Time-integrated sampling of glyphosate in natural waters

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Highlights

- Glyphosate losses are likely in monitoring programs using time-integrated sampling
- Losses are partly due to degradation, partly to binding to metals or humus
- Refrigerated time-integrating samplers reduces degradation and increases recovery
- Acidification during sampling increases recovery by reversing metal and humus binding
- Labelled surrogate standards are imperative to identify false negatives

Abstract

Environmental monitoring of pesticide residues in surface water is often done with time-integrated sampling where a specified volume is sampled each hour during, e.g., a week, thus avoiding at momentary high or low extreme concentrations. However, sampling over an extended period of time can result in losses of easily degradable analytes, why the stability of the target analytes over the timespan of the sampling must be checked. Glyphosate is one of the most widely used herbicides. Because of its chemical complexity, glyphosate binds differently to metals and colloids at different pH, and the degradation may also be affected. Recovery of glyphosate from spiked natural waters after one and three weeks of storage was higher when the samples were acidified to approximately pH 2 rather than at their natural pH. Keeping the samples refrigerated to 4 °C in darkness also enhanced recovery, while glyphosate losses were substantial from samples kept at their natural pH at 20 °C. Total loss of glyphosate was observed in some samples kept at natural pH, 20 °C, and daylight; a loss partly due to binding to metals or colloids that could only partially be reversed by acidification. For one-week time-integrated sampling a small amount of hydrochloric acid in a piece of heat-sealed hydrophobic micro-porous tubing is added to the sampling bottles before deployment, a procedure that acidifies the samples during collection keeping them below pH 2 until analysis, thus minimizing losses of glyphosate. The method also allows determination of the primary degradation product aminomethylphosphonic acid (AMPA).

Key words

N-(phosphonomethyl)glycine, AMPA, aminomethylphosphonic acid, environmental monitoring, sample preservation, sample integrity

1. Introduction

The Swedish national environmental monitoring programme for pesticides in agricultural areas currently includes some 126 different compounds (Graaf et al., 2010; SLU, 2012a). In addition, various regional or local monitoring programmes essentially include the same analytes, but sometimes with less elaborate sampling regimes (SEPA, 2004; Törnquist et al., 2005a, 2005b). Sampling within these monitoring programmes is usually based on one-week time-integrated sampling, where a specified volume is sampled each hour (SEPA, 2004). Although problems with momentary high or low concentrations at the time of sampling is avoided by time-integrated sampling, it introduces another problem; pesticides may degrade during sampling and transport as more than one week will pass between the start of the sampling and the arrival of the sample at the laboratory. Within the national environmental monitoring

programme, the samplers are refrigerated so that the samples are kept below +4 °C during the sampling period to minimize analyte degradation, but regional or local monitoring programmes at times use samplers that are not refrigerated (Törnquist et al., 2005). Irrespective if the individual monitoring programme uses refrigerated samplers or not, transport from the sampling site to the laboratory will take at least one day, during which time the sample temperature, in the worst case, may rise to ambient.

Glyphosate [*N*-(phosphonomethyl)glycine] is one of the most widely used herbicides in Sweden, and constitutes 40-45% of the total amount of herbicides sold nationally (KemI, 2010). It is, therefore, an important compound to include in environmental monitoring. However, glyphosate chemistry is complex. The molecule has four ionizable functional groups (Smith et al., 1989), is a zwitterion at the pH of most natural waters (Sheals et al., 2001), and has different propensity for binding to metal ions or colloids at different pH (Barja and dos Santos Afonso, 2005; Daniele et al., 1997; Piccolo et al., 1996; Sheals, 2002; Subramanian and Hoggard, 1988). The whole series of acid dissociation constants includes pK_{a1} 0.8 (1st phosphonic), pK_{a2} 2.3 (carboxylate), pK_{a3} 6.0 (2nd phosphonic), and pK_{a4} 11.0 (amine) (Virtual Museum, 2012). However, the zwitterionic character of glyphosate, where one proton leaves the phosphonic moiety and binds to the amine, makes pK_{a1} difficult to measure (Smith et al., 1989), and most sources (e.g., Bleeke, 1998; Mackay et al., 2006) only give the three acid dissociation constants corresponding to pK_{a2} to pK_{a4} here. Because of complications such as these, predicting how glyphosate will behave in a specific environment is difficult.

