


Occurrence of the herbicide glyphosate and its metabolite AMPA in surface waters in Switzerland determined with on-line solid phase extraction LC-MS/MS

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Abstract Glyphosate is currently one of the most important herbicides worldwide. Its unique properties provide for a wide range of uses in agriculture but also in non-agricultural areas. At the same time, its zwitterionic nature prevents the inclusion in multi-residue analytical methods for environmental monitoring. Consequently, despite its extensive use, data on occurrence of glyphosate in the aquatic environment is still scarce. Based on existing methods, we developed a simplified procedure for the determination of glyphosate and its main metabolite aminomethylphosphonic acid (AMPA) in water samples using derivatization with fluorenylmethyl chloroformate FMOC-Cl, combined with on-line solid phase extraction and liquid chromatography-tandem mass spectrometry (LC-MS/MS) detection. This method was extensively tested on over 1000 samples of surface water, groundwater, and treated wastewater and proved to be simple, sensitive, and reliable. Limits of quantification of 0.005 µg/L were routinely achieved. Glyphosate and AMPA were detected in the vast majority of stream water samples in the area of Zurich, Switzerland, with median concentrations of 0.11 and 0.20 µg/L and 95th percentile concentrations of 2.1 and 2.6 µg/L, respectively. Stream water data and data from treated wastewater indicated that non-agricultural uses may significantly contribute to the overall loads of glyphosate and AMPA in surface waters. In the investigated groundwater samples, selected specifically because they had shown presence of other herbicides in previous monitoring programs,

glyphosate and AMPA were generally not detected, except for two monitoring sites in Karst aquifers, indicating that these compounds show much less tendency for leaching.

Keywords Glyphosate · AMPA · Surface water · Groundwater · Wastewater · LC-MS/MS

Introduction

Glyphosate is a non-selective, herbicidally active compound that acts on the shikimate pathway by blocking the EPSP synthase (Schönbrunn et al. 2001). The compound is highly polar and carries a positive as well as several negative charges when in solution at around neutral pH (Smith et al. 1989). Adsorption to soil is strong, primarily by interaction with iron oxides and clay minerals (see Vereecken 2005 and references cited therein). Consequently, uptake of glyphosate through plant roots is negligible and glyphosate thus solely acts on weeds which have emerged prior to application (no residual activity in soil) (Franz et al. 1997). The combination of properties, i.e., broad spectrum of susceptible weeds, rapid translocation within plants, and lack of residual activity in soil, provides for its use in many agricultural and non-agricultural applications. After the widespread introduction of glyphosate-tolerant field crops like corn, soybean, and canola, the compound has become the most used pesticide worldwide with an estimated annual use of 8.3×10^8 kg active substance in 2014 (Benbrook 2016).

Glyphosate is metabolized in soil, natural waters, and plants to aminomethylphosphonic acid (AMPA, Fig. 1) which is further metabolized. However, glyphosate is not the only precursor of AMPA in the environment. A number of phosphonates, e.g., those used as chelating agents in detergents, are also degraded to AMPA (Nowack 2003).

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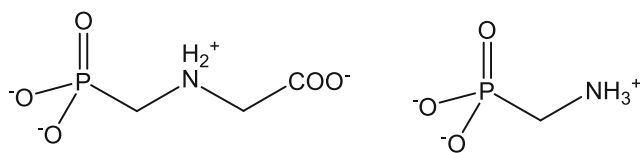


Fig. 1 Structures of glyphosate (*left*) and its main metabolite aminomethylphosphonic acid (AMPA, *right*) with likely speciation in water at neutral pH

Consequently, the presence of AMPA in environmental samples can only be related to glyphosate in locations where other precursors are absent, e.g., in edge of field situations, headwater streams in rural areas, and drainage water from railway tracks, but not in streams with inputs of domestic or industrial wastewater.

A remarkable number of analytical methods for glyphosate in water are available in the literature, including methods based on HPLC with precolumn and postcolumn derivatization and detection by UV, fluorescence, and mass spectrometry, GC-MS after multiple derivatization, and ELISA (for a comprehensive, recent review, see Koskinen et al. (2016)). Despite the range of available methods, analysis of glyphosate and AMPA remains challenging. Both molecules are small, highly polar, multiply (positively and negatively) charged, and carry no chromophore. These properties prevent inclusion of glyphosate and AMPA in multi-residue analytical methods for pesticides in water. Consequently, data on occurrence of glyphosate and AMPA in the environment are still scarce.

