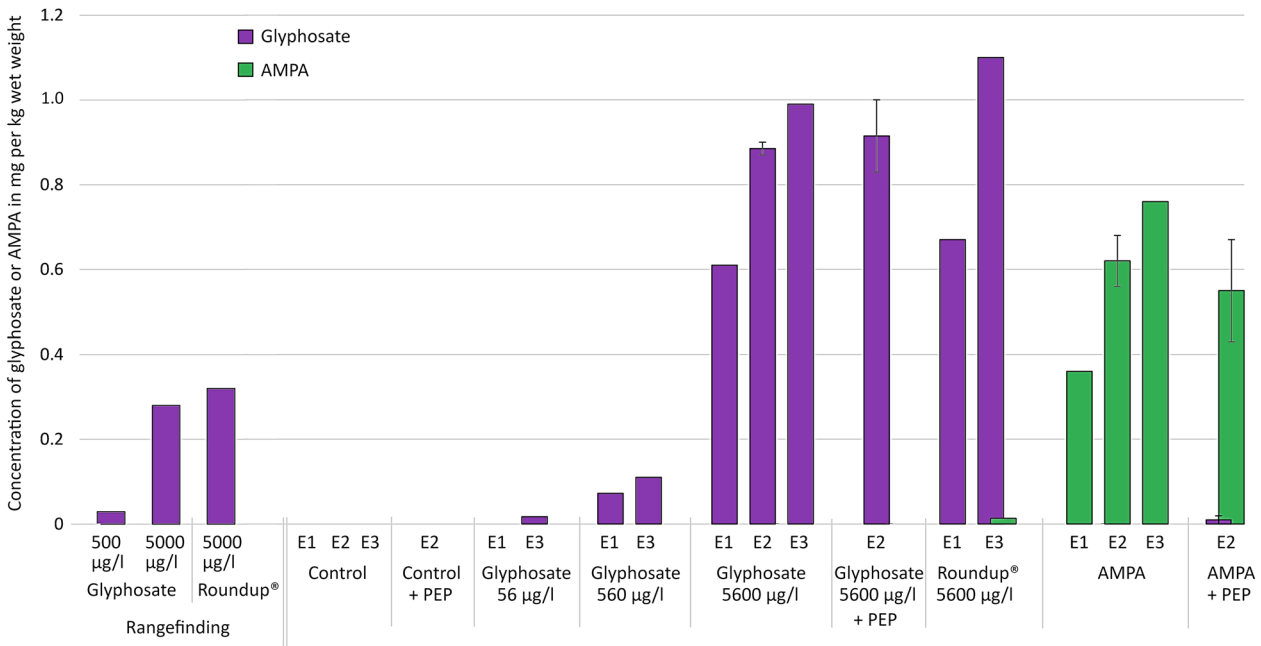

Fig. 2 Concentration of glyphosate (purple) and AMPA (green) in the fillets of brown trout of the range-finding experiment, and in the samples consisting of muscle, heads, backbones, and caudal fin tissue. X-axis: exposure concentrations in the range-finding experiment (left; exposure at 7 °C), in experiment 1 (E1; exposure at 7 °C), experiment 2 (E2; exposure at 7 °C), and experiment 3 (E3; exposure at 15 °C); Y-axis: concentration of glyphosate or AMPA after exposure to the respective test concentration. Data are calculated as the means of three respective replicates of each treatment

time' (n.s.), 'age of fish' (n.s.), and the intercept (n.s.). In this context, the AMPA concentration in the water of the aquaria was positively correlated with the internal AMPA concentration and, alone, explained 88.75% ($p=0.0005$) of the variation in the concentrations of this compound in fish tissues. In our experiments, the highest AMPA concentration measured in fish tissues also was associated with the highest exposure temperature (15 °C) but, again, due to this single observation, the contribution of the parameter 'temperature' to the model was not significant.

After brown trout larvae in E3 had been exposed to glyphosate, Roundup® LB Plus, and AMPA for two weeks at 15 °C, about half of the fish were transferred to new, clean tanks and kept in pure filtered tap water (iron, particle, and activated carbon filters) completely lacking test solutions for three more weeks as a 'recovery experiment' (E4). Glyphosate and AMPA uptake was determined in the same tissue portions as directly after chemical exposure. It was evident that glyphosate or AMPA detected in the tissues directly after the two-week exposure persisted in the fish tissues after recovery in pure water for a longer period of time than they had previously been exposed to the test solutions (Fig. 3). At the lowest, environmentally relevant glyphosate concentration (56 µg/l), no more glyphosate was detected after three weeks of recovery, whereas at the highest glyphosate concentration (5600 µg/l), 42.42% of the previously measured concentration remained in the tissues. In the Roundup® treatment, 31% of the previously measured glyphosate concentration in the Roundup®-treatment remained in the tissues, and after AMPA exposure, 34.2% of the previously measured concentration remained. A small amount of AMPA was also detected after two weeks of exposure to 5600 µg/l Roundup®, suggesting that glyphosate is metabolized to AMPA [71], and therefore, tissue uptake of AMPA may have been possible.

Figure 4 shows the bioconcentration factors (BCFs) for glyphosate and AMPA in the respective exposure experiments. All BCFs were well below 1, indicating that glyphosate does not bioconcentrate or bioaccumulate in fish. However, while the glyphosate concentrations detected in the tissues of brown trout increased with increasing exposure concentrations (Fig. 2), it was apparent that higher exposure concentrations of glyphosate generally resulted in lower BCF values. The BCFs for glyphosate provided as Roundup® were always slightly higher than those for the parent compound. At 15 °C in the third exposure experiment E3, the BCF values for glyphosate were much higher than at 7 °C. In the first exposure experiment E1, the BCF for 56 µg/l glyphosate exposure was calculated 0 because the concentration in the tissues was below the detection limit, but in E3 with younger fish and at a higher exposure temperature, the BCF for 56 µg/l glyphosate was the highest of all. The BCFs for AMPA were not only in a similar range compared with those for glyphosate but also higher in younger fish and at a higher exposure temperature.