The complex chemistry makes glyphosate difficult to include in multi-residue methods, why glyphosate and its primary degradation product aminomethylphosphonic acid (AMPA) are usually determined with a separate method. But AMPA also has other sources than glyphosate degradation (Jaworska et al., 2002; Nowack, 2003). The presence of AMPA without the simultaneous detection of glyphosate, therefore, is an ambiguous indicator of the release of glyphosate into the environment. Hence, the stability of the mother compound during sampling and storage is imperative to draw firm conclusions about residues of glyphosate in the environment. Stability is particularly important in monitoring programmes that use time-integrated sampling; glyphosate should not degrade during the time-span of the sampling or the concentrations will be underestimated.

This paper describes a study of the stability of glyphosate in natural waters during time-integrated sampling based on the sampling and analytical protocol developed for the Swedish national environmental monitoring programme. Based on the stability test, during which light regime, temperature, and pH varied, a method to acidify the samples during time-integrated sampling was developed.

2. Material and Methods

2.1 Chemicals

2.1.1 Standards

Native glyphosate and AMPA, both >99%, and their isotope-labelled analogues (glyphosate-1,2-¹³C₂¹⁵N, AMPA-¹³C¹⁵N) used as surrogate standards were from Dr Ehrendorfer (Augsburg, Germany).

2.1.2 Other chemicals

Pesticide grade methanol and pesticide grade ethyl acetate were from Labscan (Stillorgan, County Dublin, Eire). Analytical grade hydrochloric acid (HCl), analytical grade sodium hydroxide (NaOH), analytical grade potassium hydroxide (KOH), HPLC-grade water, and analytical grade cotton wool were all from VWR-International (Spånga, Sweden). Trifluoroacetic anhydride (TFAA) and trifluoroethanol (TFE) were from Sigma-Aldrich (Haninge, Sweden). Ethanol (96%) was from Solveco (Rosersberg, Sweden). Ion exchange resin (AG 1-X8 formate form, 100-200 mesh, 1.2 meq mL⁻¹) Bio-Rad Laboratories, Hercules, CA, USA). Isolute C18 solid-phase extraction (SPE) cartridges (3-mL polypropylene reservoir) were from Biotage (Uppsala, Sweden).

2.2 Water samples

2.2.1 Stability test

The design of the stability test was based on a previous exploratory study with water from River Vendelån (60° 2.4' N, 17° 40.0' E) that runs through an agricultural area north of Uppsala, Sweden (Kreuger, 2003). The larger stability test described here was performed using water from River Stångån (at Vimmerby, 57° 37.7' N, 15° 51.2' E), River Helge å (at Yngsjö, 55° 52.5' N, 14° 13.2' E), Lake Havagyl (in Blistorp, at 56° 12.5' N, 14° 26.7' E), and a groundwater well close to Lake Havagyl. The locations of the different test sites are shown in Fig. 1. The three surface water locations were chosen to be representative of different types of surface water in Sweden. River Stångån is a medium-size river typical for southern Sweden with both forested and agricultural areas in the catchment. River Helge å is a larger river with forest in the upper reaches of the catchment, but the intense agriculture in the lower reaches (where the sample was taken) makes this part of the river rich in nutrients and microbes. Lake Havagyl is a small lake with a little low-intensive pastureland (<5% of the area), but mainly forest and bog in the catchment. The catchment also holds a small disused iron mine and the bedrock is rich in various metals. Due to the low intensity of agriculture with almost no fertilizer use within the catchment, Lake Havagyl has lower levels of nutrients than the two rivers. On the other hand, the metal rich bedrock and high percentage of forest and bog in the catchment leads to higher concentrations of metals and dissolved organic carbon in the lake than in the rivers. Information on the long term variation of the water chemistry at the three sites can be found in the Swedish Environmental Monitoring data base for surface water (SLU, 2012b).