New sensitive and selective methods for glyphosate and AMPA based on liquid chromatography-tandem mass spectrometry (LC-MS/MS) usually rely on precolumn derivatization with fluorenylmethyl chloroformate (FMOC-Cl), followed by sample preconcentration (on-line or off-line) (Battaglin et al. 2005; Daouk et al. 2013; Hanke et al. 2008; Ibanez et al. 2005, 2006; Landry et al. 2005; Ramirez et al. 2014; Sanchis et al. 2012). Derivatization eliminates the positive charge in the molecule and renders the compound more lipophilic and thus amenable to reversed-phase HPLC. Tandem mass spectrometry, in contrast to other detection options (e.g., fluorescence detection), allows use of stable isotope-labeled internal standards and is less sensitive to the chemical background present.

In this paper, we report on a simplified procedure for the analysis of glyphosate and AMPA in water samples, using a miniaturized sample preparation process combined with on-line preconcentration, thus minimizing the number of sample preparation and transfer steps. The method was applied in numerous studies on the occurrence and behavior of glyphosate and AMPA in Swiss surface waters, groundwater, and wastewater treatment plants (WWTPs) and proved to be simple (in comparison to other currently available methods), robust, and sensitive. The analyses show the ubiquitous occurrence of glyphosate and AMPA in surface water and treated

wastewater while in groundwater, only very few detections were observed.

Materials and methods

Chemicals

Glyphosate (purity 98 %), glyphosate-FMOC (99.5 %), and AMPA-FMOC (97 %) were from Dr. Ehrenstorfer, Augsburg, Germany. $^{13}\text{C}_2^{15}\text{N}$ -glyphosate (internal standard, 98 %) was from Toronto Research Chemicals, North York, Canada. AMPA (99 %) was from Aldrich Chemicals, Milwaukee, WI, and $^{13}\text{C}^{15}\text{ND}_2$ -AMPA (100 mg/L in water) was from LGC Promochem GmbH, Wesel, Germany. Stock solutions of the unlabeled compounds were prepared at concentrations of 500 mg/L in acetonitrile/water (7:3). To aid dissolution of glyphosate and AMPA, 100 μL 1 M aqueous NaOH solution was added to 20 mL of stock solution. Diluted mixed standards were prepared from these stock solutions in the same solvent mixture as needed. A 2 mM solution of FMOC-Cl (>97 % from Fluka, Buchs, Switzerland) was prepared in acetonitrile (51.7 mg in 100 mL) for derivatization. Borate buffer was prepared by dissolving 381 mg sodium tetraborate decahydrate (>99.5 %, from Sigma-Aldrich, Seelze, Germany) in 10 mL purified water to give a 0.1 M solution. For the HPLC eluent buffer, a stock solution was prepared by dissolving 960 mg ammonium carbonate (Fluka) in 80 mL purified water. This stock solution was adjusted to pH 9.5 with ammonia (25 %, Merck, Darmstadt, Germany) before the volume was brought to 100 mL to yield a concentration of 0.1 M with respect to carbonate. To prepare fresh eluent daily, this stock solution was diluted to 1:100 with purified water. Dichloromethane, acetonitrile, and methanol were from Merck; all solvents were of HPLC grade.

Water samples

Grab samples from various streams in the area of Zurich, Switzerland, were collected during routine samplings by the Office for Waste, Water, Energy, and Air of the Canton of Zurich (AWEL) from 2006 to 2013. Further grab samples from a small stream in the Canton of Vaud were provided by the water protection laboratory of the canton from 2011 to 2014. Groundwater samples were collected by the Federal Office for the Environment (FOEN) at selected monitoring sites of the NAQUA National Groundwater Monitoring Program during a pilot study in 2010 and 2011, and by the official food control authority of the Canton of Zurich in 2006, 2007, and 2012. Grab samples and 24-h flow proportional composite samples of treated wastewater from various WWTPs in Switzerland were obtained from the personnel of these plants. All samples were collected in 125-mL high-

density polyethylene (HDPE) flasks, shipped in ice-cooled containers (not frozen), and stored at 4 °C after addition of internal standard (see below). Storage time was usually less than 2 weeks.

