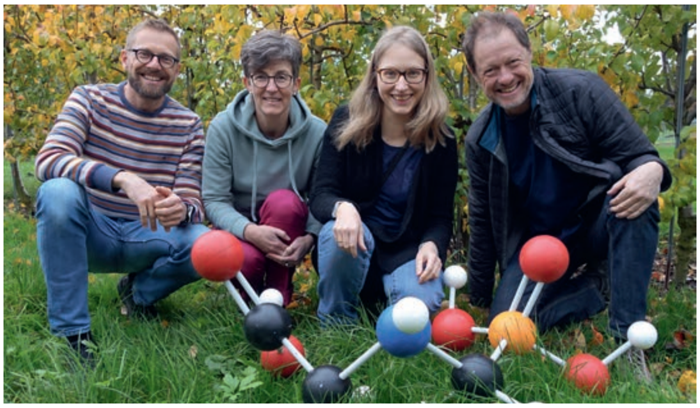
doi:10.2533/chimia.2020.156 Chimia 74 (2020) 156–160 © T. Poiger, M. Keller, I. J. Buerge, M. E. Balmer

Behavior of Glyphosate in Wastewater Treatment Plants

Thomas Poiger*[a], Martina Keller[b], Ignaz J. Buerge[a], and Marianne E. Balmer[a]

Abstract: The herbicide glyphosate is frequently detected in surface waters and its occurrence is linked to agricultural as well as urban uses. Elevated concentrations downstream of wastewater treatment plants (WWTPs) suggest that municipal wastewater is an important source of glyphosate in surface waters. We therefore conducted a study at a typical municipal WWTP in Switzerland to characterize the seasonality of glyphosate occurrence, the removal efficiency, and the processes involved in glyphosate removal. Glyphosate was present in raw (mechanically treated) wastewater during the whole study period (April to November). A lab incubation experiment with activated sludge indicated negligible degradation of glyphosate. Lack of degradation combined with strong adsorption lead to substantial enrichment of the compound in the sludge. Due to this enrichment and the long residence time of activated sludge (several days, compared to hours for wastewater itself), concentrations in treated wastewater show comparatively little variation, whereas concentrations in raw wastewater may fluctuate considerably. Overall removal efficiencies were in the range of 71–96%. This behavior could be described qualitatively using a numerical model that included input of glyphosate *via* raw wastewater, adsorption to activated sludge, and export *via* treated wastewater and excess sludge, but excluded degradation processes.

Keywords: Activated sludge · Adsorption · Glyphosate · Wastewater treatment



Ignaz Bürge studied environmental sciences at the Swiss Federal Institute of Technology in Zurich (ETH) and obtained his PhD in 1999 at the Swiss Federal Institute of Environmental Science and Technology (Eawag). Since 2000, he works as an expert for the environmental fate of pesticides at Agroscope, for the national assessment of pesticides and with own research projects.

Marianne Balmer studied environmental sciences at the Swiss Federal Institute of Technology in Zurich (ETH) and obtained her PhD in 1999 at the Swiss Federal Institute of Environmental Science and Technology (Eawag). Since 2001, she works as an expert for the environmental fate of pesticides at Agroscope, for the national assessment of pesticides and since 2013 she is head of the Plant Protection Chemistry group at Agroscope.

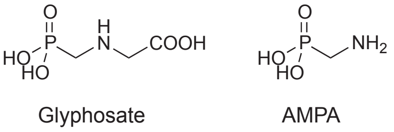
Martina Keller studied agricultural sciences at the Swiss Federal Institute of Technology in Zurich (ETH). She worked from 2008 to 2009 in the Plant Protection Chemistry group at Agroscope. She obtained her PhD in 2014 at the University of Hohenheim (Germany) in the field of weed science. Since 2013, she works as a weed scientist and as an efficacy evaluator in the Vegetable-Production Extension group at Agroscope.