The well-water was included in the tests to separate sequestration of glyphosate by cations from sequestration by humic matter or loss due to microbial degradation. The well-water had passed a dolomite filter to increase the pH, it had low concentrations of microbes (potable, not sterile), and the colour indicated very low concentrations of humic matter, but several metals, including iron, aluminium, and copper were present at similar concentrations as in the lake. All water samples were tested for the presence of the analytes before use. None of the water samples had detectable levels of glyphosate, but the samples from River Stångån and River Helge å, contained appreciable concentrations (>1 µg L⁻¹) of AMPA. AMPA was, therefore, excluded from the stability test.

A schematic overview of the glyphosate loss studies is given in Fig. 2 and Table 1 shows the pH measured at the individual stages. Two 10-L batch samples were taken at each sampling

site at 15-30 cm below surface in ethanol-rinsed high-density polyethylene containers (VWR-International, Spånga, Sweden). Surface water samples were taken from an aluminium boat by lowering the closed containers to the desired depth, opening the containers under water and then sealing them again before breaking the surface. In the rivers the samples were taken on the upstream side of the boat. Well-water was sampled from a tap. After measuring the natural pH of each water sample with a calibrated PHM 210 Standard pH-meter (Radiometer, Copenhagen, Denmark), with a glass electrode (Thermo Electron Orion AquaPro 9102AP, Scandinovata, Bromma, Sweden) at 20 °C, the amount of hydrochloric acid necessary to acidify one of the 10-L samples to pH 2 was calculated and added. After the addition of the water, the containers were shaken vigorously for 20 min on a shaking table and the resultant pH measured. The two batches from each site, one at the natural pH and one acidified, were subsequently spiked with glyphosate to an estimated concentration of 1 µg L⁻¹ by adding the relevant amount of a standard solution and after 20 min of vigorous shaking, aliquotes (n=5) were taken to determine the initial glyphosate concentrations. Each batch sample was then divided into 15 subsamples (600 mL) in polyethylene bottles (Vwr-International, Spånga, Sweden) for the stability test.

Treatments and number of replicates are shown in Fig. 2. Treatments included different storage time (7, and 21 days), pH, temperature (4 and 20 °C), and light regime (dark and daylight) as controlled variables. In addition, subsamples (100 mL) from the treatments at original pH of water from River Stångån and the well were on Day 21 acidified to approximately pH 1.5 by drop wise addition of conc. HCl, after which the samples were shaken vigorously for 4 h and the glyphosate concentration determined once again.

2.2.2 Acidification of samples in time-integrated sampling

In the Swedish national environmental monitoring programme of pesticides time-integrated sampling is preferred to discrete batch sampling (SEPA, 2004). To lower the pH of the samples during collection and transport, hydrochloric acid (6 mL, 6 mol L⁻¹) in a 12-cm length of heat-sealed Spectra/Por Biotech polyvinylidene difluoride (PVDF) tubing (cut-off 250 000 D, Spectrum Laboratories, Rancho Dominguez, CA, USA) is added before shipping the containers to the sampling site (Kylin, 2003). The samples usually have a pH of approximately 2 on return to the laboratory after one week of sampling. See Electronic Supplement for further information.

2.3 Extraction

As far as possible, the work was carried using polyethylene containers and polypropylene SPE-cartridges. When use of glassware could not be avoided, it was checked to be free from scratches and notches after which it was filled with ethanol saturated with KOH and sonicated in an ultrasonic bath for three minutes before rinsing thoroughly with deionized water. Scratched glassware will lead to losses of glyphosate and AMPA (Mogadati et al., 1996). The KOH solution should be prepared in small batches (40 g KOH in 300 mL ethanol) in a high-density polyethylene bottle and used within five weeks.