Derivatization with FMOC-Cl

Upon arrival at the laboratory, exactly 100 mL of each water sample was retained in the HDPE container while the rest was discarded. Samples were fortified with 100 µL of a solution of $^{13}\text{C}_2\text{ }^{15}\text{N}$ -glyphosate and $^{13}\text{C}^{15}\text{ND}_2$ -AMPA (0.1 ng/µL each) in acetonitrile/water (7:3) to yield concentrations of 100 ng/L in the samples. Spiked samples were kept for at least 24 h at 4 °C to allow for equilibration between dissolved and particulate phase.

Derivatization was done in 10-mL headspace glass vials (Brechtbühler, Dielsdorf, Switzerland). To an aliquot of 5 mL water sample, 100 µL of 0.1 M borate buffer solution and 0.75 mL of 2 mM FMOC-Cl solution were added. After shaking, the vials were left at room temperature overnight. To remove excess reagent and side products, as well as a substantial fraction of the acetonitrile, 2 mL of dichloromethane was then added to the derivatized samples. The samples were shaken and left undisturbed until the phases were completely separated. No removal of the organic phase was necessary prior to injection of the samples. However, the rubber septa were removed from the caps as they provided too much resistance to the autosampler syringe needle.

“Matrix matched” standards were prepared in “fossil” groundwater (Aqui, Zurich), which was also used for blank determination. Concentrations ranged from 10 to 2000 ng/L glyphosate and AMPA (depending on the concentrations present in the samples). The internal standards were added at the same concentration as in the water samples, and derivatization was done together with the real samples.

On-line SPE and liquid chromatography-tandem mass spectrometry

The instrumental setup was similar to the one reported earlier (Gulkowska et al. 2014) and consisted of an autosampler (HTS PAL, CTC Analytics, Zwingen, Switzerland) equipped with two six-port valves for column switching, a sample loop, and an on-line extraction cartridge. The HPLC system included a pump used solely for sample loading (Jasco PU-980, Gross-Umstadt, Germany) and a binary gradient HPLC pump (Agilent 1100 series, Palo Alto, CA) for the mobile phase.

Preconcentration of the derivatized analytes was achieved using a column switching technique (see supporting information, Fig. S1, in Gulkowska et al. (2014)). A 1-mL PEEK loop was loaded with 1 mL of the derivatized sample solely from the upper, aqueous layer

via the autosampler syringe. The sample was then transferred from the loop to the SPE cartridge (two stacked Gemini-NX C18 cartridge precolumns, 4×3.0 mm i.d., 5 µm; Phenomenex, Torrance, CA) with purified water at a flow rate of 1 mL/min. Total transfer time was 90 s, thus providing for enrichment of the analytes (60 s) as well as cleanup of the samples from highly polar components, including the borate buffer needed for derivatization (additional 30 s). After valve switching, the enriched analytes were eluted backward directly on to a Gemini-NX C18 column (150×2.0 mm i.d., 5 µm; Phenomenex) equipped with a guard column filled with the same stationary phase (4×3 mm i.d.).

The LC conditions were as follows: saturation of the column with 100 % ammonium carbonate buffer, linear gradient from 0 to 30 % methanol within 1 min and then to 100 % methanol within 20 min, followed by an isocratic phase of 5 min, all at a flow rate of 200 µL/min.

The HPLC column was connected to an API 4000 triple quadrupole mass spectrometer (Sciex, Framingham, MA) equipped with a turbo ion spray (TIS) source operated in negative mode (ion spray voltage, -4.5 kV, 450 °C) and multiple reaction monitoring (MRM) with the following ion transitions: glyphosate-FMOC, m/z 390 \rightarrow 168 with a collision energy of -18 eV (and for confirmatory purposes: m/z 390 \rightarrow 150, -34 eV); $^{13}\text{C}_2\text{ }^{15}\text{N}$ -glyphosate-FMOC, m/z 393 \rightarrow 171, -18 eV (m/z 393 \rightarrow 153, -34 eV); AMPA-FMOC, m/z 332 \rightarrow 110, -12 eV (m/z 332 \rightarrow 136, -22 eV); and $^{13}\text{C}^{15}\text{ND}_2$ -AMPA-FMOC, m/z 336 \rightarrow 114, -12 eV (m/z 336 \rightarrow 140, -22 eV). The characteristic fragmentation reaction for the primary transition was the cleavage of the FMOC moiety from the derivatized molecule.

