GLYPHOSATE IS POLLUTING OUR WATERS - ALL ACROSS EUROPE. PAN EUROPE'S WATER REPORT, SEPTEMBER 2023





Acknowledgements: This report was commissioned using the financial support of The Greens/EFA group in the European Parliament and was written by Pesticide Action Network (PAN) Europe.

Lead author (coordination and research): Gergely Simon

Editor and reviewer: Dr. Angeliki Lysimachou (PAN Europe)

Assistant: Lysiane Copin (PAN Europe)

Pesticide Action Network Europe, 2023. Rue de la Pacification 67, 1000 Brussels, Belgium tel.: +32 2 318 62 55; info@pan-europe.info; www.pan-europe.info

The Greens/EFA group in the European Parliament. 60 Rue Wiertz/Wiertzstraat 60, 1047 Brussels, Belgium; <u>contactgreens@ep.europa.eu</u>; <u>www.greens-efa.eu</u>

TABLE OF CONTENTS

EXECUTIVE SUMMARY	4
1. INTRODUCTION	6
The impact of glyphosate: widespread use, contamination, and concerns for aquatic ecosystems	6
The EU regulatory framework	8
Presence and concentrations of glyphosate in aquatic environments	10
Glyphosate in aquatic environments	10
Maximum concentrations in aquatic environments	12
Potential risks to aquatic ecosystems	13
Aim of the study	13
2. METHODOLOGY	14
Sampling and analysis	14
Description of water bodies, location of samples	14
3. RESULTS	19
4. DISCUSSION: IMPACT OF GLYPHOSATE AND AMPA ON AQUATIC ECOSYSTEMS	21
Water unsuitable for human consumption	21
Impacts on aquatic environments	22
Policy relevance	24
CONCLUSIONS	28
Policy recommendations	28

EXECUTIVE SUMMARY

The current levels of glyphosate use in Europe have resulted in widespread contamination of the environment, with the herbicide being detected in human urine, house dust, soils, and surface waters. Scientific evidence indicates that the concentrations of pesticides, including glyphosate, found in the environment negatively impact the quality of our water resources and put aquatic ecosystems at risk. According to EU pesticide legislation, the use of pesticides should not have any adverse impacts on human and animal health or the environment, in that the approval process for pesticides and their "sustainable use" should cause no harm, including to biodiversity and ecosystems. This study challenges the validity of this assumption by demonstrating it to be wrong.

In order to raise awareness about the extensive pollution resulting from the current utilisation of glyphosate-based herbicides in Europe, the Pesticide Action Network Europe, along with its members and the Stop-Glyphosate Coalition, conducted a water sampling exercise across 12 EU countries in October 2022, in the period immediately following the agricultural season. Organisations from these 12 countries sampled 23 flowing freshwater (rivers/streams) and five lake samples.

The samples were analysed for glyphosate and for its metabolite AMPA, and the limit of quantification was set at 0.2 μ g/L (LOQ).

Glyphosate and/or AMPA were detected in 17 out of 23 samples (74%), in 11 out of the 12 countries. Considering that the <u>drinking water safety limit for pesticide active substances</u> <u>and their relevant metabolites is 0.1 μ g/L, five out of 23 water samples (22%), collected in Austria, Spain, Poland, Portugal, contained glyphosate at levels not suitable for human consumption. A Portuguese sample contained 3 μ g/L glyphosate, which is 30 times higher than the safety limit for human consumption. In Austria, Belgium, Poland, Spain and Portugal, samples showed concentrations of glyphosate or AMPA above 1 μ g/L. Alarmingly, three of the samples contained AMPA levels exceeding 3 μ g/L.</u>

Both glyphosate and AMPA <u>pose risks</u> to the aquatic environment, and glyphosate is already <u>classified</u> as being toxic to aquatic life with long-lasting effects (Aquatic Chronic 2; H411). However, based on the data from the scientific literature, a stricter classification would be justified. Currently, there is no environmental quality standard (EQS) for glyphosate or AMPA at the EU level. In its recent <u>proposal</u>, the European Commission revised the list of priority substances for surface water and included an extremely high EQS value for glyphosate which would allow a higher level of contamination compared to the drinking water safety standards. In the same proposal, a threshold limit of 0.5 μ g/L (AA-EQS - Annual Average of Environmental Quality Standard) is included for the combined concentration of pesticide active substances or relevant metabolites, degradation and reaction products. Yet, out of the 23 samples analysed, AMPA was found at levels equal to or exceeding the 0.5 μ g/L threshold in 10 samples and glyphosate in one sample. At the time of writing, it is not yet defined by the

Commission and EU member states whether metabolites such as AMPA, which pose a risk to the aquatic environment, would be included in this threshold limit.

These results underline that exposure to glyphosate is unavoidable emphasising the urgent need for EU-wide measures to eliminate this hazardous substance from our environment.

1. INTRODUCTION

The impact of glyphosate: widespread use, contamination, and concerns for aquatic ecosystems

Since it was first introduced in the US market as Roundup[™] in 1974, the active ingredient glyphosate (N-(phosphonomethyl)-glycine) and its formulations have become the most commonly and intensively used herbicides in the EU and globally (Benbrook, 2016). As a non-selective, broad spectrum, systemic herbicide, it kills all plants and has been used extensively in agriculture both on conventional crops and those genetically modified to be glyphosate-resistant [Box 1]. In 2012, Europe represented around 16.6% of the global glyphosate market, and in 2017, glyphosate represented 33% of the total herbicide market in the EU (Antier et al., 2020).

Box 1. Main uses of glyphosate

In conventional chemical agriculture, glyphosate-based herbicides are applied before the crops are sown, to kill weeds and facilitate crop establishment. They are also used in chemical no-till farming to clear the land of weeds and previous crops, as a replacement for tillage/cultivation. In glyphosate-tolerant crops (genetically modified to be tolerant to glyphosate), the herbicide is used post crop emergence to kill the weeds but leave the crop unharmed. Glyphosate-based herbicides are also used to clear the ground beneath perennial crops such as fruit trees and grape vines. Another use of glyphosatebased herbicides is as a crop desiccant on cereals and grains, to facilitate harvest. It is applied close to harvest to accelerate the ripening process and dry the seeds while the crop plant dies. Post-harvest, glyphosate is used in conventional cropping systems to kill the remains of the crop plants and any weeds present. The use of glyphosate as a pre-harvest desiccant has become a common practice, particularly in regions where humidity levels are higher. However, since this practice results in the highest accumulation of glyphosate residues in seeds and grains, certain Member States have implemented strict rules regarding its usage.

Glyphosate exerts its herbicidal action by blocking the synthesis of certain essential plant nutrients (amino acids) via the shikimate enzymatic pathway; devoid of nutrients, the plant gradually dies. Since this pathway is present only in plants and certain microorganisms such as bacteria and certain kinds of parasites and fungi, there is a misconception that glyphosate is relatively safe for other species. Nevertheless, glyphosate-based herbicides have been shown to cause toxic effects on animal species such as invertebrates, fishes, amphibians, reptiles, birds, and mammals including humans (Gill et al., 2017).

Once it reaches the environment, glyphosate gradually breaks down to its main metabolite Aminomethylphosphonic acid (AMPA), as well as other degradation compounds. AMPA, according to the European Food Safety Authority (EFSA), presents a similar toxicological profile to glyphosate and therefore exposure to both glyphosate and/or AMPA is an issue of concern.

The widespread use of glyphosate has resulted in its ubiquitous presence as a contaminant in the environment, including in aquatic ecosystems, a phenomenon that has been observed globally. Therefore, significant concerns have arisen about the impact of glyphosate on both the quality of our water resources and the inevitable exposure of the different aquatic species to glyphosate. Both glyphosate and its main metabolite AMPA have been identified as <u>posing risks</u> to aquatic environments. Moreover, glyphosate has been <u>classified</u> as being toxic to aquatic life, with long-lasting effects (Aquatic Chronic 2; H411).

Box 2. Aquatic toxicity CLP hazard classification of glyphosate

Glyphosate has been classified as being toxic to aquatic life with long-lasting effects (Aquatic Chronic 2; H411) according to the EU's Regulation 1272/2008 on classification, labelling and packaging of substances and mixture (CLP Regulation).

According to the CLP regulation, a substance can be classified as having two levels of chronic toxicity in aquatic environments:

- The highest toxicity level is Category Chronic 1 in this case the highest concentration of the substance where no effect on the sample organisms (fish / crustaceans / algae or other aquatic plants) is observed is ≤ 0.1mg/L.
- The next highest toxicity level is Category Chronic 2 in this case the highest concentration of the substance tested shows no effects on fish / crustacea / algae or other aquatic plants is between 0.1 mg/L and 1 mg/L.

The classification is based mainly on laboratory experiments in three different trophic levels (mainly on algae, invertebrates, and fish). Typical endpoints are mortality, growth, and survival as well as fertility and fecundity, all of which are considered relevant to the population survival of the species.

For glyphosate, ECHA states in its opinion "The lowest reliable aquatic chronic value was a 7d No-Observed-Effect-Concentration of 1 mg /L for zebrafish Danio rerio. With Glyphosate being not rapidly degradable, an Aquatic Chronic 2; H411 classification was considered...."

However, this hazard classification is based mainly on experiments carried out by agro-chemical companies according to international protocols (e.g. OECD) that do not measure all potential adverse effects, nor examine all levels of exposure seen in real-life environments.