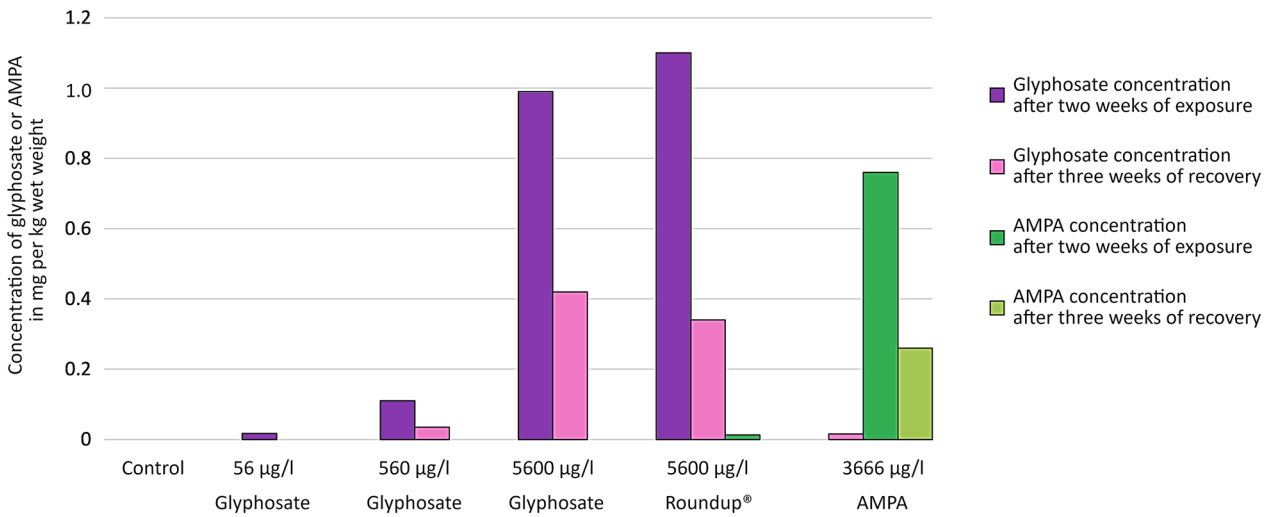

Fig. 3 Concentration of glyphosate (purple and pink) and AMPA (dark and light green) in the samples consisting of muscle, heads, backbones, and caudal fin tissue after an initial two-week exposure experiment followed by a three-week recovery period in pure filtered tap water. X-axis: exposure concentrations in experiment 4 (E4; recovery at 15 °C); Y-axis: concentration of glyphosate or AMPA after exposure to the respective test concentration and after three weeks of recovery, respectively

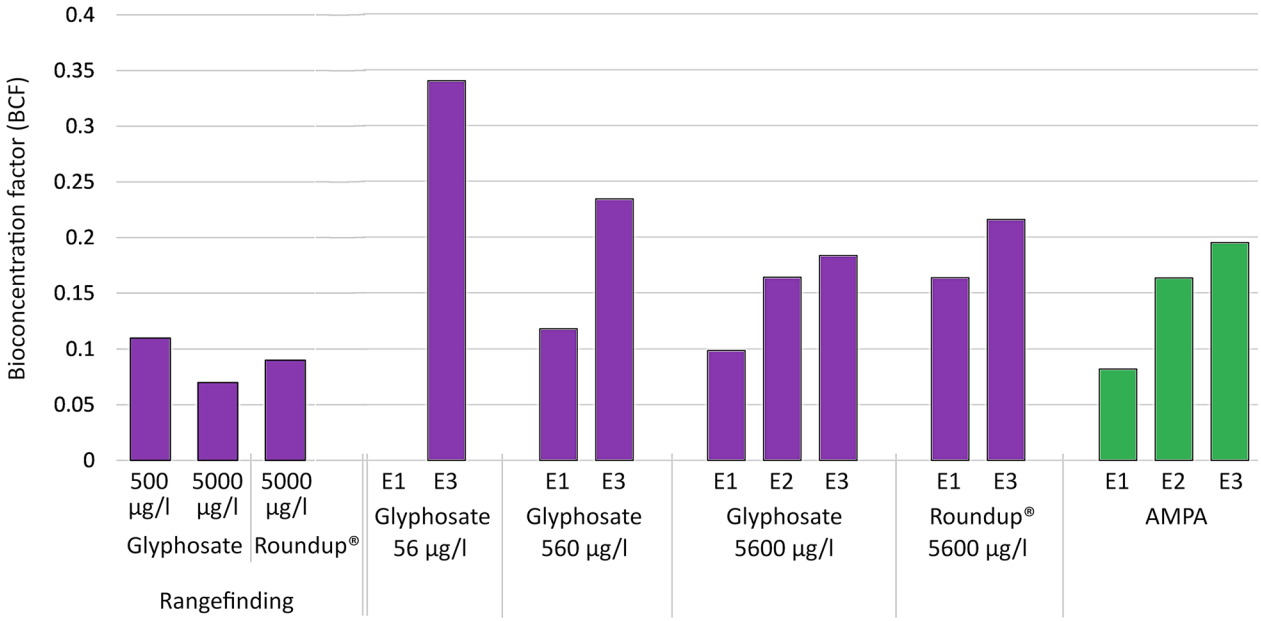

Fig. 4 Bioconcentration factors (BCF values) of glyphosate (purple) and AMPA (green) in the fillets of brown trout of the range-finding experiment and in the samples consisting of muscle, heads, backbone, and caudal fin tissue. X-axis: exposure concentrations in the range-finding experiment (left), in experiment 1 (E1; exposure at 7 °C), experiment 2 (E2; exposure at 7 °C), and experiment 3 (E3; exposure at 15 °C); Y-axis: Bioconcentration factors for glyphosate and AMPA, after exposure to the respective test concentration

Discussion

Although neither strong bioconcentration nor bioaccumulation of glyphosate in animal tissues is expected due to the high water solubility of this chemical, and as confirmed by the low BCF values, glyphosate is generally taken up by fish into their tissues and it is statistically proven that increasing concentrations of glyphosate and AMPA in the surrounding media lead to increased concentrations of these substances in brown trout tissue. The presence of glyphosate in tissues is relevant for the transfer into the food chain and for consumption of fish. Also for other mobile and hydrophilic substances similar BCFs in fish were reported, e.g., for sodium 4,4′-diaminostilbene-2,2′-disulphonate (CAS: 25394-13-2; Log K_{ow} = − 3.99; BCF = 0.200), 2,7-naphthalenedisulfonic acid (CAS: 5460-09-3; Log K_{ow} = − 6.33; BCF = 0.302), and 2-Naphthol-3,6-disulfonic acid (CAS: 15883-57-5; Log K_{ow} = − 4; BCF = 0.350) [72].

Other previously conducted experiments with glyphosate have shown that both glyphosate and AMPA can accumulate to rather high levels in biota. For example, Wang et al. [73] measured the radioactivity after exposure of fish to different concentrations of radiolabeled glyphosate (50 µg/l and 5 µg/l, respectively) and thus the presence of glyphosate in the tissues of two fish, common carp (*Cyprinus carpio*) and tilapia (*Oreochromis mossambicus*), and showed that the maximum radioactivity was reached five to seven days after exposure (660 µg/l in carp and 1300 µg/l in tilapia, respectively), although the radioactivity in the surrounding water had decreased continuously. The bioconcentration factors (BCF) published in this study for common carp (22 ± 12) and tilapia (25 ± 19) exceeded the BCFs calculated in the present study by far. In addition to the general problem with radiolabeling, that the radiolabeled atom may have transferred to other molecules [74], the reason for this difference could be that the experiments of Wang et al. [73] were conducted at 22 °C, whereas brown trout in the present study were exposed only at 7 °C and 15 °C, respectively. This assumption is supported by our finding that more glyphosate or AMPA was detected in the tissues of animals exposed at 15 °C than in those exposed at 7 °C, and that bioconcentration factors were higher at 15 °C in the present study than at 7 °C. The ability of pollutants to bioaccumulate in organisms has been shown to depend on temperature, among other physicochemical properties, i.e., substances may behave differently with increasing temperature. Thus, the toxicity of organic and inorganic contaminants may increase with increasing water temperature [75, 76]. This is particularly important in the context of environmental warming with temperature projections indicating that if climate change will continue unabatedly, water temperatures will increase as much as 4 °C by the year 2050 [77]. Furthermore, plant protection products are used throughout the year for different purposes and therefore also at different