Quantification was based on peak area ratios of analyte versus internal standard in reference to standards in spiked fossil groundwater. Concentrations were determined separately using the primary (Q) and secondary ion transitions (q), and measurements were flagged when the concentration ratio Q/q was not within 0.8–1.2. However, the reported concentrations were always based on the primary transition (Q).

Relative response, method precision, and recovery in different matrices

The influence of the sample matrix on the intensity of MRM transitions for glyphosate and AMPA was studied in groundwater (Aqui), surface water (Sagentobelbach, sampled on August 18, 2015), WWTP effluent (Dübendorf, August 18, 2015), and purified water containing 1 mM calcium chloride. Standards were prepared in these matrices by appropriate dilution of a stock solution of the isotopically labeled surrogate compounds (concentrations, 62.5, 125, 250, 500, 1000, and 2000 ng/L), followed by derivatization. By using the

isotopically labeled surrogate compounds, a possible influence of background levels could be excluded. The slopes of the respective calibration curves were used to calculate responses in matrix relative to purified water (Table 1).

Method precision was determined by replicate analysis ($N = 6$) of WWTP effluent (Villars-sous-Yens, August 11, 2015), surface water (Boiron, July 17, 2015), and groundwater (Aqui, spiked with glyphosate and AMPA at concentrations of 25 and 250 ng/L, respectively). Recoveries were determined in surface water (Sagentobelbach, August 18, 2015) and WWTP effluent (Villars-sous-Yens, August 11, 2015) relative to calibration standards in groundwater (Aqui) at two spike levels each (Table 1).

Quality assurance in routine monitoring

Several measures were used to assure accurate determination of glyphosate and AMPA during monitoring campaigns. Blank samples (fossil groundwater) and a control standard of spiked groundwater were analyzed with each batch of samples. Selected samples were analyzed in triplicate to determine precision (RSD values were in the range of those reported in Table 1). In longer campaigns, selected samples from previous sampling events were reanalyzed to determine intermediate precision (measured concentrations, usually within $\pm 10\%$) and storage stability (peak area of internal standards over time, usually within $\pm 10\%$). Storage stability varied between 1 and 2 months (groundwater) and 2 weeks (WWTP effluent). During storage, concentrations of the analytes and internal standards did not decrease continuously but rather dropped rapidly after a certain “lag phase.” However, peak area ratios (analyte vs. internal standard) remained constant.

Results and discussion

Optimization of the analytical procedure

Derivatization of glyphosate and related compounds with FMOCCl offers several advantages. First, the reaction proceeds directly in aqueous samples (with a certain amount of acetonitrile as co-solvent) so that there is no need for preconcentration or solvent exchange prior to derivatization. Second, the derivative is significantly more lipophilic than the underivatized test substances and thus more easily concentrated from water and more suited for reversed-phase HPLC. Third, the main by-product of the derivatization, the FMOCCl alcohol, can easily be removed as it is far more lipophilic than the derivatives.

Our goal for optimization of the analytical procedure was to simplify the existing methods based on derivatization with FMOCCl, on-line preconcentration, and HPLC-MS/MS (Battaglin et al. 2005; Daouk et al. 2013; Hanke et al. 2008; Ibanez et al. 2005, 2006; Landry et al. 2005; Ramirez et al. 2014; Sanchís et al. 2012). The result is a procedure that requires a minimal amount of sample (5 mL) and no transfer steps during or after derivatization. On-line preconcentration requires no dedicated equipment except a dual injection valve on a standard PAL autosampler and an auxiliary HPLC pump for transfer of the sample from a sample loop to a cartridge precolumn used for preconcentration (note that preconcentration of one and elution of another sample cannot be done simultaneously with this setup).

In chromatograms from natural water samples, some of the mass traces also contain substantial signals other than those of

Table 1 Influence of sample matrix on responses of glyphosate and AMPA in groundwater, river water, and WWTP effluent; method precision; and recovery

	Relative response [%] ^a		Precision				Recovery		
	Glyphosate	AMPA	Concentration (ng/L)		RSD ^b (%) ($N = 6$)		Spike level (ng/L)	Recovery ^c (%)	
			Glyphosate	AMPA	Glyphosate	AMPA		Glyphosate	AMPA
Groundwater	72 %	111 %	25	25	3.2	1.7			
			250	250	1.5	1.1			
Surface water	70 %	100 %	66	102	2.3	3.6	100 ^d	97 %	103 %
							500 ^d	91 %	99 %
WWTP effluent	85 %	89 %	105	1560	1.2	1.4	500 ^e	91 %	93 %
							2000 ^e	100 %	97 %