Scientific evidence (Fiorino et al., 2018, Uren Webster & Santos, 2015) shows that glyphosate can be toxic to organisms in aquatic environments at lower concentrations, so aquatic chronic category 1 classification would be justified. Other species than the model species used for the hazard classification of aquatic toxicity (fish, aquatic

invertebrates and algae), such as tadpoles of amphibians, are similarly sensitive to glyphosate and glyphosate products (Bach et al., 2016, Babalola & Wyk, 2017, Navarro-Martín et al., 2014).

Furthermore, according to Regulation 1107/2009, the assessment report should include the evaluation of not only the active substance but also the representative formulation. However, this requirement is often not met in practice. In natural aquatic environments, species are exposed to all the ingredients present in formulated products. The toxicity levels of these products, especially towards amphibians, can often be higher than glyphosate alone. However, ecotoxicological studies of glyphosate-based products are not adequately taken into account in the CLP classification.

The EU regulatory framework

The protection of water resources from pesticides in Europe is governed by different pieces of EU legislation.

Regulation (EC) 1107/2009 (EU pesticides law) acknowledges that the use of pesticides can cause harm to humans, other animals and the environment and has set strict rules for their authorisation to ensure a high level of protection. Under these rules, the active ingredients of pesticides (active substances) and pesticide product formulations can only be approved if it is demonstrated that their use does not adversely affect human or animal health or the environment. Particular attention is given to the protection of the vulnerable groups of the population, such as pregnant women and children, as well as to biodiversity and ecosystems. The assessment must consider the potential toxicity of all the pesticide product ingredients and metabolites, the whole product formulation as well as the resulting residues on food, drinking water and the environment [Box 3].

The protection of the water resources is overseen by the **EU Water Framework Directive 2000/60/EC (WFD)**, and its daughter directives, [drinking water (EU) 2020/2184, groundwater 2006/118/EC and Environmental Quality Standards (EQS) 2008/105/EC]. The overall aim of these directives is to prevent and reduce pollution of the aquatic environment and ensure that EU waters are in good chemical and ecological status.

The **Drinking Water Directive (EU) 2020/2184** and the **Groundwater Directive 2006/118/EC**, which is under revision, set strict criteria to prevent water pollution from dangerous chemicals, including pesticides. For drinking water or groundwater to be considered of good quality, the pesticide thresholds have been set at $0.1 \mu g/L$ for individual pesticide active substances and their relevant metabolites¹, and at $0.5 \mu g/L$ for the sum of all individual pesticides ("pesticides total"). Therefore, according to the directives, if glyphosate is detected in water above $0.1 \mu g/L$, the water cannot be considered adequate for

¹ According to the Drinking Water Directive (EU) 2020/2184, "a pesticide metabolite shall be deemed relevant for water intended for human consumption if there is reason to consider that it has intrinsic properties comparable to those of the parent substance in terms of its pesticide target activity or that either itself or its transformation products generate a health risk for consumers." (Annex I, Part B).

human consumption. AMPA is not considered a relevant metabolite for drinking water and groundwater at EU level. However, AMPA is <u>more persistent in the environment</u> and EFSA (2015) considers that the metabolite shows a <u>toxicological profile similar to glyphosate</u>. Some countries like <u>Denmark</u>, <u>Hungary</u> and <u>France</u> apply the 0.1 μ g/L limit for AMPA in drinking water.

According to the Water Framework Directive, surface waters are considered of good water status if they comply with the **Environmental Quality Standards (EQS)** set in the Directive 2008/105/EC for several 'priority' pollutants that are considered hazardous. A number of these pollutants are pesticides, many of which have been banned from use in the EU because of their highly hazardous properties. At the time of writing, there is no established EQS for glyphosate or its primary metabolite, AMPA, at the EU level. However, the European Commission recently adopted a <u>proposal</u> for a revised list of priority substances, which includes glyphosate. To our concern, the proposed EQS for glyphosate is alarmingly high. While the annual average (AA) for freshwater used for the preparation of drinking water is set at 0.1 μ g/L, for other inland waters it is set at 86.7 μ g/L, a level which is close to 800 times higher than the safety limit for human consumption and which has been reported to cause adverse effects on aquatic organisms.

The proposal also includes a threshold limit of 0.5 μ g/L (AA-EQS) for the total of pesticide active substances or relevant metabolites, degradation and reaction products. It is not decided yet whether metabolites such as AMPA, which pose risks to the aquatic environment, would be included in this limit.

The EU pesticide law (Reg.1107/2009) specifically calls to protect drinking and groundwater from exposure to pesticide active substances, relevant metabolites and whole products, and requests compliance with the WFD and its daughter directives. In this respect, if this compliance is compromised, the Commission and Member States may review and withdraw the authorisation of a pesticide active substance or product (Article 21 and 44, Reg.1107/2009).

Moreover, the **Sustainable use of pesticides directive 2009/128/EC**, takes into consideration that the aquatic environment is particularly sensitive to pesticides and requests from Member States to set measures to prevent pollution of surface water and groundwater (Article 11). These measures consist of establishing appropriately-sized 'buffer zones' for the protection of non-target aquatic organisms and 'safeguard zones' for surface and groundwater used for the abstraction of drinking water, where pesticides must not be used or stored, as well as mitigation measures to prevent contamination. Nevertheless, the overall enforcement of the directive to reduce pesticide use and protect vulnerable ecosystems and populations has been weak and the Commission has proposed an upgrade of the directive to a regulation (more binding legally). The Sustainable use of pesticides regulation (SUR) proposal aims to prohibit the use of pesticides in all surface waters and within 3 metres of such waters. However, the 3 metres buffer zone is considered too low to ensure protection of the aquatic environment.

Box 3. **Regulation (EC) 1107/2009 (**EU pesticides law) on the protection of the environment:

Article 4.3 of the Regulation clearly states that an active substance can only be approved if the following criteria are met:

(e) it shall have no unacceptable effects on the environment, having particular regard to the following considerations where the scientific methods accepted by the Authority to assess such effects are available:

(i) its fate and distribution in the environment, particularly contamination of surface waters, including estuarine and coastal waters, groundwater, air and soil, taking into account locations distant from its use following long-range environmental transportation;

(ii) its impact on non-target species, including on the ongoing behaviour of those species;

(iii) its impact on biodiversity and the ecosystem

Article 4.2 on residues:

(b) they shall not have any unacceptable effect on the environment. For residues which are of toxicological, ecotoxicological, environmental or drinking water relevance, there shall be methods in general use for measuring them. Analytical standards shall be made available to the public.

Presence and concentrations of glyphosate in aquatic environments

Glyphosate in aquatic environments

Due to the extensive use of glyphosate-based products and the high water solubility (11.6 g/L at 25°C) and mobility of the herbicide, glyphosate has become an ubiquitous contaminant in the aquatic environment. Glyphosate reaches the aquatic environment mainly through atmospheric drift following its application and through runoff during rainfall. Although its persistence in water is relatively low, when it is combined with soil particles it can remain in water systems for longer periods. According to EFSA (2015), the half-life of glyphosate in aquatic environments varies between 13.82 to 301 days depending on environmental conditions. The frequency and the magnitude of detected residue levels are highly dependent on e.g., application rates, hydrological conditions, and rainfall intensity (Coupe et al., 2012). Since glyphosate products have various pre- and post-harvest uses, environmental exposure takes place at different times of the year, and may result in continuous exposure of ecosystems.

There is a high variability in detected and reported glyphosate residue levels across European surface waters (Székács & Darvas, 2018). The main metabolite of glyphosate is AMPA, which has a higher mobility compared to the parent compound (Duke & Powles, 2008) and slower degradation rates, and so of the two compounds, is the one most frequently detected in the environment.

Concentration of Glyphosate and AMPA in surface waters

Several independent and national monitoring studies have investigated surface water concentrations of glyphosate around the world. The table below summarises the results from European and global surface water studies.

Country	Region	Substance	Mean [µg/L]	Maximum [µg/L]	Study & year
		AMPA	1.464	30.922	
Delaisan	Wallonia Region	Glyphosate	0.347	5.256	<u>Frippiat et al., 2018</u>
Belgium	Brussels Capital	AMPA	1.0097	2.754	(data in Annex)
	region	Glyphosate	0.1462	0.35	
	Černičí	AMPA	0.160	1	
	Cernici	Glyphosate	0.103	1	
Czech	Němčice	AMPA	0.336	1	Konečná et al.,
Republic		Glyphosate	0.055	1	<u>2023</u>
		AMPA	0.481	1	
	Uhřice	Glyphosate	0.037	1	
		AMPA	0,45	164	
France		Glyphosate	0,22	558	Ineris, 2020
	Lake Balaton,	AMPA	0.3	2.0	
······	Western Hungary	Glyphosate	0.85	3.0	<u>Tóth et al., 2022</u>
	River Veneto, North-East Italy.	AMPA	0.18	1	
Italy		Glyphosate	0.17	/	<u>Masiol et al., 2018</u>

Table 1. Concentrations of glyphosate recorded in globally.

				1	
	Lobith	AMPA	0.207	0.30	RIWA-Rijn report, 2021
		Glyphosate	<	0.0706	
	Nieuwegein	AMPA	0.475	0.781	
		Glyphosate	<	0.032	
Netherlands		AMPA	0.485	0.811	
	Nieuwersluis	Glyphosate	<	0.039	
		AMPA	0.223	0.316	
	Andijk	Glyphosate	<	<	
_	Pampas region, centre of	АМРА	0.66	1.03	_
Argentina	Buenos Aires province	Glyphosate	1.88	4.36	<u>J. Pérez et al., 2021</u>
	Melbourne	AMPA	0.8	1	
	Rural streams	Glyphosate	≤ 0.3	1	
Australia	Melbourne	AMPA	1.1	4.3	<u> Okada et al., 2020</u>
	Urban streams	Glyphosate	1.6	4.8	
Mexico	Jalisco state	Glyphosate	/	510.46	Silva-Madera et al., 2021
United States		AMPA	/	5.6	
		Glyphosate	/	8.1	<u>Medalie et al., 2020</u>

Maximum concentrations in aquatic environments

The concentrations of glyphosate recorded in Europe are in the range of a few μ g/L [Table 1], with some exceptions. For example, in a study in France the maximum concentration of glyphosate recorded was 558 μ g/L and for AMPA, 164 μ g/L.