temperatures. Rising temperatures in combination with environmental pollution represents a major challenge for the future, and it is likely that the mutual effects will be additive or synergistic [78–80]. Recently, increased toxicity was observed after exposure of several GBHs at elevated temperatures (20 °C and 30 °C, respectively) in various marine crustaceans, such as *Artemia franciscana* or *Sphaeroma serratum,* and in the American oyster *(Crassostrea virginica)* [81, 82]. But on the other hand, significant effects of glyphosate on the development of Common toads (*Bufo bufo*) were observed at lower temperatures (15 °C) [83]. In the present study, mortality and tissue uptake of glyphosate were significantly higher at 15 °C compared to 7 °C. With increasing temperature, the mortality increased, and this was already the case for 56 µg/l glyphosate, an environmentally relevant concentration. The high mortality in E3 is due to the interaction between glyphosate and temperature, as mortality in E4 decreased substantially when glyphosate was no longer used in the water. The effect of glyphosate at 7 °C on mortality in E2 seems rather small, as mortality remained constant at the control level. Therefore, it is obvious that temperature can alter potential non-target effects of glyphosate or pollutants in general. This is notable because ecotoxicological risk assessment studies are typically conducted at one standard temperature, thereby perhaps not adequately examining effects at natural conditions above or below the standard temperature. The effects of temperature are also important in the approval of hazardous substances.

It became evident that 10-month-old fish (E1) took up less glyphosate, glyphosate formulated in Roundup®, and AMPA in their tissues compared to the 6-month-old fish (E2), even when the latter were exposed for one week less (E3). This may probably be due to a generally higher metabolic activity in older fish with higher body mass compared to younger ones [84]. In addition, glyphosate was still detectable in fish tissues after a three-week recovery period. The mechanism, by which glyphosate is processed in the body, is not yet fully known. It has been shown that it is excreted primarily as the unchanged parent chemical via the kidneys and urine [71, 85], with only a small fraction being metabolized. In general, the biotransformation of lipophilic xenobiotics is a biphasic process involving phase I and phase II enzymes, e.g., cytochrome P450 or glutathione-S-transferase. These enzymes work together to increase the body´s ability to eliminate mostly organic, hydrophobic contaminants, thereby preventing harmful accumulation in the body. The highly water soluble glyphosate is likely to be eliminated by a different pathway. However, there is evidence that glyphosate can inhibit cytochrome P450 enzymes and thus alter their detoxification potential for other substances [86–88]. An important enzyme of the phase II biotransformation system, glutathione-S-transferase (GST), also protects cells from effects of xenobiotics and endogenous substances and is considered to be beneficial in coping with stressful conditions like oxidative stress [89]. Studies conducted with several fish species have shown that glyphosate also affects this detoxification enzyme. In *Anabas testudineus*, *Heteropneustes fossilis*, *Rhamdia quelen,* and *Carassius auratus*, a significant reduction of GST activity has been shown after exposure to glyphosate [90–92]. In silver barb (*Barbonymus gonionotus*), however, GST activity was not altered after exposure to 10 mg/kg glyphosate for several days [93], and this was also the case in a study on *Prochilodus lineatus,* in which no change in GST activity was observed after exposure to 7.5 mg/l and 10 mg/l glyphosate for 24 and 96 h, respectively [94]. These different results make evident that further investigations concerning pathways of detoxification of glyphosate in fish are urgently required.

Our results clearly showed that the BCF values decrease with increasing glyphosate concentrations. Similar observations were made in a study conducted by Contardo-Jara et al. [39] with the earthworm *Lumbriculus variegatus*, where the BCF was significantly lower in the treatments containing 5 mg/l glyphosate (pure or as active ingredient in Roundup Ultra) than in the treatment with concentrations of 0.05 mg/l and 0.1 mg/l in the exposure medium. The authors suggest that the decreasing BCF value at high concentrations is associated with an increase in the activity of the biotransformation enzyme GST [39], which, however, as previously discussed, is unlikely to explain the result for fish in the present study. The BCF values for the metabolite AMPA were similar to the BCF values for the corresponding glyphosate concentration, whereas the BCF values for the Roundup® treatment were higher. In mammals, amino acid transporters have been reported to be involved in the transport of glyphosate across epithelial tissues [45]. Due to the surfactants and adjuvants in the glyphosate-based formulation, the membrane permeability increases, allowing for easier cellular uptake [51, 95, 96]. This may account for the higher bioconcentration potential of glyphosate when applied in the Roundup® formulation, as seen in the present study.

Farmed animals were shown to take up glyphosate via their food resulting in residues in various organs like intestines, liver, muscles, spleen, and kidneys [41–43, 62]. In muscle tissues, the accumulation of glyphosate was always lowest. Due to the small size of the brown trout, it was not possible to analyze solely fillets in the exposure experiments 1–3 due to analytical limitations. However, in the range-finding experiment prior to the other exposure experiments, pure fillets also exhibited lower

glyphosate concentrations resulting in lower BCF values than in the bone-containing samples from the following experiments. So, the bioconcentration in our samples containing muscle tissue and skin, head, backbone, and caudal fin tissue was higher than it would be expected in fillets alone. This sounds plausible since it has already been shown that after oral administration of glyphosate to rats, most of the administered compound was found in their bones [97]. This bone-specific accumulation of glyphosate may result from its interaction with calcium [98, 99], possibly resulting in a negative impact of glyphosate on bone mineral density and quality in female *Wistar* rats as reported by Hamdaoui et al. [100]. At first glance, the lower concentrations of glyphosate in the fillets of fish compared to those in fillets plus head, bone, and caudal fin tissue may imply a lower risk for consumers of fish. However, on one hand, there are fish that are eaten as a whole (e.g., anchovies), and on the other hand, glyphosate residues in fillets and bones are relevant for fish-eating animals, and, consequently, the transfer of chemicals along the food chain. This relevance of glyphosate for terrestrial and aquatic food chains has been emphasized already by Gill et al. [101] or Thanomsit et al. [19], which is in contrast to previous statements of the glyphosate producer [102].