^a Slope of calibration curve in matrix relative to purified water (containing 1 mM CaCl₂)

^b Relative standard deviation

^c Relative recoveries calculated using calibration standards prepared in groundwater

^d Background concentrations of glyphosate and AMPA were 12 and 39 ng/L, respectively; recoveries were calculated after correction for background

^e Background concentrations of glyphosate and AMPA were 145 and 1720 ng/L, respectively; recoveries were calculated after correction for background

the target compounds (Fig. 2). This is due to the fact that the major transitions observed in negative ion tandem mass spectrometry result from loss of the FMOC moiety. Therefore, we optimized the chromatographic separation between target compounds and possible interferences by using a rather high pH eluent (≈ 9.15) in combination with an HPLC column that is sufficiently stable at this high pH. As can be seen in Fig. 2, glyphosate and AMPA elute earlier than any of the interferences. At even higher pHs, the target compounds elute very early so that the separation between target and potentially interfering compounds increases. This would come, however, at the cost of lower sensitivity and shortened column lifetime.

Signal responses in different matrices, detection limits, precision, and recoveries

Derivatization of samples necessarily adds to the naturally present sample matrix (buffer, organic solvents, unreacted derivatization reagent, and reaction by-products). It is thus expected that significant LC-MS/MS signal suppression occurs when compared to pure reference compounds dissolved in water. While these derivatization-related sample components clearly have an impact on the observed MRM signal intensities, they are likely the same in natural water samples and in (derivatized) calibration standards. Signal suppression due to natural sample components, however, is expected to vary. Besides affecting the LC-MS/MS response directly, natural

sample components are also expected to influence the derivatization yield and the extraction efficiency during on-line enrichment. However, the relative responses (expressed in % of the response in purified water) showed fairly narrow variation between different matrices (Table 1).

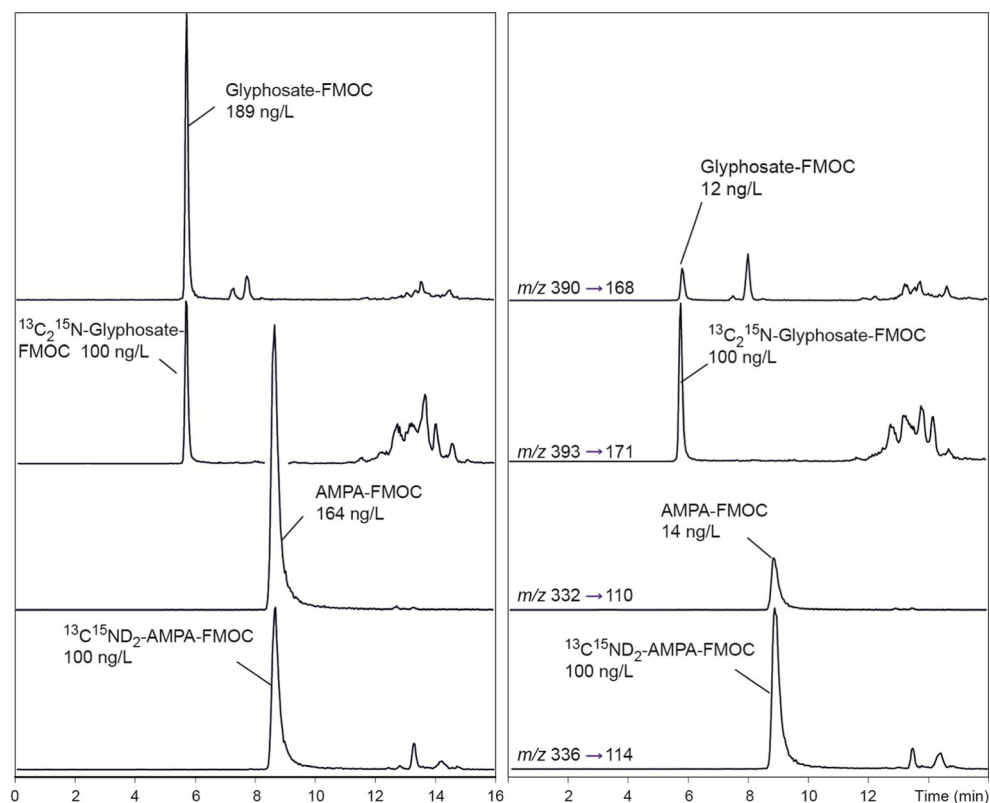
Limits of quantification (LOQs) for glyphosate and AMPA of 5 ng L^{-1} (signal/noise ratio of >10 for the ion trace used for quantification and $S/N >3$ for the ion trace used for confirmation) were achieved under most circumstances, except for surface water samples with high particle loads and groundwater samples with low pHs (see below). Method precision was excellent with relative standard deviations for replicate analyses ranging from 1.1 to 3.6 % with no clear trend with regard to matrix or substance. Recoveries in spiked surface water and WWTP effluent ranged from 91 to 103 % (Table 1).