There are also some exceptional cases where very high concentrations of glyphosate have been recorded. In a study from 1980, Edwards *et al.* found maximum concentrations reaching up to 5200 μ g/L in waters receiving agricultural runoff. In more recent studies aiming to investigate the impact of glyphosate on foraging bees, the concentration of glyphosate and AMPA in runoff and in puddle waters near agricultural areas was exceptionally high.

<u>Farina et al</u>. (2019) observed that "For the worst case scenario in small water bodies (ponds or puddles), a median expected environmental concentration of 3.49 mg/L was calculated". In another example, <u>Herbert et al</u>. (2014) reported concentrations of glyphosate in puddles and runoff waters in the range of 1.4 to 7.6 mg/L as environmentally relevant concentrations.

Potential risks to aquatic ecosystems

Freshwater ecosystems are rich in minerals and nutrients and provide habitat for many species including aquatic plants, invertebrates, fishes, amphibians, birds, and mammals. The interaction of these species not only forms dynamic food webs but also plays a key role in ecosystem processes such as nutrient cycling, production of organic matter, release or capture of greenhouse gases and filtering pollutants. All these organisms, including humans, depend on the quality of the water.

The structure and function of freshwater ecosystems depends on the equilibrium of the abundances of the different species. Therefore, changes in the structure of the communities of species not only alter the food web structure and population dynamics, but can have negative consequences on all related ecosystem processes.

Glyphosate has already been classified as a substance that can cause long-term harm to aquatic species. Therefore, direct exposure to glyphosate or glyphosate-based products, e.g. while being used/applied, will have a negative impact on aquatic species and can put their populations at risk.

Scientific studies indicate that lower levels of glyphosate can also cause harm to nontarget species, particularly during the vulnerable early life stages and when the exposure is prolonged. Furthermore, it is important to consider that glyphosate-based products can be more toxic than glyphosate alone, as they contain glyphosate together with co-formulants or adjuvants that are added to increase the absorption of glyphosate by the target plants and augment the overall efficacy of the product as an herbicide.

Aim of the study

The widespread use of glyphosate-based products has resulted in the contamination of EU waters with this chemical, lowering the quality of our water resources and putting aquatic ecosystems at risk. In anticipation of the 2023 vote on the reauthorisation of glyphosate, the Pesticide Action Network Europe, together with its members and those from the Stop-Glyphosate Coalition, conducted a water sampling campaign across the EU. Based on the results, this report aims to provide information on the widespread exposure of the aquatic environment to glyphosate and AMPA, and alert decision-makers to take the actions necessary to protect our aquatic environment and its vulnerable ecosystems from exposure to this chemical.

2. METHODOLOGY

Sampling and analysis

PAN Europe and its partners collected surface water samples across 12 EU countries in late October 2022, after the agricultural season. The latest use of glyphosate-based herbicides in early autumn is either for desiccation (pre-harvest) or for post-harvest purposes. Samples were taken by members of PAN Europe and of the Stop-Glyphosate Coalition (list of partners in Annex 1). Overall, 23 river/stream samples and 5 lake water samples were collected. Samples were sent in cooling boxes with ice packs to the Eurofins laboratory in Budapest for analysis to detect glyphosate and AMPA. The limit of quantification (LOQ) was set at 0.2 μ g/L, which is twice the safety threshold under which water is considered of good quality for human consumption (0.1 μ g/L) for individual active ingredients or for relevant metabolites.

Description of water bodies, location of samples

The samples collected and analysed for the purpose of this study originate from rivers and streams located in 12 European countries: Austria, Belgium, Bulgaria, Croatia, France, Germany, Hungary, the Netherlands, Slovenia, Spain, Poland and Portugal.

River/stream water samples				
Country	Water body	Size of river	GPS	
Slovenia	River Savinja	Large	<u>46.242540, 15.137991</u>	
Slovenia		Large	<u>46°14'33.1"N 15°08'16.8"E</u>	
			Sample 1	
	Germany River Erft Med		<u>51.168215, 6.704045</u>	
Cormony		Medium	<u>51°10'05.6"N 6°42'14.6"E</u>	
Germany		Medium	Medium	Sample 2
			<u>51.182481, 6.730497</u>	
			51°10'56.9"N 6°43'49.8"E	
Germany	Piver Lippo	Medium	<u>51.643528, 6.675861</u>	
Germany	River Lippe	Medium	51°38'36.7"N 6°40'33.1"E	
Gormany	River Gera	Medium	<u>50.924639, 10.987667</u>	
Germany	RIVEL DELA	Medium	50°55'28.7"N 10°59'15.6"E	

Table 2. Collection sites of the surface water samples.

	Austria Diver Mühlhash Orașili		48.318102, 16.564445
Austria	River Mühlbach	Small	48°19'05.2"N 16°33'52.0"E
Orestia		1	46.360611, 16.326500
Croatia	River Drava	Large	46°21'38.2"N 16°19'35.4"E
Pulgorio	Maritza River, Varbitsa	Lorgo	42.040889, 25.372222
Bulgaria	Maritza River, Varbitsa	Large	42°02'27.2"N 25°22'20.0"E
Pulgorio	Bivolare/Pleven, Vit	Medium	<u>43.493778, 24.565722</u>
Bulgaria	Divolare/Fleven, vit	Medium	<u>43°29'37.6"N 24°33'56.6"E</u>
Poland	Pilica River, Sulejów	Large	<u>51.354879, 19.882903</u>
		Large	<u>51°21'17.6"N 19°52'58.5"E</u>
Poland	Opocznianka River	Small	<u>51.359204, 20.254507</u>
		Sman	51°21'33.1"N 20°15'16.2"E
Poland	Rykolanka River	Small	<u>51.662500, 20.846110</u>
		Sman	51°39'45.0"N 20°50'46.0"E
Spain	Lleida Aigua Panta	Small	<u>41.497145, 0.513258</u>
Span		Sman	<u>41°29'49.7"N 0°30'47.7"E</u>
Spain	Canal perimetral San	Small	<u>37.843281, -0.767189</u>
Spain	Pedro del Pinata	Smail	<u>37°50'35.8"N 0°46'01.9"W</u>
Spain	Agua superficial Rambla	Small	<u>37.716286, -0.861044</u>
Span	del Albujón	Smail	<u>37°42'58.6"N 0°51'39.8"W</u>
Portugal	Stream, Herdade da Fonte	Small	<u>39.880780, -7.247578</u>
Tortugar	Insonsa Idanha-a-Nova	Sman	<u>39°52'50.8"N 7°14'51.3"W</u>
Portugal	Duoro river	Large	<u>41.072450, -8.464370</u>
T OI CUGAI		Laige	41°04'20.8"N 8°27'51.7"W
Hungary	River Little Danube,	Medium	<u>47.352778, 19.069028</u>
	Dunaharaszti,	Mediain	<u>47°21'10.0"N 19°04'08.5"E</u>
France	La Chapelle aux pots /	Small	<u>49.437407, 1.919286</u>
Tance	l'Avelon	Sillan	<u>49°26'14.7"N 1°55'09.4"E</u>
France	Rochy Condé/ le Therain	Small	<u>49.398526, 2.182851</u>
	Trance Rochy Condey le merain Small		<u>49°23'54.7"N 2°10'58.3"E</u>
France	rance St Leu d'Esserent / l'Oise Large		<u>49.213308, 2.422123</u>
		Large	49°12'47.9"N 2°25'19.6"E
Netherlands	River Veengoot R.	Small	<u>52.086944, 6.364556</u>
inecite latius	Niver veengoot K.		<u>52°05'13.0"N 6°21'52.4"E</u>

Netherlands	River Slinge, Borculo	Small	52.107611. 6.491056 52°06'27.4"N 6°29'27.8"E
Belgium	La Mehaigne	Small	50.628207, 5.083869 50°37'41.6"N 5°05'01.9"E
Lake water s	amples		
A	De de ve de vé		47.855679, 16.825008
Austria	Padersdorf		47°51'20.5"N 16°49'30.0"E
Croatia	Datanajia Čankovao		<u>46.417891, 16.410886</u>
Croatia	Retencija Šenkovec		46°25'04.4"N 16°24'39.2"E
Croatia	Datanajia Mačkovao		<u>46.428528, 16.425000</u>
Croatia	Retencija Mačkovec		46°25'42.7"N 16°25'30.0"E
Portugal	Albufeira da Barragem do		<u>37.932500, -8.080100</u>
Portugal	Roxo, Portugal		<u>37°55'57.0"N 8°04'48.4"W</u>
Hundony			<u>47.259778, 19.095972</u>
Hungary	Délegyháza lake		47°15'35.2"N 19°05'45.5"E