As residues of glyphosate and AMPA in food can be of potential toxicological concern, the so-called maximum residue limits (MRLs) have been introduced and recommended by the European Food Safety Authority (EFSA) [103], ranging from 0.05 to 30 mg/kg for various food sources. The glyphosate MRLs for animal products such as milk and eggs, and muscle, fat tissue, liver, and kidney of pigs, cattle, sheep, goats, horses, and poultry range from 0.05 to 2 mg/kg. Surprisingly, there are no MRLs for fish tissues consumed by humans [104], most likely because glyphosate is only rarely applied directly to water. Furthermore, its hydrophilicity does not urgently call for testing the bioconcentration of glyphosate in fish within regulatory requirements [105]. At 56 µg/l, the environmentally relevant concentration of glyphosate, the measured glyphosate concentration in fish did not reach the above mentioned MRLs for animal products. However, at the highest concentration tested (5600 µg/l), the values were in the range of 0.05 to 2 mg/kg. Due to the increasing consumption of fish [106], however, MRLs for fish samples should be established, especially since in many cultures small fish are eaten as a whole, i.e., with skin and bones.

It has been shown that the herbicide glyphosate and its major metabolite AMPA are present in juvenile brown trout after exposure and persist in the tissues even after three weeks of recovery. This is the most important finding of this study along with the evidence that these chemicals are associated with the skeleton. BCF determination should be used to determine whether bioaccumulation or bioconcentration is actually occurring. Although the BCF in this study was less than 1, we were able to demonstrate that glyphosate enters the tissues and is still detectable after three weeks.

Conclusion

Tissue analysis show that the controversially discussed herbicide glyphosate and its main transformation product AMPA do not bioaccumulate or bioconcentrate but are nevertheless taken up into the body of brown trout and remain detectable even after three weeks of recovery in clean water. The combination of glyphosate and elevated temperature resulted in significantly increased tissue uptake and significantly increased mortality. Although there is a vast number of data especially on the presence of glyphosate in humans and on the short-term effects of high and environmentally irrelevant concentrations of this herbicide in biota, long-term effects resulting from exposure to environmentally relevant concentrations are far from being understood and should be in the focus of future studies in order to realistically assess the risk that glyphosate poses to humans and the environment. Little data are available on AMPA in this regard, although the present study suggests a similar toxicity to the parent compound. In the context of the precautionary principle, the present data for glyphosate and AMPA toxicity and their residues in fish tissue, and their interaction with temperature should be regarded as a warning signal for the—so far largely disregarded—environmentally relevance of glyphosate and AMPA.

Abbreviations

AA	Averaged allowed concentration
AMPA	Aminomethylphosphonic acid
BCF	Bioconcentration factor
DL	Detection limit
E1–E4	Experiment 1–4
EFSA	European Food Safety Authority
EPSPS	5-Enolpyruvylshikimate-3-phosphate synthase
EQS	Environmental quality standard
f.	Forma
Fmoc-Cl	Fluorenylmethyloxycarbonyl chloride
GBH	Glyphosate-based herbicide
GST	Glutathione-S-transferase
LAWA	Bund/Länder-Arbeitsgemeinschaft Wasser
log K_{ow}	Octanol/water partition coefficient
MRL	Maximum residue limits
MS-222	Tricaine methanesulfonate
n.a.	Not analyzed
n.s.	Not significant
NOEC	No effect concentration
OECD	Food and Agriculture Organization of the United Nations
PEP	Phosphoenolpyruvate
POEA	Polyethoxylated tallow amine
PVC	Polyvinyl chloride
RAC	Regulatory acceptable concentration

Supplementary Information

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Additional file 1: Table S1: Properties of the different experiments. **Table S2**: Glyphosate concentrations at the end of the range-finding experiment.

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Author contributions
VD supervised and performed the experiments, analyzed the data, prepared the figures and tables, and drafted the manuscript. SK performed the experiments and contributed substantially to the drafting of the manuscript. KP performed the experiments and prepared the figures in the manuscript. MZ performed the range-finding experiment and provided the data. HK and RT conceived and designed the experiments, contributed to the data analysis, and revised drafts of the paper. All authors read and approved the final manuscript.

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Availability of data and materials
All data generated or analyzed during this study are included in this published article and its additional files.

Declarations

Ethics approval and consent to participate
All experiments were approved by the Animal Welfare Committee of the Regional Council of the administrative district of Tübingen, Germany (approval/ authorization numbers ZO 2/16 and ZO 02 /21 G).

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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References