Field testing of the analytical procedure

The optimized procedure was extensively tested on a total number of more than 1000 samples of groundwater, surface water from different streams and lakes, and effluents from WWTPs and proved to be robust and sensitive. Some of the experiences during application of the method are discussed hereafter.

In some groundwater samples, fairly low signals were obtained for internal standards as well as the test substances. This phenomenon was reported by other researchers and attributed

Fig. 2 Typical chromatograms of glyphosate and AMPA in samples from the river Aabach at Mönchaltorf (weekly composite sample, September 30 to October 6, 2013, *left*) and from Lake Greifensee (1 m depth, October 7, 2013, *right*)



to possible complexation of the test substances with metals (Ibanez et al. 2006). In our experience, low signals were limited to groundwater samples with low pHs (<6.5) and low calcium content. In these cases, adjusting the pH to ≥ 7 and addition of 1 mM CaCl₂ prior to derivatization resulted in a substantial improvement in signal intensity.

Samples were normally analyzed without prior filtration. After addition of the internal standard, the particles were allowed to settle and a subsample from the supernatant was subjected to derivatization and analysis. Assuming that the time between addition of internal standard and derivatization (24 h) was long enough to allow for an equilibration between aqueous and particulate phases, the measured concentration thus reflects the total amount in the sample, including the fraction which is adsorbed to particles.

Surface water samples from storm events with very high content of organic matter (particulate and/or dissolved) sometimes also yielded low signal intensities. Regardless of whether this was due to signal suppression, low derivatization yield, or both, dilution with blank (fossil) groundwater improved the situation.

Glyphosate in groundwater samples

A total of 141 groundwater samples from 14 monitoring sites in Switzerland in 2010 and 2011 were analyzed as part of an intensive campaign conducted by the FOEN to determine the concentration dynamics of pesticides at these stations. Concentration dynamics were expected to be high at these locations due to high vulnerability. Glyphosate was detected twice above the LOQ of 0.005 µg/L at one location (0.009 and 0.025 µg/L, respectively). AMPA was regularly detected at two locations above the LOQ of 0.005 µg/L in concentrations of 0.08–0.65 and 0.017–0.070 µg/L, respectively. Both monitoring sites are located in vulnerable Karst aquifers with a shallow soil cover. During 2006, 2007, and 2012, further single groundwater samples from eight locations were analyzed with no detections above the LOQ of 0.005 µg/L. Some of these locations are known to receive substantial amounts of river bank infiltration (Buerge et al. 2009). Overall, these results confirm the low potential of glyphosate and AMPA for leaching to groundwater which is due to strong sorption to soil particles combined with fairly rapid dissipation (European Food Safety Authority (EFSA) 2015).

Occurrence of glyphosate and AMPA in rivers and streams

From 2006 to 2013, glyphosate and AMPA were analyzed in numerous water samples from various locations in Switzerland, particularly in the canton of Zurich. In the following, we present results from monitoring campaigns

where monthly grab samples were taken between March and November (no sampling during winter and early spring) as part of the pesticide monitoring program of the canton of Zurich (AWEL 2016). Both compounds were regularly detected in the investigated streams with median concentrations of 0.11 and 0.20 µg/L and 95th percentile concentrations of 2.1 and 2.6 µg/L, respectively (Fig. 3). Only 40 out of 583 samples showed glyphosate concentrations below the LOQ of 0.005 µg/L (27 for AMPA). On average, concentrations of AMPA were higher than those of glyphosate. On a sample-by-sample basis, in only 28 % of samples, concentrations of AMPA were lower than those of glyphosate. Nevertheless, the highest overall concentrations were found for glyphosate. Widespread occurrence in streams as well as the detected concentrations compare well to findings in other studies (Battaglin et al. 2014; Daouk et al. 2013; European Glyphosate Environmental Information Sources (EGEIS) 2009; Hanke et al. 2008; Hanke et al. 2010; Kolpin et al. 2006).