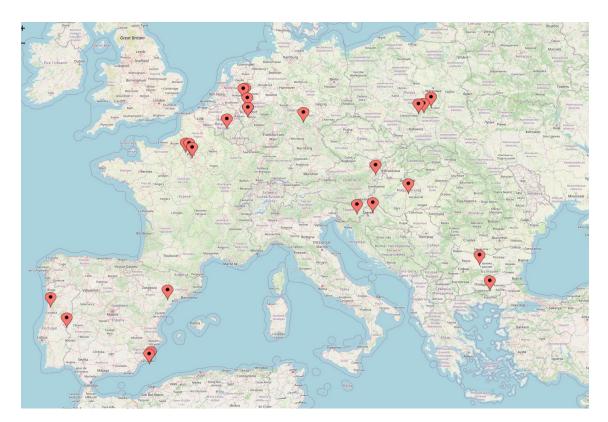
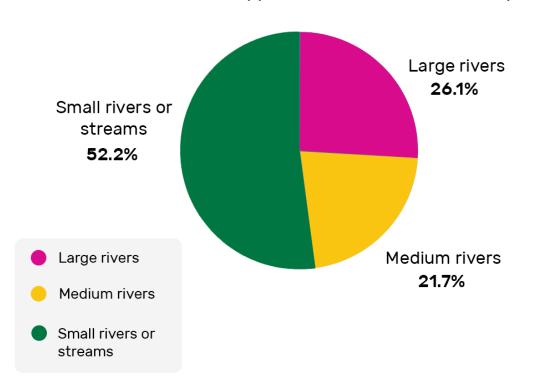


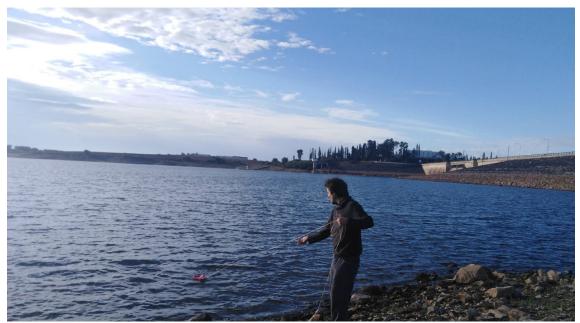
Figure 1. Location of rivers and streams from which samples were collected. Map data ©<u>OpenStreetMap</u>

The samples were collected from large, medium and small rivers and streams. Out of the 23 samples we collected across Europe, 8 samples originate from small rivers and streams. 6 were collected from large rivers (Maritsa in Bulgaria, Oise in France, Savinja in Slovenia, Pilica in Poland, Duoro in Portugal, Drava in Croatia) and 9 originate from medium-size rivers (Vit in Bulgaria, Lippe, Gera & Erft in Germany, Ponsul in Portugal, Ráckevei-Duna in Hungary).



Classification of the types of water bodies sampled

Figure 2. Proportions of water body types and sizes in the samples



Sampling location: Albufeira da Barragem do Roxo, Portugal



Sampling location: Vit river, Bivolare, Bulgaria.

As part of the study, lake water samples were also collected in Austria, Croatia, Hungary and Portugal.

3. RESULTS

Out of the 23 river/stream surface water samples collected, 17 (74%) of them, in 11 out of the 12 countries, had detectable levels of glyphosate or AMPA above the 0.2 μ g/L limit of qualification (LOQ). As the samples were taken after the agricultural season, the glyphosate metabolite AMPA was more frequently detectable in the river samples, rather than glyphosate itself. Hence, AMPA was present in 17 samples, whereas glyphosate was detected in just five of them.

Therefore, AMPA was detected in 74% of cases of the river/stream samples, while glyphosate was detected in 22% of cases. The highest concentration measured was 3.9 μ g/L for AMPA in Poland and 3 μ g/L for glyphosate in Portugal. Of the samples that had detectable residues of AMPA, approximately 22% showed measurements exceeding 1 μ g/L. Five out of 23 water samples (22%), collected in Austria, Spain, Poland, Portugal, contained glyphosate at levels exceeding the 0.1 μ g/L threshold limit for human consumption.

Moreover, the drinking water safety limit for the total amount of pesticides and their relevant metabolites, and the one currently proposed for surface waters (AA-EQS) is 0.5 μ g/L. In this respect AMPA was detected at levels above or equal to 0.5 μ g/L in 10 sites, and glyphosate was detected above this threshold in one site. Hence, taking both glyphosate and AMPA into account, this threshold was breached in 44% (10 out of 23) of river/stream sites sampled in Austria, Belgium, France, Germany, Netherlands, Poland, Portugal and all 3 samples from Spain. However, at the time of writing it has not been clarified whether the EQS limit includes AMPA, at EU level AMPA is considered relevant for aquatic toxicity but not relevant for human consumption. Furthermore, an extremely high EQS value for glyphosate is currently being proposed, which would allow a higher level of glyphosate contamination.

In contrast to other countries, the samples collected in Slovenia did not contain glyphosate or AMPA above the detection limit of $0.2 \mu g/L$. At least one of the tested river water samples from Austria, Belgium, Bulgaria, Croatia, France, Germany, Hungary, the Netherlands, Spain, Poland and Portugal did contain detectable amounts of glyphosate or AMPA. The concentrations of glyphosate and AMPA of our random water samples were comparable, or even higher than, those reported in the monitoring data and scientific literature presented in Table 1. All detailed results can be found in Annex 2.

No residues of glyphosate or AMPA were detected in the 5 lake water samples.

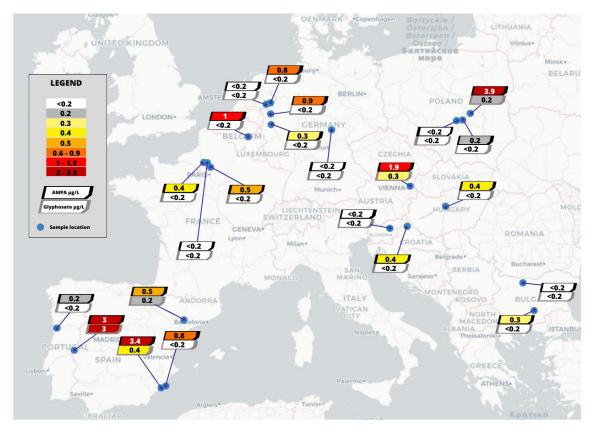


Figure 3. Concentration of AMPA and glyphosate in μ g/L; PAN EUROPE, 2022 October measurements

The highest concentrations recorded:

- Poland, Rykolanka River: AMPA 3.9 µg/L
- Spain, Agua superficial Rambla del Albujón: AMPA 3.4 µg/L
- Portugal, Idanha-a-Nova: AMPA 3.0 µg/L and glyphosate 3.0 µg/L
- Austria, River Mühlbach in Deutsch Wagram: AMPA 1.9 µg/L

4. DISCUSSION: IMPACT OF GLYPHOSATE AND AMPA ON AQUATIC ECOSYSTEMS

EU law aims to ensure, on one hand, that authorised pesticides do not harm the environment and its ecosystems, and, on the other hand, to achieve good surface water quality across the EU, under the WFD. This has led to a false assumption that pesticides that have been approved to be used in agriculture and food do not contaminate the aquatic environment and do not cause any harm to its ecosystems. However, our report shows that glyphosate and its metabolite AMPA reach the surface waters long after the application of glyphosate pesticides, and due to their toxic properties, they adversely impact water quality and put aquatic ecosystems at risk.

The detection of both AMPA and glyphosate in certain European rivers suggests that the real level of the active substance glyphosate was previously even higher than the one measured, since part of glyphosate had been metabolised to AMPA and we are measuring the tail end of the exposure curve. Therefore, we can assume that the aquatic environment was previously exposed to higher levels than the ones measured.

Water unsuitable for human consumption

In our study we set the detection limit for glyphosate and AMPA at 0.2 μ g/L, which is twice above the EU standard for individual pesticides and for relevant metabolites in drinking water. Therefore, the five water samples out of the 23 (22%) with detectable levels of glyphosate at or above 0.2 μ g/L, collected in Austria, Spain, Poland, Portugal, contained glyphosate at levels not suitable for human consumption. The water of all the 17 rivers where the samples tested positive for AMPA would be a concern for human consumption in some Member States, and would breach drinking water safety limits. Moreover, in 10 sites, the concentration of AMPA on its own exceeded the 0.5 μ g/L threshold set for the total amount of pesticides or relevant metabolites in drinking and surface water. Therefore, additional pesticides used in the surrounding fields would be adding to the pollution of glyphosate and AMPA reaching even higher contamination levels. From the scientific literature, we know that higher glyphosate concentrations can be found in puddles and runoff waters in agricultural areas. In our study, samples were taken from running rivers, the water of which is diluted by rainwater, but significant contamination was still found.

Based on the conclusion drawn by EFSA (EFSA, 2015) "AMPA presents a similar toxicological profile to glyphosate and the reference values of the latter apply to its metabolite AMPA", yet AMPA is not currently recognised as a metabolite of toxicological significance in relation to the EU's drinking water standard for human consumption. Therefore, the 0.1 μ g/L limit (as established by the Drinking Water Directive 2020/2184 for individual pesticide active substances and their relevant metabolites) does not apply to AMPA at EU level. However, some Member States, including Denmark, Hungary and France, apply the 0.1 μ g/L threshold

for both AMPA and glyphosate. We are of the opinion that other Member States should adopt a similar precautionary approach by applying a $0.1 \,\mu$ g/L safety limit for each of these compounds.

In Austria, Belgium, Poland, Spain and Portugal, AMPA concentrations were even above 1 μ g/L. The highest glyphosate concentration in a sample from Portugal was 30 times above drinking water safety limit.