1. Powles SB (2008) Evolved glyphosate-resistant weeds around the world: lessons to be learnt. Pest Manag Sci 64(4):360–365. https://doi.org/10.1002/ps.1525
2. Duke SO (2018) The history and current status of glyphosate. Pest Manag Sci 74(5):1027–1034. https://doi.org/10.1002/ps.4652
3. Duke SO, Powles SB (2008) Glyphosate: a once-in-a-century herbicide. Pest Manag Sci 64(4):319–325. https://doi.org/10.1002/ps.1518
4. Antier C, Andersson R, Auskalnienė O, Baric´ K, Baret P, Besenhofer G, Calha I, Carrola Dos Santos S, De Cauwer B, Chachalis D, Dorner Z, Follak S, Forristal D, Gaskov S, Gonzales Andujar JL, Hull R, Jalli H, Kierzek R, Kiss J, Kudsk P, Leonhardt C, Leskovsek R, Mennan H, Messéan A, Necajeva J, Mullins E, Neve P, Pedraza V, Pintar A, Reboud X, Redl M, Riemens M, Ringselle B, Ruuttunen P, Sattin M, Simic´ M, Soukup J, Stefanic E, Steinkellner S, Storkey J, Ulber L, Weickmans B, Wirth J (2020) A survey on the uses of glyphosate in European countries
5. Benbrook CM (2016) Trends in glyphosate herbicide use in the United States and globally. Environ Sci Eur 28(1):3. https://doi.org/10.1186/s12302-016-0070-0
6. Boocock MR, Coggins JR (1983) Kinetics of 5-enolpyruvylshikimate-3-phosphate synthase inhibition by glyphosate. FEBS Lett 154(1):127–133. https://doi.org/10.1016/0014-5793(83)80888-6
7. Schönbrunn E, Eschenburg S, Shuttleworth WA, Schloss JV, Amrhein N, Evans JNS, Kabsch W (2001) Interaction of the herbicide glyphosate with its target enzyme 5-enolpyruvylshikimate 3-phosphate synthase in atomic detail. Proc Natl Acad Sci USA 98(4):1376–1380. https://doi.org/10.1073/pnas.98.4.1376
8. Steinrücken HC, Amrhein N (1980) The herbicide glyphosate is a potent inhibitor of 5-enolpyruvyl-shikimic acid-3-phosphate synthase. Biochem Biophys Res Commun 94(4):1207–1212. https://doi.org/10.1016/0006-291x(80)90547-1
9. Vivancos PD, Driscoll SP, Bulman CA, Ying L, Emami K, Treumann A, Mauve C, Noctor G, Foyer CH (2011) Perturbations of amino acid metabolism associated with glyphosate-dependent inhibition of shikimic acid metabolism affect cellular redox homeostasis and alter the abundance of proteins involved in photosynthesis and photorespiration. Plant Physiol 157(1):256–268. https://doi.org/10.1104/pp.111.181024
10. Gomes MP, Smedbol E, Chalifour A, Hénault-Ethier L, Labrecque M, Lepage L, Lucotte M, Juneau P (2014) Alteration of plant physiology by glyphosate and its by-product aminomethylphosphonic acid: an overview. J Exp Bot 65(17):4691–4703. https://doi.org/10.1093/jxb/eru269
11. Aristilde L, Reed ML, Wilkes RA, Youngster T, Kukurugya MA, Katz V, Sasaki CRS (2017) Glyphosate-induced specific and widespread perturbations in the metabolome of soil *Pseudomonas* species. Front Environ Sci 5:1–13. https://doi.org/10.3389/fenvs.2017.00034
12. Bellec L, Le Du-Carré J, Almeras F, Durand L, Cambon-Bonavita M-A, Danion M, Morin T (2022) Glyphosate-based herbicide exposure: effects on gill microbiota of rainbow trout (*Oncorhynchus mykiss*) and the aquatic bacterial ecosystem. FEMS Microbiol Ecol 98(8):1–12. https://doi.org/10.1093/femsec/fiac076
13. Van Bruggen AHC, He MM, Shin K, Mai V, Jeong KC, Finckh MR, Morris JG (2021) Indirect effects of the herbicide glyphosate on plant, animal and human health through its effects on microbial communities. Front Environ Sci. https://doi.org/10.1016/j.scitotenv.2017.10.309
14. Hembach N, Drechsel V, Sobol M, Kaster A-K, Köhler H-R, Triebskorn R, Schwartz T (2024) Effect of glyphosate, its metabolite AMPA, and the glyphosate formulation Roundup® on brown trout (Salmo trutta f. fario) gut microbiome diversity. Front Microbiol 14:1271983. https://doi.org/10.3389/fmicb.2023.1271983
15. Kanissery R, Gairhe B, Kadyampakeni D, Batuman O, Alferez F (2019) Glyphosate: its environmental persistence and impact on crop health and nutrition. Plants 8(11):1–11. https://doi.org/10.3390/plants8110499
16. Maggi F, la Cecilia D, Tang FHM, McBratney A (2020) The global environmental hazard of glyphosate use. Sci Total Environ 717:137167. https://doi.org/10.1016/j.scitotenv.2020.137167
17. Singh S, Kumar V, Datta S, Wani AB, Dhanjal DS, Romero R, Singh J (2020) Glyphosate uptake, translocation, resistance emergence in crops, analytical monitoring, toxicity and degradation: a review. Environ Chem Lett 18(3):663–702. https://doi.org/10.1007/s10311-020-00969-z
18. Lanzarin GAB, Félix LM, Fontaínhas-Fernandes A, Monteiro SM, Venâncio C (2023) Effects of glyphosate or glyphosate-based herbicide during the zebrafish life cycle: a review addressing the mechanisms of toxicity. Water 15(12):2276. https://doi.org/10.3390/w15122276
19. Thanomsit C, Saowakoon S, Wattanakornsiri A, Nanuam J, Prasatkaew W, Nanthanawat P, Mongkolvai P, Chalorcharoenying W (2020) Glyphosate (roundup): fate in aquatic environment, adverse effect and toxicity assessment in aquatic organisms. Naresuan Univ J Sci Technol 28(28):1