From acidified samples a subsample (200 mL) was taken out and the pH adjusted to 7-8 by adding NaOH (0.5 mL, 12 mol L⁻¹), checking the pH with a pH-meter. If the pH was not appropriate, it was further adjusted either by drop-wise addition of NaOH (0.2 mol L⁻¹) or the acidic sample. The added NaOH solution changes the volume of the subsample with less than

0.5% and is considered to have a negligible effect on the glyphosate concentration. An aliquot (10–30 g) was weighed in a 100-mL polyethylene container (VWR-International, Spånga, Sweden). The remaining subsample was placed in a freezer until the aliquot had been successfully analysed and the results passed quality control. The natural pH of the samples used for the stability test was within the permissible range for extraction without pH adjustment, but the pH of samples from Lake Havagyl was adjusted with NaOH (0.2 mol L^{-1}).

Individual ion-exchange columns were used for each sample with ten extractions operated simultaneously on a stand using check valves from a vacuum-manifold to adjust the flow and ensure that the surface of the resin was never dry (see Fig. S1 in the Electronic Supplement). An ion exchange column was prepared using an empty SPE-reservoir (polypropylene, 6 mL, Biotage, Uppsala, Sweden) with a small tuft of cotton wool at the bottom. The resin (2 g) was made up to a slurry in HPLC-grade water (10 mL), poured into the reservoir, the water allowed to drain off by gravity adjusting the flow to no more than 1 drop s^{-1} , and the resin washed with additional HPLC-grade water (10 mL). The Isolute C18-cartridge was activated with one reservoir volume of methanol and rinsed with an equal volume of HPLC-grade water before coupling the SPE-cartridge on top of the ion-exchange column with a syringe adaptor (Biotage, Uppsala, Sweden). On top of the C18-cartridge, an empty polypropylene reservoir (70 mL, Biotage, Uppsala, Sweden) was attached. To obtain adequate mixing of the surrogate standards into the sample a small part (approximately 5 mL) of the sample was transferred to the 70-mL reservoir, then the surrogate standard solution, and then the rest of the sample. When the sample and standards have been added to the reservoir, the check valve was opened and the sample passed through the C18 and ion exchange columns by gravity. When the sample had passed through the columns these were rinsed with HPLC-grade water (10 mL). The C18-cartridge was discarded and the ion exchange column was eluted with hydrochloric acid (10 mL, 0.6 mol L^{-1}) into a pear-shaped flask. The eluate was evaporated using a rotary evaporator until approximately 1 mL was left and transferred into test tubes with Teflon-lined screw caps and evaporated to dryness in a heating block ($25\text{--}30^\circ\text{C}$) under a slow stream of nitrogen. The flask was rinsed with methanol:water (8:2) which was also evaporated to dryness in the test tube.

2.4 Derivatization

To the test tube TFAA (1.5 mL) and TFE (0.75 mL) were added. The tubes were capped immediately with Teflon-lined screw caps and sonicated (3 min) in an ultrasonic bath, after which they were transferred to a heating block (100°C , 1 h). After cooling to room temperature, the remaining derivatization reagents were evaporated in a heating block ($25\text{--}30^\circ\text{C}$) under a slow stream of nitrogen. To ensure total removal of the derivatization reagents, ethyl acetate (2 mL) was added twice and blown to dryness, blowing at least 2 h each time. The residue was dissolved in ethyl acetate (0.5 mL) and transferred to a 2-mL GC-vial with 100- μL glass inserts into which the ethyl acetate was transferred successively and the solvent reduced to approximately 50 μL after which the vial was sealed with a Teflon-lined crimp cap.

2.5 Instrumental analysis

Quantification was by coupled gas chromatography-mass spectrometry (GC-MS), using an Agilent 6890 GC coupled with an Agilent 5973 MS (Agilent, Santa Clara, CA, USA). The GC was equipped with a fused-silica capillary column (HP-5 for GC-MS, 30 m x 0.25 mm, 0.25

µm film thickness), a split/splitless injector with a Siltek deactivated bottom plug and liner (Restek, Bellfonte, PA, USA) with bottom plug and a plug of silanized glass wool (Coricon, Knivsta, Sweden). The data was collected and processed using the MSD Productivity ChemStation Revision D.01.02 software (Agilent). Samples were injected (1 µl) in the splitless mode at 260 °C, oven temperature 70 °C. After 1 min, the injector split was opened and the oven temperature raised first to 160 °C at 15 °C min⁻¹, and then at 60 °C min⁻¹ to 300 °C. Helium (N47 grade, 99.997%, AirLiquid, Malmö, Sweden) was used as the carrier gas, flow rate 0.8 mL min⁻¹. The mass spectrometer was operated in the electron ionisation mode; the auxiliary temperature was 260 °C and the MS source temperature 230 °C.