Contamination of surface waters with pesticides has been a great challenge for water companies who need access to adequate and reliable water resources to guarantee that EU citizens have access to safe drinking water. Water companies must increasingly resort to expensive and energy-intensive additional treatments to reach drinking water safety standards, while the consumers paying the water bills bear the costs. As a result, <u>European Water companies</u> have requested strict rules for the protection of EU freshwater ecosystems from pesticides. In our study, there was no indication whether the rivers from where the samples were taken were used to extract drinking water or not.

Impacts on aquatic environments

The observation that glyphosate or/and AMPA was detected above 0.2 μ g/L in 74% of the river sites outside the agricultural season indicates prolonged exposure of the aquatic environment to glyphosate. This extended exposure puts aquatic ecosystems at risk. In 22% of the sites, concentrations exceeded 1 μ g/L, which has been shown to cause adverse effects in certain aquatic organisms. The concentrations we found after the agricultural season are still comparable, and often even higher than what is found in monitoring studies from scientific literature [Table 1].

Academic scientific literature confirms that glyphosate and glyphosate-based herbicides can affect biochemical, physiological, endocrine, and behavioural pathways in fish , invertebrates, and amphibians (<u>Gonçalves et al., 2019</u>). Glyphosate is also toxic to plants and algae which are primary producers and provide food and shelter for other species in the higher trophic levels of the ecosystem. Therefore, chronic exposure of the aquatic environment to glyphosate can gradually alter the structure of the whole ecosystem.

To our concern, some of the effects of glyphosate can be observed at concentrations below the ones corresponding to the Category 2 chronic aquatic toxicity hazard class (< 0.1 mg/L), which are comparable to the levels detected in the environment and the ones found in our study [Box 2]. Moreover, glyphosate's degradation product AMPA has also been found to be toxic in various aquatic organisms, and in certain cases where both substances are present, they may result in more pronounced toxic effects than the individual substances acting on their own.

For example, low concentrations of glyphosate (50 μ g/L) may significantly alter the growth of aquatic plants and algae, whereas inhibition of chlorophyll synthesis has been observed at 16 μ g/L, and effects were more prominent when AMPA was also present. In fact, AMPA has

been shown to inhibit chlorophyll synthesis at between 6 and 1.6 μ g/L, depending on the species.

Fish, particularly fish embryos, are sensitive to low levels of glyphosate exposure, as delays in growth and development have been observed at concentrations in the range of 5-10 μ g/L. Changes in cell membrane permeability and expression of genes regulating key developmental processes in embryo and juvenile fish have been found at exposures of 1-10 μ g/L. On the other hand, amphibians appear to be particularly sensitive to glyphosate-based herbicides during their early development, possibly due to their surfactants as well as AMPA (<u>Annett et al., 2014</u>). Effects on development of tadpoles have been observed at concentrations as low as 0.7 μ g/L (expressed as glyphosate); exposures of as low as 0.07 to 3.57 μ g/L of AMPA decrease embryonic survival. Similarly, aquatic invertebrates are sensitive to glyphosate-based products. Interestingly, the combination of glyphosate and AMPA together, at the very low concentration of 0.1 and 1 μ g/L respectively, disrupt the cytoprotective and detoxification mechanisms of the Mediterranean mussel, indicating the combined toxic effect of these two substances acting together [Box 3].

Delays in the growth of embryos or embryonic malformations lengthens the time that they are vulnerable to aquatic predators and may increase mortality rate impacting the populations of species. Amphibians for example, have been victims of some of the sharpest population crashes in recent decades, indicating that pesticide exposure may be among the factors leading to their decline.

Therefore the levels of glyphosate and its metabolite AMPA detected in our study indicate that this pesticide is putting the aquatic ecosystem at risk, particularly because of its adverse effects on growth and development during the early life stages and during reproduction of non-target organisms.

Although the harmful effects caused by glyphosate-based herbicides (GBHs) to aquatic organisms are relatively well documented in the scientific literature, there are many gaps regarding the toxicity of AMPA. Nevertheless, the current state of research suggests that AMPA may also cause adverse effects to aquatic organisms and therefore its impact should be taken into account.

Moreover, the active substance glyphosate is not the only problem, as GBHs are composed of multiple, often unknown, constituents, each with a unique level of toxicity. Co-formulants such as surfactants that increase herbicidal efficacy also increase the toxicity of the products for non-target species, particularly amphibians (Annett et al., 2014).

The impact of glyphosate on the different trophic levels should not be considered in isolation from one another, as changes in one trophic level will inevitably affect the others, leading to alterations in the function and structure of the ecosystem. Moreover, this impact should be estimated together with the presence of other pesticides and pollutants (rather than considering each in isolation, which does not reflect real life situations). There are also other anthropogenic activities that should be taken into account as they might be putting the aquatic environment under stress, e.g. habitat destruction, climate change, urbanisation and other kinds of pollution.

Policy relevance

Currently there is no environmental quality standard (EQS) for glyphosate or AMPA on the EU level. In the recent <u>proposal of the European Commission</u> for a revised list of priority substances for surface water, there is a proposal for AA-EQS (average value - inland surface waters) for glyphosate, but not AMPA, and for two different values:

- 0.1 µg/l for freshwater used for the abstraction and preparation of drinking water.
- 86.7 µg/l for freshwater not used for the abstraction and preparation of drinking water.

In the same proposal, the Commission has introduced a threshold limit of 0.5 μ g/L (AA-EQS²) for the total of all active substances in pesticides, including their relevant metabolites, degradation and reaction products in surface waters. In 10 of the PAN testing sites (44% of river/stream sites), AMPA was detected at levels above or equivalent to 0.5 μ g/L and glyphosate was detected above or equal to 0.5 μ g/L in 1 site. At the time of writing, it is not yet defined by the Commission and EU member states whether metabolites such as AMPA, which pose a risk to the aquatic environment, would be included in this threshold limit. This 0.5 μ g/L value is much lower and clearly contradicts the 86.7 μ g/L threshold for glyphosate. In fact, it does not make sense for the EQS of an individual pesticide and its metabolites (glyphosate/AMPA) to exceed the threshold value established for the total EQS of all detected pesticides and their metabolites (AA-EQS).

Additionally, such a large distinction in the water quality values between surface waters that are intended for drinking water and those that are not is difficult to understand: the high standards are needed for both use scenarios – humans and other non-target organisms will still be exposed regularly during their normal activities to "non-drinking standard" water, so a differentiated approach does not make sense. In the case of glyphosate, setting such a high safety threshold for water not intended for human consumption diminishes the impact of adding glyphosate in the priority substances list in the first place. The EQS for glyphosate and AMPA should be set at $0.1 \,\mu$ g/L in all surface waters based on the harm these pesticides may cause.

² Annual Average of Environmental Quality Standard, as proposed for Total active substances

Box 4. Ecotoxicological studies

Below we summarise scientific studies showing harmful effects to aquatic ecosystems at low GBH or glyphosate exposure levels as well as AMPA.

Aquatic plants, algae and other microorganisms

Because of its properties, glyphosate can alter the growth of plants and algae, and the structure of certain microorganism communities (phytoplankton, microbes). These are considered primary producers and provide food and shelter for other species at different trophic levels of the ecosystem. Therefore, chronic exposure of the aquatic environment to glyphosate can gradually alter the structure of the whole ecosystem.

Alterations in the growth of macrophyte *Vallisneria natans* and phytoplankton *Chlorella vulgaris*, have been observed at concentrations as low as 50 μ g/L of glyphosate. At that level of exposure, glyphosate, particularly when combined with AMPA, inhibited the growth of aquatic plants but induced the growth of phytoplankton indicating that glyphosate may alter the structure of freshwater ecosystems to an algal-based one (Qu et al., 2022). Glyphosate and AMPA have been reported to decrease the rate of photosynthesis in the aquatic macrophyte *Salvinia molesta* at 16 and 6 μ g/L, respectively, whereas in combination their impact increased (Mendes et al., 2021). In another study, negative effects on the photosynthesis of the macrophyte *Lemna minor*, through chlorophyll biosynthesis inhibition, were observed at concentrations as low as 1.3 μ g/L of AMPA (Gomes et al., 2022).

A study that tested the impact of five glyphosate-based formulations on aquatic microbial communities found differential effects on microphytoplankton and picoplankton community structures, suggesting that co-formulants have an important role in the toxicity of these glyphosate products for primary producers (Garcia et al., 2022).

Aquatic vertebrates

Fish species

Scientific literature shows that glyphosate exposure may lead to adverse effects in fish below the established level of exposure of 0.1 mg/L (or 100ug/L) and at concentrations comparable to the ones found in the environment.

Exposure of carp fish *Cyprinus carpio* embryos to different concentrations of glyphosate (0.005-50 mg/L) during their development resulted in higher malformation disorders and late development in all exposure groups, including at the lower concentration of 5 μ g/L. Malformations on zebrafish embryos of *Danio rerio* were also apparent but above 0.05 mg/L of glyphosate exposure although reduced hatching rates and mortality were observed at the lowest level of exposure 5 μ g/L by Fiorino et al. (2018). Delays in the growth of embryos or malformations lengthens the time that they are vulnerable to aquatic predators and may increase mortality rate.

In a different experiment, zebrafish of *Danio rerio* embryos exposed to glyphosatebased herbicide Roundup or glyphosate alone (0.01, 0.065 and 0.5 mg/L) altered their swimming behaviour, resulting in reduced distance travelled and altered swimming patterns even at 10 μ g/L exposure level. However, in zebrafish adults, changes in swimming behaviour were observed at 0.5 mg/L of glyphosate and above 0.065mg/L of roundup, indicating that larvae are much more sensitive to glyphosate exposure than adults (<u>Bridi et al., 2017</u>).