The seasonal variation of glyphosate and AMPA concentrations in weekly, flow-proportional composite samples was monitored in various streams in the canton of Zurich from spring to fall. For example, in the Furtbach, a small stream in the north of Zurich (long-term mean discharge, 655 L/s; Q₃₄₇ = 208 L/s), receiving inputs from agricultural land as well as from three municipal WWTPs serving a total population of approximately 32,000, glyphosate was already present in the water samples in April and increased to a maximum of 1.9 µg/L at the end of May (Fig. 4 (top)). Thereafter, the concentrations remained relatively high until mid-September, consistent with its main application window in August, and then dropped to below 0.1 µg/L at the beginning of November. The minima in

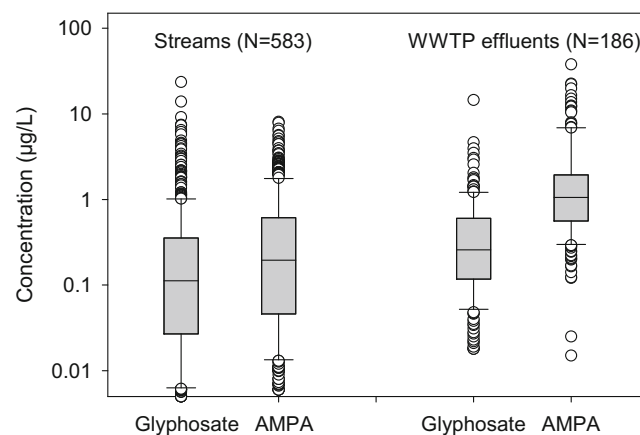


Fig. 3 Distribution of glyphosate and AMPA concentrations in rivers and streams ($N = 583$) and WWTP effluents ($N = 186$), analyzed from 2006 to 2013. The boxes indicate median and 25th and 75th percentiles, the whiskers the 5th and 95th percentiles. Values outside this range are plotted individually

June, August, and end of September correspond to weeks with no precipitation. Glyphosate was thus detected at elevated concentrations for a much longer part of the year than other herbicides that are applied in large quantities such as isoproturon or metolachlor and which are typically found primarily during a narrow time window during and immediately following the application period.

Concentrations of AMPA in the same samples varied much less. While, overall, higher concentrations were observed in summer, the differences between summer and spring/fall were smaller than for glyphosate. Particularly, the minima in June and August were not observed for AMPA.

Concentration ratios AMPA/glyphosate were calculated for all weekly composite river water samples analyzed from 2006 to 2013. To eliminate some of the variability due to different meteorological conditions, the calculated values were grouped monthly. Concentration ratios varied greatly as indicated by the wide range that is spanned by the 5th and 95th percentile whiskers in Fig. 4 (bottom). Nevertheless, there is a trend toward lower ratios in summer, when glyphosate concentrations are at their maximum.