The mass spectrometer was operated in the selected ion monitoring mode, using four quantification fragments at m/z (mass over charge) 411 for glyphosate, m/z 414 for glyphosate-1,2-¹³C₂¹⁵N, m/z 302 for AMPA, and m/z 303 for AMPA-¹³C¹⁵N. To confirm the identity of the respective compounds were used fragments m/z 384 and 511 for glyphosate, m/z 386 and 514 glyphosate-1,2-¹³C₂¹⁵N, m/z 272 and 371 for AMPA, and m/z 273 and 372 for AMPA-¹³C¹⁵N. The analytes and surrogate standards were considered positively identified if the ratios between the area of the quantification fragment and identification fragments were within ±30% of the ratios in an appropriate standard. The analytes were quantified by comparison with their respective isotope labelled analogue. Quantification was done with a five-point calibration curve, separate for each of the two analytes, covering the expected concentration range of the samples. The calibration curve was constructed by preparing a dilution series from a stock solution of the analytes with an equal amount of the surrogate standards added to each dilution, derivatized in the same way as the samples. The response is usually linear over a range of at least four orders of magnitude. Should any sample fall outside of the calibration curve, a new subsample may be taken out from the frozen sample and reanalysed at a different dilution. The derivatized calibration standards were used to create calibration curves for several sets of samples, but to check the derivatization procedure; at least one standard in the lower range of the expected concentrations and a procedural blank was included in each set of samples derivatized. The limit of quantification was set to the lowest standard concentration and the limit of detection to half this concentration (always more than three times the noise of the blanks). Every fifth injection on the GC-MS was a standard.

2.6 Quality assurance and control

A mixture of HPLC-grade and tap water (1:1) was used for blanks. The tap water was included to add some metal ions to the blanks as metal ions often plays a crucial role for the results. Within the environmental monitoring programme, the quantifications of glyphosate and AMPA are continuously monitored with a control chart (Miller and Miller 1993), and the recovery is usually 100-110% with a reproducibility of ±10-20% (n=20). Further, the method was tested within an international round robin study with reference samples of surface, ground, and tap water (Reichert, 2005), in which this analytical protocol performed well both for glyphosate and AMPA.

In the stability tests, the lowest standard concentration used to construct the calibration curve was 0.01 µg L⁻¹ which was also defined as the limit of detection (LOD). This concentration was chosen as it was 1% of the added glyphosate concentration. The LOD is discussed further in Section 4.3.

2.7 Data treatment

Statistical evaluations were performed using Prism 5.04 (GraphPad Software Inc., La Jolla, California, USA)

3. Results

Fig. 3 shows changes in concentrations over the 21 days of the stability test using all replicate data (not means) and additional statistics are presented in Table 2. In all cases the replicate data within each treatment were normally distributed. All regressions were linear when using a runs-test to test departure from linearity. All slopes were significantly ($P < 0.05$) different from zero (Table 2) except for one treatment (River Stångån: pH 2, darkness, 20 °C) indicating glyphosate losses irrespective of treatment, albeit small in most of the acidified incubations. Within treatments, regression slopes were equal for treatments at 4 °C (both acidified and at natural pH) and for acidified samples at 20 °C in darkness. On the other hand regression slopes were not equal within treatments in the daylight treatments or at natural pH and 20 °C (Table 2). These results indicate that glyphosate breaks down and/or is sequestered differently in different waters except when acidified and kept in darkness. Half-lives based on the linear regressions (Table 2) were calculated as half X-axis intercept.

By acidification on Day 21 of samples incubated at natural pH, part of the lost glyphosate was regained. When the samples had been incubated at 20 °C in daylight the measured concentrations of glyphosate were significantly higher ($P < 0.001$) after acidification than before, indicating that at least some of the glyphosate loss is due to reversible pH-dependent complexation with cations or colloids. It is noteworthy, though, that it was not possible to fully reverse the glyphosate loss by acidification on Day 21.