Similarly, in a different study zebrafish *Danio rerio* embryos were exposed to 1, 10, 100, and 700 μ g/L of glyphosate or AMPA for 72 hours. A series of developmental abnormalities were observed, including embryo hatching inhibition, spinal curvature, abnormal blood circulation, and abnormal heart shape and function. These changes were significant at concentrations of 10 μ g/L, although at 1 μ g/L both glyphosate and AMPA reduce the activity sodium–potassium pump, which controls ion exchange in cells, whereas AMPA upregulated a key gene related to heart development (Zhang et al., 2021).

Even in cases where glyphosate exposure is not high enough to cause significant mortality in laboratory experiments, it can still lead to biological effects through alterations in gene expression of components of the antioxidant defence system, which can gradually lead to disease. In this respect, exposure of juvenile female brown trout to the glyphosate-based herbicide Roundup or glyphosate alone (0.01 - 10 mg/L) for 14 days, resulted in alternations in the expression of genes that encode for components of the antioxidant system, a number of stress-response proteins and molecules of proapoptotic signalling, among others, across all exposure levels (Uren Webster & Santos, 2015). The results were shown to be consistent with a cellular response to oxidative stress, as it has been previously reported, even at lower exposure levels of 10 µg/L.

<u>Le Du-Carrée et al. (2022)</u> found that exposure to glyphosate or two glyphosate-based products at environmental concentrations of 1 µg/L reduced the level of an important protein involved in the immune response (interleukin-1 β). The researchers observed that glyphosate co-formulants can modulate fish viral susceptibility, meaning that the presence of other chemicals in the GBH can affect how susceptible the fish are to viral infections.

Amphibians

Studies on amphibians show that they are more sensitive to glyphosate-based pesticides than glyphosate alone, particularly during early developmental stages possibly due to the action of surfactants. For example, significant effects on the development of tadpoles *Leptodactylus latrans* (at Gosner stage 25) were observed following exposure to $0.7 \mu g/L$ (expressed as glyphosate acid equivalent) of Roundup ULTRA MAX® whereas such a significant effect was only apparent with pure glyphosate following exposure to 15 mg/L (Bach et al., 2016).

AMPA has been observed to affect embryonic development in the European common toad. <u>Cheron et al. (2020)</u> found that exposure to concentrations spanning the range found in natural water bodies (0.07 to 3.57 μ g/L) decreased embryonic survival, increased developmental time, and influenced hatchling morphology.

Aquatic invertebrates

Study by <u>Cuhra et al. (2012)</u> suggests that even environmental concentrations of glyphosate can have an impact on the growth and development of juvenile *Daphnia Magna*. The findings show that exposure of juvenile *D. Magna* to low concentrations of 50 µg/L glyphosate or Roundup resulted in a significant reduction in their size.

<u>Ferreira-Junior et al. (2017)</u> observed that the glyphosate-based herbicide Roundup had deleterious effects on the growth and development of tropical aquatic diptera, *Chironomus xanthus* (*C. xanthus*) at environmentally relevant concentrations of 700 μ g/L. Furthermore, exposure to glyphosate caused females to emerge later (at 1.53 mg/L) and males to emerge earlier (at 0.49 mg/L) than in the control group, suggesting that glyphosate exposure can affect the reproductive development of *C. xanthus* in sex-dependent manner.

In their investigation on the impact of glyphosate and AMPA on cytoprotective/ detoxification mechanisms expressed in haemocytes of the Mediterranean mussel (*Mytilus galloprovincialis*) <u>Wathsala et al. (2022</u>) showed that exposure to a mixture of glyphosate and AMPA at 0.1 μ g/Land 1 μ g/L respectively was enough to decrease cellular defence mechanisms.

Overall, these concentration values of glyphosate and AMPA that are reported in scientific literature to cause adverse effects in aquatic species, are comparable to PAN's test results for AMPA samples (range 0.2–3.9 ug/L).

CONCLUSIONS

The majority of our water samples collected from EU rivers and streams contained glyphosate or its main metabolite AMPA after the agricultural season. This observation emphasises the widespread contamination of the aquatic environment resulting from glyphosate use, even outside the agricultural season. Much higher glyphosate and AMPA concentrations can be found around agricultural areas. Scientific literature shows that the concentrations we found already pose risk to different aquatic species, and continuous exposure to glyphosatebased herbicides not only lowers the water quality but also endangers our ecosystems and their functioning. Glyphosate-based herbicide products as such (active substances acting together with co-formulants) can be more toxic to aquatic life than the active substance alone.

We can conclude that the current extent of glyphosate-based herbicide use translates to an unacceptable risk to the aquatic environment, and it should be discontinued. Accordingly, and in light of the recent European Commission proposal on a revised list of priority substances for surface water, strict EQSs below 0.1 μ g/L environmental quality standards are needed for both glyphosate and AMPA in all European surface waters. It is now time for all regulations to be ambitious. It goes without saying that the issue of glyphosate and AMPA's water contamination has to be addressed at the stage of approval of the active substance; however, strict and ambitious EQSs effectively protecting aquatic biodiversity and human health must also be enforced.

Policy recommendations

In light of the findings from the report regarding the widespread contamination of our waters with glyphosate, and recognising the importance of protecting our European waters and their ecosystems, we recommend the following:

- Adopting the Commission's proposal on the Sustainable Use of Plant Protection Products Regulation to set legally binding targets to halve the use and risk of chemical pesticides by 2023, and ban the use of all chemical pesticides in sensitive areas used by the general public and of ecological importance. Pesticides should not be used at a distance of 50m from these areas, to ensure their protection.
- Setting the EQS for glyphosate and AMPA in surface waters at 0.1 μ g/L, to ensure protection of human health and biodiversity in the aquatic ecosystems.
- Include both AMPA and glyphosate in national monitoring programs, as AMPA is also toxic to aquatic organisms.
- Include all scientific literature studies in the assessment of glyphosate's toxicity and take into consideration that glyphosate-based products are much more toxic for certain species than glyphosate alone.

- Increase the chronic aquatic toxicity classification from Category 2 to Category 1 since glyphosate can cause adverse effects to aquatic organisms below 0.1 mg/L.
- The European Commission and Member States should issue a non-renewal of glyphosate's licence and phase out the use of glyphosate-based products, as their use lowers the quality of EU waters, and exposure to glyphosate has been linked to adverse effects in a wide range of species including humans.

References

Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail (ANSES). (2017). Synthèse des données de surveillance Appui scientifique et technique n°2017-04. https://www.anses.fr/fr/system/files/Fiche_PPV_Glyphosate.pdf

Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail (ANSES). (2023, March 15). *Pesticides in tap water*. <u>https://www.anses.fr/en/content/pesticides-tap-water</u>

Annett, R., Habibi, H. R., & Hontela, A. (2014). Impact of glyphosate and glyphosate-based herbicides on the freshwater environment. *Journal of Applied Toxicology*, *34*(5), 458–479. <u>https://doi.org/10.1002/jat.2997</u>

Antier, C., Kudsk, P., Reboud, X., Ulber, L., Baret, P. V., & Messéan, A. (2020). Glyphosate use in the European agricultural sector and a framework for its further monitoring. *Sustainability*, *12*(14), 5682. <u>https://doi.org/10.3390/su12145682</u>

Association of River water companies (RIWA-Rijn). (2021, October 11). *Annual Report 2020 The Rhine*. <u>https://www.riwa-rijn.org/wp-content/uploads/2021/10/RIWA-2021-EN-Anual-Report-2020-The-Rhine.pdf</u>

Babalola, O. O., & Van Wyk, J. H. (2017). Comparative early life stage toxicity of the African clawed frog, *Xenopus laevis* following exposure to selected herbicide formulations applied to eradicate alien plants in South Africa. *Archives of Environmental Contamination and Toxicology*, 75(1), 8–16. <u>https://doi.org/10.1007/s00244-017-0463-0</u>

Bach, N. C., Natale, G. S., Somoza, G. M., & Ronco, A. E. (2016). Effect on the growth and development and induction of abnormalities by a glyphosate commercial formulation and its active ingredient during two developmental stages of the South-American creole frog, *Leptodactylus latrans*. *Environmental Science and Pollution Research*, *23*(23), 23959–23971. https://doi.org/10.1007/s11356-016-7631-z

Bastos Gonçalves, B., Cardoso Giaquinto, P., dos Santos Silva, D., de Melo e Silva Neto, C., Alves de Lima, A., Antonio Brito Darosci, A., & Lopes Rocha, T. (2019). Ecotoxicology of Glyphosate-Based Herbicides in the Aquatic Environment. IntechOpen. DOI : <u>10.5772/intechopen.85157</u>

Benbrook, C. M. (2016). Trends in glyphosate herbicide use in the United States and globally. *Environmental Sciences Europe*, *28*(1). <u>https://doi.org/10.1186/s12302-016-0070-0</u>

Bonansea, R., Filippi, I., Wunderlin, D., Marino, D., & Amé, M. (2017). The fate of glyphosate and ampa in a freshwater endorheic basin: An ecotoxicological risk assessment. *Toxics, 6(1), 3.* <u>https://doi.org/10.3390/toxics6010003</u>

Bridi, D., Altenhofen, S., Gonzalez, J. B., Reolon, G. K., & Bonan, C. D. (2017). Glyphosate and Roundup® Alter Morphology and behaviour in zebrafish. *Toxicology*, *392*, 32–39. <u>https://doi.org/10.1016/j.tox.2017.10.007</u>

Carles, L., Gardon, H., Joseph, L., Sanchís, J., Farré, M., & Artigas, J. (2019). Meta-analysis of glyphosate contamination in surface waters and dissipation by biofilms. *Environment International*, 124, 284–293. <u>https://doi.org/10.1016/j.envint.2018.12.064</u>

Cheron, M., & Brischoux, F. (2020). Aminomethylphosphonic acid alters amphibian embryonic development at environmental concentrations. *Environmental Research*, *190*, 109944. <u>https://doi.org/10.1016/j.envres.2020.109944</u>

Coupe, R.H., Kalkhoff, S.J., Capel, P.D., & Gregoire, C. (2012) Fate and transport of glyphosate and aminomethylphosphonic acid in surface waters of agricultural basins, Pest Manag. Sci. 68:16–30.