20. Bastos Gonçalves B, Cardoso Giaquinto P, dos Santos Silva D, de Melo e Silva Neto C, de Lima A, Antonio Brito Darosci A, Laço Portinho J, Fernandes Carvalho W, Lopes Rocha T (2019) Ecotoxicology of glyphosate-based herbicides on aquatic environment. Biochem Toxicol Heavy Met Nanomater. https://doi.org/10.5772/intechopen.85157
21. De Maria M, Kroll KJ, Yu F, Nouri M-Z, Silva-Sanchez C, Perez JG, Moraga Amador DA, Zhang Y, Walsh MT, Denslow ND (2022) Endocrine, immune and renal toxicity in male largemouth bass after chronic exposure to glyphosate and Rodeo®. Aquat Toxicol 246:106142. https://doi.org/10.1016/j.aquatox.2022.106142
22. Liu J, Dong C, Zhai Z, Tang L, Wang L (2021) Glyphosate-induced lipid metabolism disorder contributes to hepatotoxicity in juvenile common carp. Environ Pollut 269:116186. https://doi.org/10.1016/j.envpol.2020.116186
23. Ames J, Miragem AA, Cordeiro MF, Cerezer FO, Loro VL (2022) Effects of glyphosate on zebrafish: a systematic review and meta-analysis. Ecotoxicology 31(8):1189–1204. https://doi.org/10.1007/s10646-022-02581-z
24. Marques A, Guilherme S, Gaivão I, Santos MA, Pacheco M (2014) Progression of DNA damage induced by a glyphosate-based herbicide in fish (*Anguilla anguilla*) upon exposure and post-exposure periods–insights into the mechanisms of genotoxicity and DNA repair. Comp Biochem Physiol Toxicol Pharmacol 166:126–133. https://doi.org/10.1016/j.cbpc.2014.07.009
25. Lopes AR, Moraes JS, de Martins C, MG, (2022) Effects of the herbicide glyphosate on fish from embryos to adults: a review addressing behavior patterns and mechanisms behind them. Aquat Toxicol 251:106281. https://doi.org/10.1016/j.aquatox.2022.106281
26. Bastos Gonçalves B, Nascimento NF, Santos MP, Bertolini RM, Yasui GS, Giaquinto PC (2018) Low concentrations of glyphosate-based herbicide cause complete loss of sperm motility of yellowtail tetra fish *Astyanax lacustris*. J Fish Biol 92(4):1218–1224. https://doi.org/10.1111/jfb.13571
27. Lopes FM, Varela Junior AS, Corcini CD, da Silva AC, Guazzelli VG, Tavares G, da Rosa CE (2014) Effect of glyphosate on the sperm quality of zebrafish *Danio rerio*. Aquat Toxicol 155:322–326. https://doi.org/10.1016/j.aquatox.2014.07.006
28. Webster TMU, Laing LV, Florance H, Santos EM (2014) Effects of glyphosate and its formulation, roundup, on reproduction in zebrafish (*Danio rerio*). Environ Sci Technol 48(2):1271–1279. https://doi.org/10.1021/es404258h
29. Brkić Ž (2023) Increasing water temperature of the largest freshwater lake on the Mediterranean islands as an indicator of global warming. Heliyon 9(8):e19248. https://doi.org/10.1016/j.heliyon.2023.e19248
30. Liu S, Xie Z, Liu B, Wang Y, Gao J, Zeng Y, Xie J, Xie Z, Jia B, Qin P, Li R, Wang L, Chen S (2020) Global river water warming due to climate change and anthropogenic heat emission. Glob Planet Change 193:103289. https://doi.org/10.1016/j.gloplacha.2020.103289
31. Noyes PD, Lema SC (2015) Forecasting the impacts of chemical pollution and climate change interactions on the health of wildlife. Curr Zool 61(4):669–689. https://doi.org/10.1093/czoolo/61.4.669
32. van Vliet MT, Franssen WH, Yearsley JR, Ludwig F, Haddeland I, Lettenmaier DP, Kabat P (2013) Global river discharge and water temperature under climate change. Glob Environ Chang 23(2):450–464. https://doi.org/10.1016/j.gloenvcha.2012.11.002
33. Vázquez DP, Gianoli E, Morris WF, Bozinovic F (2017) Ecological and evolutionary impacts of changing climatic variability. Biol Rev Camb Philos Soc 92(1):22–42. https://doi.org/10.1111/brv.12216
34. Zaller JG, Kruse-Plaß M, Schlechtriemen U, Gruber E, Peer M, Nadeem I, Formayer H, Hutter H-P, Landler L (2022) Pesticides in ambient air, influenced by surrounding land use and weather, pose a potential threat to biodiversity and humans. Sci Total Environ 838(Pt 2):156012. https://doi.org/10.1016/j.scitotenv.2022.156012
35. Kazmi SSUH, Wang YYL, Cai YE, Wang Z (2022) Temperature effects in single or combined with chemicals to the aquatic organisms: an overview of thermo-chemical stress. Ecol Indic 143:109354. https://doi.org/10.1016/j.ecolind.2022.109354
36. Neubauer P, Andersen KH (2019) Thermal performance of fish is explained by an interplay between physiology, behaviour and ecology. Conserv Physiol 7(1):coz025. https://doi.org/10.1093/conphys/coz025
37. Raths J, Švara V, Lauper B, Fu Q, Hollender J (2023) Speed it up: how temperature drives toxicokinetics of organic contaminants in freshwater amphipods. Glob Chang Biol 29(5):1390–1406. https://doi.org/10.1111/gcb.16542
38. Annett R, Habibi HR, Hontela A (2014) Impact of glyphosate and glyphosate-based herbicides on the freshwater environment. J Appl Toxicol 34(5):458–479. https://doi.org/10.1002/jat.2997
39. Contardo-Jara V, Klingelmann E, Wiegand C (2009) Bioaccumulation of glyphosate and its formulation Roundup Ultra in *Lumbriculus variegatus* and its effects on biotransformation and antioxidant enzymes. Environ Pollut 157(1):57–63. https://doi.org/10.1016/j.envpol.2008.07.027
40. Van Eenennaam AL, Young AE (2017) Detection of dietary DNA, protein, and glyphosate in meat, milk, and eggs. J Anim Sci 95(7):3247–3269. https://doi.org/10.2527/jas.2016.1346
41. Krüger M, Schledorn P, Schrödl W, Hoppe H-W, Lutz W, Shehata AA (2014) Detection of glyphosate residues in animals and humans. J Environ Anal Toxicol. https://doi.org/10.4172/2161-0525.1000210
42. Shehata AA, Schrödl W, Schledorn P, Krüger M (2014) Distribution of glyphosate in chicken organs and its reduction by humic acid supplementation. J Poult Sci 51(3):333–337. https://doi.org/10.2141/jpsa.0130169
43. Krüger M, Schrödl W, Pedersen I, Shehata AA (2014) Detection of glyphosate in malformed piglets. J Environ Anal Toxicol 04(05):4–5. https://doi.org/10.4172/2161-0525.1000230
44. Fu H, Qiu S, Yao X, Gao F, Tan P, Teng T, Shi B (2020) Toxicity of glyphosate in feed for weanling piglets and the mechanism of glyphosate detoxification by the liver nuclear receptor CAR/PXR pathway. J Hazard Mater 387:121707. https://doi.org/10.1016/j.jhazmat.2019.121707
45. Xu J, Li G, Wang Z, Si L, He S, Cai J, Huang J, Donovan MD (2016) The role of L-type amino acid transporters in the uptake of glyphosate across mammalian epithelial tissues. Chemosphere 145:487–494. https://doi.org/10.1016/j.chemosphere.2015.11.062
46. Mesnage R, Benbrook CM, Antoniou MN (2019) Insight into the confusion over surfactant co-formulants in glyphosate-based herbicides. Food Chem Toxicol 128:137–145. https://doi.org/10.1016/j.fct.2019.03.053
47. Hess FD, Foy CL (2000) Interaction of surfactants with plant cuticles 1. Weed Technol 14(4):807–813. https://doi.org/10.1614/0890-037x(2000)014[0807:ioswpc]2.0.co;2
48. Cox C, Surgan M (2006) Unidentified inert ingredients in pesticides: implications for human and environmental health. Environ Health Perspect 114(12):1803–1806. https://doi.org/10.1289/ehp.9374
49. Defarge N, de Vendômois JS, Séralini GE (2018) Toxicity of formulants and heavy metals in glyphosate-based herbicides and other pesticides. Toxicol Rep 5:156–163. https://doi.org/10.1016/j.toxrep.2017.12.025
50. Folmar LC, Sanders HO, Julin AM (1979) Toxicity of the herbicide glyphosphate and several of its formulations to fish and aquatic invertebrates. Arch Environ Contam Toxicol 8(3):269–278. https://doi.org/10.1007/BF01056243
51. Mesnage R, Defarge N, Spiroux de Vendômois J, Séralini GE (2015) Potential toxic effects of glyphosate and its commercial formulations below regulatory limits. Food Chem Toxicol 84:133–153. https://doi.org/10.1016/j.fct.2015.08.012
52. Geissen V, Silva V, Lwanga EH, Beriot N, Oostindie K, Bin Z, Pyne E, Busink S, Zomer P, Mol H, Ritsema CJ (2021) Cocktails of pesticide residues in conventional and organic farming systems in Europe–Legacy of the past and turning point for the future. Environ Pollut 278:116827. https://doi.org/10.1016/j.envpol.2021.116827
53. Grandcoin A, Piel S, Baurès E (2017) AminoMethylPhosphonic acid (AMPA) in natural waters: Its sources, behavior and environmental fate. Water Res 117:187–197. https://doi.org/10.1016/j.watres.2017.03.055
54. Sidoli P, Baran N, Angulo-Jaramillo R (2016) Glyphosate and AMPA adsorption in soils: laboratory experiments and pedotransfer rules. Environ Sci Pollut Res Int 23(6):5733–5742. https://doi.org/10.1007/s11356-015-5796-5
55. Battaglin WA, Meyer MT, Kuivila KM, Dietze JE (2014) Glyphosate and its degradation product AMPA occur frequently and widely in U.S. soils, surface water, groundwater, and precipitation. J Am Water Resour Assoc 50(2):275–290. https://doi.org/10.1111/jawr.12159
56. Giesy JP, Dobson S, Solomon KR (2000) Ecotoxicological risk assessment for roundup® herbicide. In: Ware GW (ed) Reviews of environmental contamination and toxicology, 167th edn. Springer, New York, pp 35–120