4. Discussion

4.1 Glyphosate stability

Irrespective of sampling temperature or light regime, acidification enhances the stability of the glyphosate concentrations in samples of natural water with only small losses over a three-week period. The best results were obtained with acidified samples kept at 4 °C in the dark. Both daylight and a higher temperature gave rise to increased losses of glyphosate. The results from the preliminary stability test with water from River Vendelån (Kreuger, 2003), although done with fewer replicates, are very similar to the results shown here, further strengthening that the results are general for Swedish surface waters.

The worst-case scenario is time-integrated sampling in daylight with neither refrigeration nor acidification, conditions during which a substantial part of the glyphosate will be lost. As glyphosate is stable to hydrolytic and photolytic degradation (Bleeke, 1998), the effects of light and temperature are probably due to enhanced microbial activity. But as time-integrated sampling will usually take place in the dark, light dependent microbial activity will not be a substantial problem. Even so, it is likely that glyphosate losses will occur during sampling and transport at ambient temperatures during the agricultural production season. Unless refrigeration

can be assured during the whole sampling and transport cycle, glyphosate losses seem inevitable if the samples are not acidified.

The data indicate, however, that there will be some loss of glyphosate even from acidified samples. In this study the shortest estimated half-life of glyphosate in acidified samples kept dark was 76 days (Table 2). As the half-life will vary with different waters and conditions it seems prudent to include a safety factor when sampling unknown waters to avoid undue loss of glyphosate. If a safety factor of ten is used, time-integrated sampling for glyphosate residues should continue for no longer than seven days. The shortest estimated half-life of glyphosate in samples at natural pH kept in the dark was 10 days (Table 2). Applying a safety factor of ten would in this case limit time-integrated sampling to a single day.

The increased concentrations observed after acidification on Day 21 indicate that some of the glyphosate loss is due to pH-dependent reversible glyphosate binding to metal ions, humic matter, inorganic colloids, or other agents. However, in no treatment was it possible to completely regain the lost glyphosate after four hours of shaking. This may, in part, be due to slow release of glyphosate from complexes and it cannot be excluded that more glyphosate would have been regained had the samples been left at a low pH for a longer time. But in a real-life situation letting samples stand for prolonged periods of time to release the analyte is impractical and also introduces the risk of degradation due to other processes. Keeping the samples at a low pH during the whole span of time-integrated sampling, therefore, seems to be recommended to avoid loss of glyphosate during sampling or sample transport and storage.

4.2 Particle vs. dissolved phase

It is important to point out that acidification prior to sampling will lead to the release of particle bound glyphosate during the sampling. Acidification breaks or prevents complexes between glyphosate and many cations (Ibáñez et al., 2006; Freuze et al., 2007), and also glyphosate bonds with colloids (Sheals, 2002). Thus this method will not allow the determination of particle bound and dissolved glyphosate separately. However, for the purpose of environmental monitoring within the European Union (EU), this is of little consequence, as the EU, with a few specific exceptions, recommends reporting concentrations of contaminants on a whole-water basis, i.e., dissolved and particle bound phases combined (EC, 2009). Further, due to the complex chemistry of glyphosate the separation of particle and dissolved phase will have to be made during sampling, e.g., by filtration or centrifugation in the field or very soon thereafter, if separate dissolved and particle bound glyphosate concentrations are called for. If particles and dissolved phase are not separated immediately glyphosate concentrations in the dissolved phase will likely be underestimated due to sequestration, and the results will not give a good picture of the field situation. If particles are separated from the dissolved phase in the field it will benefit the stability of glyphosate to rapidly acidify the respective phases for transport to the laboratory.