Cuhra, M., Traavik, T., & Bøhn, T. (2012). Clone- and age-dependent toxicity of a glyphosate commercial formulation and its active ingredient in daphnia magna. *Ecotoxicology*, *22*(2), 251–262. <u>https://doi.org/10.1007/s10646-012-1021-1</u>

de Brito Rodrigues, L., Gonçalves Costa, G., Lundgren Thá, E., da Silva, L. R., de Oliveira, R., Morais Leme, D., Cestari, M. M., Koppe Grisolia, C., Campos Valadares, M., & de Oliveira, G. A. (2019). Impact of the glyphosate-based commercial herbicide, its components and its metabolite AMPA on non-target aquatic organisms. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*, 842, 94–101. https://doi.org/10.1016/j.mrgentox.2019.05.002

Directive (EU) 2020/2184. On the quality of water intended for human consumption. European Parliament and Council. <u>https://eur-lex.europa.eu/eli/dir/2020/2184/oj</u>

Duke, S.O. & Powles, S.B. (2008) Glyphosate: a once-in-a-century herbicide, Pest Manag. Sci. 64:319–325.

Edwards, W.M., Triplett, G.B., & Kramer, R.M. (1980) A watershed study of glyphosate transport in runoff, J. Environ. Qual. 9:661–665.

EurEau. (2022). Position Paper on the draft Sustainable Use of Plant Protection ProductsRegulation.https://www.eureau.org/documents/drinking-water/position-papers/6796-position-paper-on-the-sustainable-use-of-pesticides-regulation/file

European Chemical Agency. (2021, March 8). *Registry of CLH intentions until outcome, Glyphosate.* <u>https://echa.europa.eu/en/registry-of-clh-intentions-until-outcome/-/dislist/details/0b0236e185e41a77</u>

European Chemical Agency. *CLP Legislation*. <u>https://echa.europa.eu/en/regulations/clp/</u> legislation

European Commission. (2022). *Proposal for a 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 final. <u>https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A52022PC0540</u>

European Food Safety Authority. (2015). Conclusion on the peer review of the pesticide risk assessment of the active substance glyphosate. *EFSA Journal*, *13*(11). <u>https://doi.org/10.2903/j.efsa.2015.4302</u>

Farina, W. M., Balbuena, M. S., Herbert, L. T., Mengoni Goñalons, C., & Vázquez, D. E. (2019). Effects of the herbicide glyphosate on honey bee sensory and cognitive abilities: Individual impairments with implications for the hive. *Insects*, *10*(10), 354. <u>https://doi.org/10.3390/insects10100354</u>

Ferreira-Junior, D. F., Sarmento, R. A., Saraiva, A. de, Pereira, R. R., Picanço, M. C., Pestana, J. L., & Soares, A. M. V. M. (2017). Low concentrations of glyphosate-based herbicide affects the development of *Chironomus xanthus*. *Water, Air, & Soil Pollution, 228*(10). <u>https://doi.org/10.1007/s11270-017-3536-9</u>

Fiorino, E., Sehonova, P., Plhalova, L., Blahova, J., Syobodova, Z., & Faggio, C. (2018) Effects of glyphosate on early life stages: comparison between *Cyprinus carpio* and *Danio rerio*, Environ. Sci. Pollut. Res. 25:8542–8549.

Frippiat, C., Bémelmans, S., Burlion, N., Carbonnelle, P., Chalon, C., Delvaux, A., Galloy, A., Marneffe, Y., Nadin, C., Nix, Ph., Nott, K., Pigeon, O., Ronkart, S., Rousseau, G., Delloye, F. & Brahy, V. (2018). Recherche de perturbateurs endocriniens et d'autres substances d'intérêt récent dans les eaux en vue de la protection de la santé publique et de l'environnement. Programme de Recherche « BIODIEN » - Rapport final. GISREAUX, rapport n° 2018-01690, 199 p. + 11 annexes. <u>http://eau.wallonie.be/IMG/pdf/2018-01690_GISREAUX_BIODIEN_</u> <u>Final_%20Rapport.pdf</u>

Gill, J. P., Sethi, N., Mohan, A., Datta, S., & Girdhar, M. (2017). *Glyphosate toxicity for animals. Environmental Chemistry Letters, 16(2)*, 401–426. <u>https://doi.org/10.1007/s10311-017-0689-</u> <u>0</u>

Gomes, M. P., Freitas, P. L., Kitamura, R. S., Pereira, E. G., & Juneau, P. (2022). How aminomethylphosphonic acid (AMPA), the main glyphosate metabolite, interferes with chlorophyll biosynthesis? *Environmental and Experimental Botany*, *203*, 105039. <u>https://doi.org/10.1016/j.envexpbot.2022.105039</u>

Herbert, L. H., Vazquez, D. E., Arenas, A., & Farina, W. M. (2014). Effects of field-realistic doses of glyphosate on honeybee appetitive behaviour. *Journal of Experimental Biology*. <u>https://doi.org/10.1242/jeb.109520</u>

Hungarian government decree on drinking water «5/2023. (l. 12.) Korm. rendelet az ivóvíz minőségi követelményeiről és az ellenőrzés rendjéről" – https://net.jogtar.hu/jogszabaly?docid=a2300005.kor

List of substances subject to the government decree from National Public Health Centre: https://www.nnk.gov.hu/attachments/article/985/E%C3%9C.%20 K%C3%B6zl%C3%B6ny_2022.%205.%20sz%C3%A1m_%20peszticid%20lista_2022.%20 %C3%A9s%202023.pdf Institut national de l'environnement industriel et des risques. (2020, March 27). *GLYPHOSATE ET SES PRINCIPAUX COMPOSES*, Verneuil-en-Halatte : Ineris - 181229 - v2.0. <u>https://substances.ineris.fr/fr/substance/getDocument/3043</u>

Konečná, J., Zajíček, A., Sáňka, M., Halešová, T., Kaplická, M., & Nováková, E. (2023). Pesticides in small agricultural catchments in the Czech Republic. *Journal of Ecological Engineering*, *24*(3), 99–112. <u>https://doi.org/10.12911/22998993/157471</u>

Laabs, V., Leake, C., Botham, P., & Melching-Kollmuß, S. (2015). Regulation of non-relevant metabolites of plant protection products in drinking and groundwater in the EU: Current status and way forward. *Regulatory Toxicology and Pharmacology*, 73(1), 276–286. <u>https://doi.org/10.1016/j.yrtph.2015.06.023</u>

Le Du-Carrée, J., Cabon, J., Louboutin, L., Morin, T., & Danion, M. (2022). Changes in defense capacity to infectious hematopoietic necrosis virus (IHNV) in Rainbow Trout intergenerationally exposed to glyphosate. *Fish & Shellfish Immunology*, *122*, 67–70. <u>https://doi.org/10.1016/j.fsi.2021.12.021</u>

Lopes, A. R., Moraes, J. S., Martinez, C., Martins, G. (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. doi: 10.1016/j.aquatox.2022.106281..

Masiol, M., Giannì, B., & Prete, M. (2018). Herbicides in river water across northeastern Italy: Occurrence and spatial patterns of glyphosate, aminomethylphosphonic acid, and glufosinate ammonium. *Environmental Science and Pollution Research*, *25*(24), 24368–24378. <u>https://doi.org/10.1007/s11356-018-2511-3</u>

Medalie, L., Baker, N. T., Shoda, M. E., Stone, W. W., Meyer, M. T., Stets, E. G., & Wilson, M. (2020). Influence of land use and region on glyphosate and aminomethylphosphonic acid in streams in the USA. *Science of The Total Environment*, 707, 136008. <u>https://doi.org/10.1016/j.scitotenv.2019.136008</u>

Mendes, E. J., Malage, L., Rocha, D. C., Kitamura, R. S., Gomes, S. M., Navarro-Silva, M. A., & Gomes, M. P. (2021). Isolated and combined effects of glyphosate and its by-product aminomethylphosphonic acid on the physiology and water remediation capacity of Salvinia Molesta. Journal of Hazardous Materials, 417, 125694. <u>https://doi.org/10.1016/j.jhazmat.2021.125694</u>

Navarro-Martín, L., Lanctôt, C., Jackman, P., Park, B. J., Doe, K., Pauli, B. D., & Trudeau, V. L. (2014). Effects of glyphosate-based herbicides on survival, development, growth and sex ratios of wood frogs (*Lithobates sylvaticus*) tadpoles. I: Chronic laboratory exposures to VisionMax®. *Aquatic Toxicology*, *154*. <u>https://doi.org/10.1016/j.aquatox.2014.05.017</u>.