57. Grunewald K, Schmidt W, Unger C, Hanschmann G (2001) Behavior of glyphosate and aminomethylphosphonic acid (AMPA) in soils and water of reservoir Radeburg II catchment (Saxony/Germany). J Plant Nutr Soil Sci 164(1):65–70. https://doi.org/10.1002/1522-2624(200102)164:1%3c65::AID-JPLN65%3e3.0.CO;2-G
58. Bento CPM, Yang X, Gort G, Xue S, van Dam R, Zomer P, Mol HGJ, Ritsema CJ, Geissen V (2016) Persistence of glyphosate and aminomethylphosphonic acid in loess soil under different combinations of temperature, soil moisture and light/darkness. Sci Total Environ 572:301–311. https://doi.org/10.1016/j.scitotenv.2016.07.215
59. Imfeld G, Lefrancq M, Maillard E, Payraudeau S (2013) Transport and attenuation of dissolved glyphosate and AMPA in a stormwater wetland. Chemosphere 90(4):1333–1339. https://doi.org/10.1016/j.chemosphere.2012.04.054
60. Tresnakova N, Stara A, Velisek J (2021) Effects of glyphosate and its metabolite AMPA on aquatic organisms. Appl Sci. https://doi.org/10.3390/app11199004
61. Reddy KN, Rimando AM, Duke SO, Nandula VK (2008) Aminomethylphosphonic acid accumulation in plant species treated with glyphosate. J Agric Food Chem 56(6):2125–2130. https://doi.org/10.1021/jf072954f
62. Fréville M, Estienne A, Ramé C, Lefort G, Chahnamian M, Staub C, Venturi E, Lemarchand J, Maximin E, Hondelatte A, Zemb O, Canlet C, Guabiraba R, Froment P, Dupont J (2022) Chronic dietary exposure to a glyphosate-based herbicide results in total or partial reversibility of plasma oxidative stress, cecal microbiota abundance and short-chain fatty acid composition in broiler hens. Front Physiol 13:974688. https://doi.org/10.3389/fphys.2022.974688
63. Fantón N, Cazenave J, Michlig MP, Repetti MR, Rossi A (2021) Biomarkers of exposure and effect in the armoured catfish *Hoplosternum littorale* during a rice production cycle. Environ Pollut 287:117356. https://doi.org/10.1016/j.envpol.2021.117356
64. Piccolo JJ, Unfer G, Lobón-Cerviá J (2018) Why conserve native brown trout? In: Lobón-Cerviá J, Sanz N (eds) Brown trout: life history, ecology and management, 1st edn. Hoboken, John Wiley & Sons Ltd, pp 643–647
65. Townsend CR (2003) Individual, population, community, and ecosystem consequences of a fish invader in New Zealand streams. Conserv Biol 17(1):38–47. https://doi.org/10.1046/j.1523-1739.2003.02017.x
66. Umweltbundesamt und Bund/Länder-Arbeitsgemeinschaft Wasser (LAWA) (2016) Rahmenkonzeption der Bund/Länder-Arbeitsgemeinschaft Wasser (LAWA)—Arbeitspapier VII: Anlage 2.1: Stoffsammlung
67. Umweltbundesamt (2020) Regulatorisch akzeptable Konzentration für ausgewählte Pflanzenschutzmittelwirkstoffe (UBA-RAK-Liste)
68. European Commission (2022) Directive of the European Parliament and of the Council amending Directive 2000/60/EC establishing a framework for Community action in the field of water policy, Directive 2006/118/EC on the protection of groundwater against pollution and deterioration and Directive 2008/105/EC on environmental quality standards in the field of water policy. COM/2022/540-Annex 1
69. European Union (2006) Council Directive 2006/88/EC on animal health requirements for aquaculture animals products thereof, and on the prevention and control of certain diseases in aquaculture. Off J Eur Union. pp 14–56
70. European Commission (2018) Analytical quality control and method validation procedures for pesticide residues analysis in food and feed-SANTE 11813/2017
71. Williams GM, Kroes R, Munro IC (2000) Safety evaluation and risk assessment of the herbicide Roundup and its active ingredient, glyphosate, for humans. Regul Toxicol Pharmacol 31(2 Pt 1):117–165. https://doi.org/10.1006/rtph.1999.1371
72. Arnot JA, Gobas FA (2006) A review of bioconcentration factor (BCF) and bioaccumulation factor (BAF) assessments for organic chemicals in aquatic organisms. Environ Rev 14(4):257–297. https://doi.org/10.1139/A06-005
73. Wang Y-S, Jaw C-G, Chen Y-L (1994) Accumulation of 2,4-d and glyphosate in fish and water hyacinth. Water Air Soil Pollut 74:397–403
74. Ma Y, Kiesewetter DO, Lang L, Gu D, Chen X (2010) Applications of LC-MS in PET radioligand development and metabolic elucidation. Curr Drug Metab 11(6):483–493. https://doi.org/10.2174/138920010791636167
75. Mehta K (2017) Impact of temperature on contaminants toxicity in fish fauna: a review. Indian J Sci Technol 10(18):1–6. https://doi.org/10.17485/ijst/2017/v10i18/112663
76. Serra-Compte A, Maulvault AL, Camacho C, Álvarez-Muñoz D, Barceló D, Rodríguez-Mozaz S, Marques A (2018) Effects of water warming and acidification on bioconcentration, metabolization and depuration of pharmaceuticals and endocrine disrupting compounds in marine mussels (*Mytilus galloprovincialis*). Environ Pollut 236:824–834. https://doi.org/10.1016/j.envpol.2018.02.018
77. Wanders N, van Vliet MTH, Wada Y, Bierkens MFP, van Beek LPH (2019) High-resolution global water temperature modeling. Water Resour Res 55(4):2760–2778. https://doi.org/10.1029/2018WR023250
78. Dinh KV, Janssens L, Stoks R (2016) Exposure to a heat wave under food limitation makes an agricultural insecticide lethal: a mechanistic laboratory experiment. Glob Chang Biol 22(10):3361–3372. https://doi.org/10.1111/gcb.13415
79. Heugens EHW, Tokkie LTB, Kraak MHS, Hendriks AJ, Van Straalen NM, Admiraal W (2006) Population growth of *Daphnia magna* under multiple stress conditions: joint effects of temperature, food, and cadmium. Environ Toxicol Chem 25(5):1399–1407. https://doi.org/10.1897/05-294r.1
80. Vilas-Boas JA, Arenas-Sánchez A, Vighi M, Romo S, Van den Brink PJ, Pedroso Dias RJ, Rico A (2021) Multiple stressors in Mediterranean coastal wetland ecosystems: Influence of salinity and an insecticide on zooplankton communities under different temperature conditions. Chemosphere 269:129381. https://doi.org/10.1016/j.chemosphere.2020.129381
81. Chowdhury A, Rahman MS (2023) Molecular and biochemical biomarkers in the American oyster *Crassostrea virginica* exposed to herbicide Roundup® at high temperature. Environ Sci Pollut Res Int 30(41):94757–94778. https://doi.org/10.1007/s11356-023-28862-6
82. Parlapiano I, Biandolino F, Grattagliano A, Ruscito A, Libralato G, Prato E (2021) Effects of commercial formulations of glyphosate on marine crustaceans and implications for risk assessment under temperature changes. Ecotoxicol Environ Saf 213:112068. https://doi.org/10.1016/j.ecoenv.2021.112068
83. Baier F, Jedinger M, Gruber E, Zaller JG (2016) Temperature-dependence of glyphosate-based herbicide's effects on egg and tadpole growth of common toads. Front Environ Sci 4:1–10. https://doi.org/10.3389/fenvs.2016.00051
84. Mohammed A (2013) Why are early life stages of aquatic organisms more sensitive to toxicants than adults? In: Gowder SJT (ed) New insights into toxicity and drug testing. InTech, London, p 254
85. Hietanen E, Linnainmaa K, Vainio H (1983) Effects of phenoxyherbicides and glyphosate on the hepatic and intestinal biotransformation activities in the rat. Acta Pharmacol Toxicol 53(2):103–112. https://doi.org/10.1111/j.1600-0773.1983.tb01876.x
86. Danielson PB (2002) The cytochrome P450 superfamily: biochemistry, evolution and drug metabolism in humans. Curr Drug Metab 3(6):561–597. https://doi.org/10.2174/1389200023337054
87. Lamb DC, Kelly DE, Hanley SZ, Mehmood Z, Kelly SL (1998) Glyphosate is an inhibitor of plant cytochrome P450: functional expression of *Thlaspi arvensae* cytochrome P45071B1/reductase fusion protein in *Escherichia coli*. Biochem Biophys Res Commun 244(1):110–114. https://doi.org/10.1006/bbrc.1997.7988
88. Samsel A, Seneff S (2013) Glyphosate's suppression of cytochrome P450 enzymes and amino acid biosynthesis by the gut microbiome: pathways to modern diseases. Entropy 15(4):1416–1463. https://doi.org/10.3390/e15041416
89. Mazari AMA, Zhang L, Ye Z-W, Zhang J, Tew KD, Townsend DM (2023) The multifaceted role of glutathione S-transferases in health and disease. Biomolecules 13(4):1–17. https://doi.org/10.3390/biom13040688
90. de Menezes CC, da Fonseca MB, Loro VL, Santi A, Cattaneo R, Clasen B, Pretto A, Morsch VM (2011) Roundup effects on oxidative stress parameters and recovery pattern of *Rhamdia quelen*. Arch Environ Contam Toxicol 60(4):665–671. https://doi.org/10.1007/s00244-010-9574-6
91. Lushchak OV, Kubrak OI, Storey JM, Storey KB, Lushchak VI (2009) Low toxic herbicide roundup induces mild oxidative stress in goldfish tissues. Chemosphere 76(7):932–937. https://doi.org/10.1016/j.chemosphere.2009.04.045