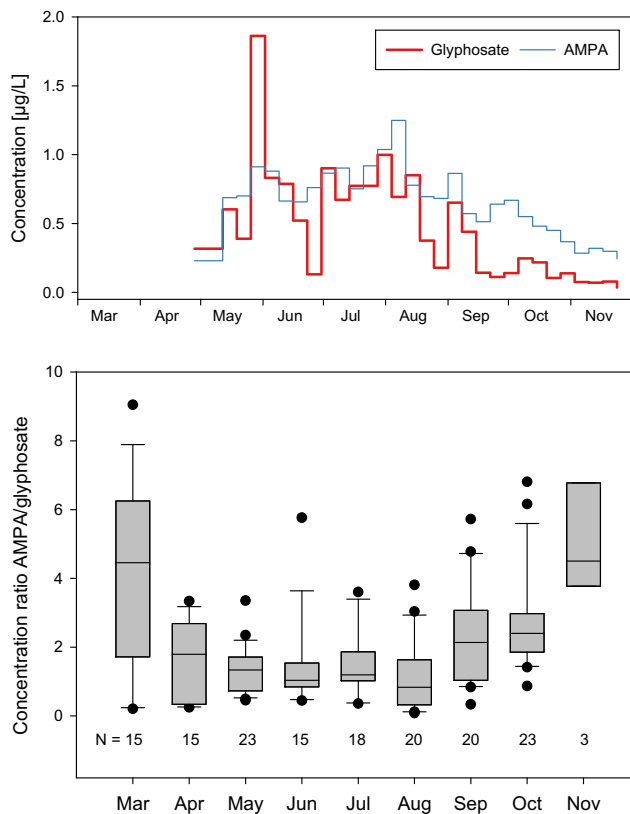


Fig. 4 Example of the seasonal variation of glyphosate and AMPA concentrations in flow-proportional weekly composite samples from a small river (Furtbach 2008, *top*) and of the AMPA/glyphosate concentration ratios in various small rivers in the Canton of Zurich, Switzerland (2008–2013, *bottom*)

Wastewater treatment plants as a source of glyphosate and AMPA in surface waters

Glyphosate and AMPA were regularly present in treated wastewater. Concentrations tended to be higher than in rivers and streams with median concentrations of 0.38 and 1.3 $\mu\text{g/L}$, respectively (Fig. 3). Comparison of glyphosate and AMPA concentrations in WWTP effluents (data not shown) do not indicate that occurrence of these two compounds is linked. Concentration ratios (AMPA/glyphosate) ranged from 1.2 to 38 and seemed to be related to the particular WWTP rather than to any other parameter (such as time of year, high or low concentrations, etc.). All these observations indicate that AMPA, although a major metabolite of glyphosate, must have other sources as well. Indeed, AMPA is also a major degradation product of a number of phosphonates used, e.g., in detergents as chelating agents (Nowack 2003).

Comparison of in-stream concentrations of glyphosate and AMPA upstream and downstream of WWTPs indicated that treated wastewater indeed is a source of these compounds in surface waters (Fig. 5). For glyphosate, contribution of WWTP effluent to downstream concentrations was predominant (>90 %) in 6 out of 47 cases, significant (20 to 90 %) in another 21, and negligible (<20 %) in 20 cases.

Urban contribution to total load of glyphosate in surface waters

Ubiquitous occurrence of glyphosate in wastewater indicates that non-agricultural uses of glyphosate may substantially contribute to the total burden to surface waters. Potential candidates are uses for weed control along highways and railroads as well as private and semi-private application such as in gardening and weed control in residential areas, parks, golf

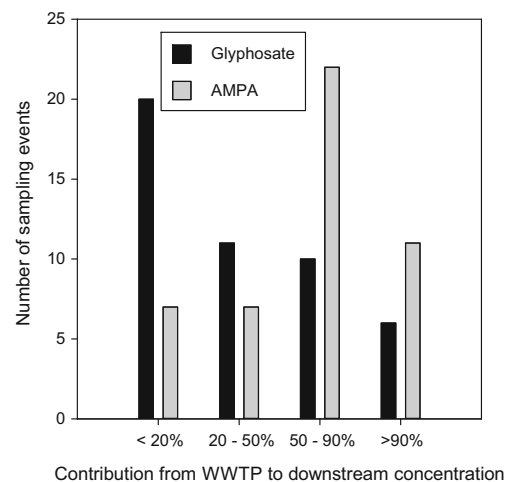


Fig. 5 Evidence of contribution of treated wastewater to total loads of glyphosate and AMPA in surface waters from a comparison of in-stream concentrations, upstream and downstream of municipal WWTPs ($N = 47$)

courses, etc. Urban contributions to total loads of glyphosate in surface waters were shown to be significant by a number of researchers either from experimental data (Botta et al. 2009; Hanke et al. 2010; Kolpin et al. 2006; Skark et al. 2004) or from calculations based on use data and experimentally determined typical losses from different types of surfaces (Blanchoud et al. 2007). Our data from streams and WWTP effluents clearly support this finding.

Due to its unique combination of properties, glyphosate has found many applications in areas where other herbicides would be expected to pose significant risk for contamination of surface and/or groundwater. Despite its favorable properties, glyphosate losses from urban uses can be quite significant (Ramwell et al. 2014) and may contribute substantially to the elevated concentrations in surface waters over extensive periods of time. Even though these concentrations are still well below the currently proposed environmental quality standards for surface waters in Switzerland and some EU Member States (Johnson 2012; Maycock et al. 2010; Oekotoxzentrum 2016), it appears to be warranted to reduce the use of glyphosate particularly in those areas (e.g., application on or along sealed surfaces) where the potential losses are high.

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