Okada, E., Allinson, M., Barral, M. P., Clarke, B., & Allinson, G. (2020). Glyphosate and aminomethylphosphonic acid (AMPA) are commonly found in urban streams and wetlands of Melbourne, Australia. *Water Research*, *168*, 115139. <u>https://doi.org/10.1016/j.watres.2019.115139</u>

Pérez, D. J., Iturburu, F. G., Calderon, G., Oyesqui, L. A. E., De Gerónimo, E., & Aparicio, V. C. (2021). Ecological risk assessment of current-use pesticides and biocides in soils, sediments and surface water of a mixed land-use basin of the Pampas Region, Argentina. *Chemosphere*, *263*, 128061. <u>https://doi.org/10.1016/j.chemosphere.2020.128061</u>

Qu, M., Wang, L., Xu, Q., An, J., Mei, Y., & Liu, G. (2022). Influence of glyphosate and its metabolite aminomethylphosphonic acid on aquatic plants in different ecological niches. Ecotoxicology and Environmental Safety, 246, 114155. <u>https://doi.org/10.1016/j.ecoenv.2022.114155 Qu et al., 2022</u>

Regulation (EU) 1107/2009. *Concerning the placing of plant protection products on the market*. European Parliament and Council. <u>https://eur-lex.europa.eu/eli/dir/2020/2184/oj</u>

Sabio y García, C. A., Vera, M. S., Vinocur, A., Graziano, M., Miranda, C., & Pizarro, H. N. (2022). Rethinking the term "glyphosate effect" through the evaluation of different glyphosatebased herbicide effects over aquatic microbial communities. *Environmental Pollution, 292*, 118382. <u>https://doi.org/10.1016/j.envpol.2021.118382</u>

Silva-Madera, R. J., Salazar-Flores, J., Peregrina-Lucano, A. A., Mendoza-Michel, J., Ceja-Gálvez, H. R., Rojas-Bravo, D., Reyna-Villela, M. Z., & Torres-Sánchez, E. D. (2021). Pesticide contamination in drinking and surface water in the Cienega, Jalisco, Mexico. *Water, Air, & Soil Pollution*, *232*(2). https://doi.org/10.1007/s11270-021-04990-y

Soukup, S. T., Merz, B., Bub, A., Hoffmann, I., Watzl, B., Steinberg, P., & Kulling, S. E. (2020). Glyphosate and AMPA levels in human urine samples and their correlation with food consumption: Results of the cross-sectional Karmen study in Germany. *Archives of Toxicology*, *94*(5), 1575–1584. <u>https://doi.org/10.1007/s00204-020-02704-7</u>

Székács, A. & Darvas, B. (2018) Re-registration challenges of glyphosate in the European Union, Front. Environ. Sci. 6:78. <u>https://www.frontiersin.org/articles/10.3389/fenvs.2018.00078/full</u>

Tóth, G., Háhn, J., Szoboszlay, S., Harkai, P., Farkas, M., Radó, J., Göbölös, B., Kaszab, E., Szabó, I., Urbányi, B., & Kriszt, B. (2022). Spatiotemporal analysis of multi-pesticide residues in the largest Central European shallow lake, Lake Balaton, and its sub-catchment area. *Environmental Sciences Europe*, *34*(1). https://doi.org/10.1186/s12302-022-00630-2

Uren Webster, T. M., & Santos, E. M. (2015). Global transcriptomic profiling demonstrates induction of oxidative stress and of compensatory cellular stress responses in brown trout exposed to glyphosate and Roundup. *BMC Genomics*, *16*(1). <u>https://doi.org/10.1186/s12864-015-1254-5</u>

Wathsala, R. H., Folgueras, E. C., luffrida, L., Candela, M., Gotti, R., Fiori, J., & Franzellitti, S. (2022). Glyphosate and its breakdown product AMPA elicit cytoprotective responses in haemocytes of the Mediterranean mussel (*Mytilus galloprovincialis*). *Environmental Toxicology and Pharmacology*, *96*, 103997. <u>https://doi.org/10.1016/j.etap.2022.103997</u>

Zhang, W., Wang, J., Song, J., Feng, Y., Zhang, S., Wang, N., Liu, S., Song, Z., Lian, K., & Kang, W. (2021). Effects of low-concentration glyphosate and aminomethyl phosphonic acid on zebrafish embryo development. *Ecotoxicology and Environmental Safety*, *226*, 112854. https://doi.org/10.1016/j.ecoenv.2021.112854

Annex 1. Partners in the water sampling

- 1. Austria: Global2000 (Robert Schwarzwald)
- 2. Belgium: Nature & Progrès
- 3. Bulgaria: AGROLINK
- 4. Croatia: Biovrt
- 5. Germany: PAN Europe
- 6. France: Générations Futures
- 7. Hungary: PAN Europe
- 8. Netherlands: PAN Europe
- 9. Poland: European Regional Centre for Ecohydrology of the Polish Academy of Sciences (Paweł Jarosiewicz)
- 10. Portugal: Zero. & Plataforma Transgénicos Fora (Graça Passos)
- 11. Slovenia: National Council of Slovenia
- 12. Spain: Ecologistas en Acción (Koldo Hernández)

Sample country / code	Location	Results µg/L
Poland ERCE Lódz River 1 Sulejov	Pilica River, Sulejów – 17.10.2022 51.354879, 19.882903 51°21'17.6"N 19°52'58.5"E	AMPA <0,2 Glyphosate <0,2
Poland ERCE Lódz River 2 Opoczno	Opocznianka River – 17.10.2022 51.359204, 20.254507 51°21'33.1"N 20°15'16.2"E	AMPA 0,2 Glyphosate <0,2
Poland ERCE Lódz River 3 Rykolanka	Rykolanka River – 18.10.2022 51.662500, 20.846110 51°39'45.0"N 20°50'46.0"E	AMPA 3,9 Glyphosate 0,2

Annex 2. Glyphosate and AMPA monitoring results, PAN, 2022 October

River, Ráckevei-D Hungary, HU2	una, Danube, <u>Dunaharaszti</u> ,
47°21'10.0"N 19°04	4'08.5"E Glyphosate <0,2 (0,03) ³
Portugal	r , GPS: 41.072450, AMPA 0,2
-8.464370	Glyphosate <0,2
Portugal Idanha-a-Nova -	Herdade da Fonte Insonsa) AMPA 3
39.880780, -7.247	Glyphosate 3
	n Deutsch Wagram AMPA 1.9
(48.3181020, 16.5	
GER1- 12.10.2022. going into Rhine,	was here, river Erft river
Germany <u>https://maps.app</u>	
GER1-GER2 GER2- Erft, below	Chuphagata (0.)
https://maps.app	.goo.gl/VB5Miog9VnoJqx576
(GER 1 and GER 2	- same river, mixed)
Germany River Gera just be	fore it enters river Unstruht AMPA <0,2
GER 3 50°55'28.7"N 10°5	Glyphosate <0,2
Germany river (Lippe) going 19.10.2022	y into Rhine (west germany) AMPA 0,9
GER 5 51°38'36.7"N 6°40	'33.1"E Glyphosate <0,2
https://goo.gl/ma	ps/sSHMaLAQDNdEh4hE9
Dimitrovgrad Mar	itza surface water AMPA 0.3
Bulgaria 42.04090 25.3722	21
https://goo.gl/ma	Glyphosate <0,2 ps/fgW7QeT1YN6CAFb79
Bivolare/Pleven V	it surface water AMPA <0,2
Bulgaria 43,493775 24,565	768
https://goo.gl/ma	ps/VCgPEEbF7LfNBU7g7 Glyphosate <0,2
Location 3 – DRA	VA
46°21'38.2"N 16°1' Croatia	9'35.4"E AMPA 0.4
46.360619, 16.326	Glyphosate <0,2

³ Glyphosate was also detected in the Hungarian small Danube sample, but in that case the laboratory applied lower LOQ, and the detected concentration was below the 0.1µg/L threshold.

France	La Chapelle aux pots / l'Avelon, 49,4374071	AMPA 0.4
S 01 F	1,9192862	Glyphosate <0,2
France	Rochy Condé/ le Therain	AMPA <0,2
S 02 F	49,398526 .2,182851	Glyphosate <0,2
France	St Leu d'Esserent . l'Ois	AMPA 0.5
S 03 F	49,2133081. 2,4221229	Glyphosate <0,2
Slovenia	River Savinja - 150 m south of the address Spodnje Roje 9, 3311 Šempeter in the	AMPA <0,2
	Savinjska dolina	Glyphosate <0,2
Spain	https://maps.app.goo.gl/ PN8ZdRPWnAVTkKRx6	AMPA 0.5
Lleida Aigua Panta	Utxesa swamp (Lleida). T	Glyphosate 0.2
Spain - Muestra 1,	Agua superficial, Canal perimetral San Pedro del Pinatar,	AMPA 0,8
	37°50'35.81"N, 0°46'1.88"W	Glyphosate <0,2
Spain Muastra 2	Agua superficial Rambla del Albujón,	AMPA 3.4
Spain - Muestra 2.	37°42'58.63"N, 0°51'39.76"W	Glyphosate 0,4
	River Veengoot R.	AMPA <0,2
Netherlands	near Ruurlo, <u>https://goo.gl/maps/</u> HuLmqX8hR9QjscM66	Glyphosate <0,2
	River Borculo	AMPA 0,8
Netherlands	Sample 2 - river, near Borculo, <u>https://goo.gl/</u> maps/2Hb7xh6pW1nSeaut7	Glyphosate <0,2
Polaium	Moxhe - surface water - La Mehaigne	AMPA 1
Belgium	Monte - Surrace water - La Merialyrie	Glyphosate <0,2

Layout: OKAY WHEN agency



60 rue Wiertz/Wiertzstraat 60 1047 Brussels, Belgium www.greens-efa.eu contactgreens@ep.europa.eu