4.3 Consequences for environmental monitoring

The determination of glyphosate (and its degradation product AMPA) was based on a method described by Mogadati et al. (1996) modified to obtain higher reproducibility and lower detection limits. Passing the sample through a C18 solid phase extraction cartridge removes compounds with large hydrophobic functional groups from the samples prior to the ion-

exchange extraction of glyphosate and reduces the background noise substantially. This modified method has been successfully used in the Swedish national environmental monitoring programme (see, e.g., Törnquist et al., 2005b; SLU, 2012a). Acidifying the samples to break glyphosate complexes adds to the repeatability and lowers the detection limits of the method. Although the samples need to be neutralized for the ion exchange extraction, the re-sequestration of glyphosate is slow (Ibáñez et al., 2006; Freuze et al., 2007) and will not normally interfere with the ion exchange extraction provided the samples are not left standing at neutral pH.

If acidification is used to preserve the integrity of the glyphosate concentrations, this should be done considering the work safety of staff engaged locally to conduct the sampling. Adding the hydrochloric acid to the sampling bottles prior to shipping them from the lab reduces the risk of exposing field staff not trained in chemistry to the acid. It also keeps handling in the field to a minimum and avoids a source of contamination at the sampling station. Adding the acid in a sealed section of PVDF-tubing is practical as it is easy to identify sampling bottles with added acid, and to some extent keeps the acid from spreading all over the bottle with the risk of exposing the field staff. The molecular cut-off of the tubing is not ideal for this purpose (too large), but other commercially available sealable tubing tested added too much background noise to the samples making quantification difficult.

It should also be noted that when adding the whole acid load prior to sampling as suggested here, the pH in the samples will be very low at the beginning of the sampling, reaching pH ~2 only at the end of the sampling period. This will probably kill many microorganisms, and leave many others deactivated or in an environment substantially below their optimal pH for most of the sampling period. Early acidification may, therefore, in itself reduce any microbial degradation during sampling. Using refrigerated samplers will additionally lower any microbial activity.

For environmental monitoring programmes an LOD $<0.01 \mu\text{g L}^{-1}$ should be strived for. This is one order of magnitude below the maximum residue limit ($0.1 \mu\text{g L}^{-1}$) in the European Union water directive (EC, 2000). However, due to the complex chemistry of glyphosate the LOD may differ substantially between waters with different chemistry (Hanke et al., 2005; Törnquist et al. 2005a). Some authors, therefore, report different LODs for different types of water (Hanke et al., 2008). In environmental monitoring programmes it is practical to instead assign the lowest concentration of the standards used for the calibration curve as the LOD. For the Swedish national environmental monitoring programme an LOD of $0.01 \mu\text{g L}^{-1}$ defined this way is used successfully (Graaf et al., 2010). When defining the LOD of the analytical method used here as three times the baseline noise will give an LOD close to $0.001 \mu\text{g L}^{-1}$ for most surface waters in Sweden, similar to LODs reported by others (Hanke et al., 2008). However, occasionally water samples with so complex chemistry are encountered that the actual LOD is two or three orders of magnitude higher than the maximum residue limit of $0.1 \mu\text{g L}^{-1}$ (Törnquist et al., 2005a). If isotope labelled analogues of the analytes are used as surrogate standards such samples are easily identified by the low recovery of the surrogates. In some cases, glyphosate may still be quantified by adjusting the extraction volume and reporting an adjusted LOD for the sample, but at times no meaningful quantification is possible. Without isotope-labelled surrogate

standards, identification of such problem samples would be difficult, and many have likely been reported as false negatives.

5. Conclusions

This study shows that the environmental fate of glyphosate is complex and far from easily understood. An analytical method should, if optimal, be able to give good results for all types of water, or at least identify such samples that are problematic and may give rise to false negatives. In the case of glyphosate, because of its complex chemistry, availability of isotope labelled surrogate standards have been of utmost importance to identify samples that may give rise to false negatives.

A well-designed experiment with several different types of natural waters, including known problem waters, and including an in-depth investigation of the water chemistry and microbiology of each water sample, may make it possible via multivariate methods to gain further understanding of the environmental fate of glyphosate. Such studies should also include the kinetics of binding reactions in natural waters. This would potentially enable us to in the future gather better data in environmental monitoring programmes and enhance models of the environmental fate of glyphosate.