92. Samanta P, Pal S, Mukherjee AK, Ghosh AR (2014) Biochemical effects of glyphosate based herbicide, Excel Mera 71 on enzyme activities of acetylcholinesterase (AChE), lipid peroxidation (LPO), catalase (CAT), glutathione-S-transferase (GST) and protein content on teleostean fishes. Ecotoxicol Environ Saf 107:120–125. https://doi.org/10.1016/j.ecoenv.2014.05.025
93. Sribanjam S, Charoenwattanasak S, Champasri T, Champasri C, Yuangsoi B (2018) Toxic effects of the herbicide glyphosate on enzymes activities and histopathological changes in gill and liver tissue of freshwater fish, Silver barb (*Barbonymus gonionotus*). Biosci Res 16(Oct):2272–2281
94. do Langiano VC, Martinez CBR (2008) Toxicity and effects of a glyphosate-based herbicide on the Neotropical fish *Prochilodus lineatus*. Comp Biochem Physiol Toxicol Pharmacol 147(2):222–231. https://doi.org/10.1016/j.cbpc.2007.09.009
95. Hedberg D, Wallin M (2010) Effects of Roundup and glyphosate formulations on intracellular transport, microtubules and actin filaments in *Xenopus laevis* melanophores. Toxicol Vitr 24(3):795–802. https://doi.org/10.1016/j.tiv.2009.12.020
96. Riechers DE, Wax LM, Liebl RA, Bush DR (1994) Surfactant-increased glyphosate uptake into plasma membrane vesicles isolated from common lambsquarters leaves. Plant Physiol 105(4):1419–1425. https://doi.org/10.1104/pp.105.4.1419
97. Brewster DW, Warren J, Hopkins WE (1991) Metabolism of glyphosate in Sprague-Dawley rats: tissue distribution, identification, and quantitation of glyphosate-derived materials following a single oral dose. Fundam Appl Toxicol 17(1):43–51. https://doi.org/10.1016/0272-0590(91)90237-x
98. Cakmak I, Yazici A, Tutus Y, Ozturk L (2009) Glyphosate reduced seed and leaf concentrations of calcium, manganese, magnesium, and iron in non-glyphosate resistant soybean. Eur J Agron 31(3):114–119. https://doi.org/10.1016/j.eja.2009.07.001
99. Mertens M, Höss S, Neumann G, Afzal J, Reichenbecher W (2018) Glyphosate, a chelating agent-relevant for ecological risk assessment? Environ Sci Pollut Res Int 25(6):5298–5317. https://doi.org/10.1007/s11356-017-1080-1
100. Hamdaoui L, Oudadesse H, Lefeuvre B, Mahmoud A, Naifer M, Badraoui R, Ayadi F, Rebai T (2020) Sub-chronic exposure to Kalach 360 SL, Glyphosate-based Herbicide, induced bone rarefaction in female Wistar rats. Toxicology 436:152412. https://doi.org/10.1016/j.tox.2020.152412
101. Gill JPK, Sethi N, Mohan A, Datta S, Girdhar M (2018) Glyphosate toxicity for animals. Environ Chem Lett 16(2):401–426. https://doi.org/10.1007/s10311-017-0689-0
102. Franz JE, Mao MK, Sikorski JA (1997) Glyphosate: a unique global herbicide. American Chemical Society, Washington
103. European Food Safety Authority (EFSA) (2018) Review of the existing maximum residue levels for glyphosate according to Article 12 of Regulation (EC) No 396/2005. EFSA J Eur Food Saf Auth 16(5):e05263. https://doi.org/10.2903/j.efsa.2018.5263
104. EFSA, Carrasco Cabera L, Di Piazza G, Dujardin B, Pastor PM (2023) The 2021 European Union report on pesticide residues in food
105. Bai SH, Ogbourne SM (2016) Glyphosate: environmental contamination, toxicity and potential risks to human health via food contamination. Environ Sci Pollut Res Int 23(19):18988–19001. https://doi.org/10.1007/s11356-016-7425-3
106. OECD/Food and Agriculture Organization of the United Nation (2019) "Fish." In: OECD-FAO Agricultural Outlook 2020–2029. Paris/Food and Rome/Agriculture Organization of the United Nations, pp 184–195

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