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Table 1

The pH at collection (natural) and after acidification of the samples at different stages of the stability tests (see Fig. 2 for an overview). In Fig. 3, the natural pH is denoted pH_n and the pH after acidification on Day 0 is denoted pH₂.

Water source	pH		
	Natural	After acidification Day 0	After acidification Day 21
Stångån	7.17	2.02	1.55 ^a ; 1.52 ^b ; 1.46 ^c
Helge å	7.36	1.97	
Havagyl	5.33	2.11	
Well	6.98	1.93	1.58 ^a ; 1.48 ^b ; 1.52 ^c

^adark/4 °C

^bdark/20 °C

^cdaylight/20 °C

Table 2

Regression results for treatments. For slopes and calculated half-lives, minima and maxima of any water source within each treatment are presented. Data from samples acidified on Day 21 were not included in the regressions.

Treatment	Slopes significantly non-zero	Linear regression deviation from linearity	Slopes equal	Slopes ^a	Half-life (days) ^a
pH2, 4 °C, Dark	All treatments	None significant	Yes P>0.2	-0.01 to -0.001	76 – 153
pH2, 20 °C, Dark	All, except Stångån	None significant	Yes P>0.7	-0.01 to -0.0001	81 – 168
pH2, 20 °C, Daylight	All treatments	None significant	No P<0.005	-0.01 to -0.002	41 – 337
pHn ^b , 4 °C, Dark	All treatments	None significant	Yes P>0.7	-0.03 to -0.008	21 – 42
pHn, 20 °C, Dark	All treatments	None significant	No P<0.01	-0.05 to -0.02	10 – 20
pHn, 20 °C, Daylight	All treatments	None significant	No P>0.6	-0.06 to -0.02	10 – 12

^aLowest and highest estimates

^bNatural pH, see Table 1

Fig. 1. Sites from which water samples were taken for stability tests. 1) River Vendelån, 2) River Stångån, 3) Lake Havagyl and groundwater Well, 4) River Helge å.

Fig. 2. Flow chart over the stability tests. Note that only samples from River Stângân and the well were acidified after incubation at original pH.

Fig. 3. Effects of different treatments on concentrations of glyphosate. Means and standard deviations are shown. Among treatments, pHn refers to incubation at the natural pH of the sample, and pH2 incubation of samples acidified to approximately 2. The actual pH at which the respective incubation took place is given in Table 1. Concentrations at 21a days are for samples from Day 21 that were acidified (see Fig. 2). Results of linear regressions are shown in Table 2.

Electronic supplemental material

Time-integrated sampling of glyphosate in natural waters

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Time-integrated sampling procedure

The standard sampling method currently used for the Swedish national environmental monitoring programme follows the recommendation of the Swedish Environmental Protection Agency that, in general, time-integrated sampling is preferable to discrete sampling for environmental monitoring of pesticides (SEPA, 2004). The sampling method is described here to show how the stability of glyphosate has been taken into account in this sampling method. Further details on other aspects of the sampling are provided by SEPA (2004) and variations are noted reports from participating laboratories.

One-week time-integrated samples are collected using one of ISCO models 2700, 2700R or 3700FR samplers (ISCO Inc., Nebraska, USA) collecting a few mL of water each hour over the sampling period. Samples for glyphosate determination are collected in 2-L ethanol-rinsed high-density polyethylene bottles. To lower the pH of the samples during collection and transport, hydrochloric acid (6 mL, 6 mol L⁻¹) in a 12-cm length of heat-sealed Spectra/Por Biotech polyvinylidene difluoride (PVDF) tubing (cut-off 250 000 D, Spectrum Laboratories, Rancho Dominguez, CA, USA) was added before shipping the sampling bottle to the sampling site (Kylin, 2003). Samples obtained for the environmental monitoring programme (acidified and refrigerated sampler) usually has a pH of approximately 2 on return to the laboratory and have been transported in a cooler.

For environmental monitoring data, refer to the yearly monitoring reports (e.g., Törnqvist et al., 2005), or the monitoring data base for pesticides (SLU, 2012).

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Fig S1. Setup for extraction of several water samples in parallel