Thomas Poiger received his degree in chemistry in 1990 from the Swiss Federal Institute of Technology (ETH), Zurich. He completed his PhD in 1994 at the Swiss Federal Institute of Environmental Science and Technology (Eawag). After 2 years as post-doctoral fellow at the U.S. Environmental Protection Agency in Athens, GA, he joined the Plant Protection Chemistry group at Agroscope.

1. Introduction

Glyphosate is the most used herbicide worldwide with an estimated annual application of 8.3×10^8 kg active substance in 2014, primarily on GMO crops.[1] In Switzerland, annual glyphosate use in agriculture reached 3.8×10^5 kg in 2011 and since then decreased to 1.5×10^5 kg in 2018,[2] with main areas of application (by area) as pre-emergence treatment in maize and post-harvest treatment in cereals. Hence, glyphosate is widely detected in surface waters. In Swiss streams, concentrations frequently exceeded 1 µg/L.[3] While arguably a large portion of glyphosate in surface waters originates from agricultural fields, primarily due to runoff during rain events, urban areas also contribute to overall loads as the compound is consistently detected in municipal wastewater.[3,4]

Aminomethylphosphonic acid (AMPA) is the main metabolite of glyphosate in soils (Scheme 1).[5] AMPA is also frequently



Scheme 1. Structures of glyphosate and its main soil metabolite aminomethylphosphonic acid (AMPA).

---

*Correspondence: Dr. T. Poiger, E-mail: thomas.poiger@agroscope.admin.ch
[a]Agroscope, Plant Protection Chemistry, Müller-Thurgau-Strasse 29, CH-8820 Wädenswil, Switzerland, [b]Agroscope, Vegetable-Production Extension, Müller-Thurgau-Strasse 29, CH-8820 Wädenswil, Switzerland

detected in surface waters and municipal wastewater at somewhat higher concentrations than glyphosate.[3b] AMPA is also a metabolite of many other phosphonate compounds that are expected to be discharged with municipal wastewater.[6] Therefore, its occurrence in surface waters may not necessarily be indicative of glyphosate use in the catchment.

Municipal wastewater as a source of glyphosate in surface waters was investigated in this study with respect to the seasonality of occurrence, the dynamics of glyphosate concentrations in in- and effluents of WWTPs, the elimination of glyphosate during treatment and the mechanisms responsible for this elimination.

2. Experimental

2.1 *Wastewater and Activated Sludge Samples*

Most samples were collected at the municipal WWTP of Horgen, Switzerland. Wastewater treatment at this WWTP consists of mechanical, biological (activated sludge treatment; hydraulic retention time excluding secondary clarification, 3.6 h; sludge retention time, 4–6 days), and chemical (phosphate removal by co-precipitation with iron oxides during activated sludge treatment) stages. The effluent from activated sludge treatment is further clarified by sand filtration.[7] Wastewater was collected on 49 days between 15. April and 12. November, 2009, as 24-h flow-proportional composite samples (7 a.m. – 7 a.m.) at the primary effluent (effluent of mechanical treatment), secondary effluent (after biological/chemical treatment) and final effluent (after sand filtration). Activated sludge was sampled on 29 days during the study (as grab samples directly from the activated sludge basin) on the same days as the water samples were collected. Water samples were spiked with internal standards (see below) upon arrival at the laboratory and then stored at 4 °C until analysis (within 2–3 d of collection). Activated sludge samples were processed within 2–3 h of collection.

Additional (grab) samples of primary effluent, activated sludge, and recycled sludge were collected for laboratory incubation experiments from the municipal WWTP Wädenswil. This WWTP is very similar to the WWTP Horgen.[8]

2.2 *Analytical Methods*

Glyphosate and AMPA were analyzed as described previously.[3b] Briefly, isotopically labelled internal standards ($^{13}C_2{}^{15}N$-glyphosate and $^{13}C^{15}ND_2$-AMPA) were added and an aliquot of the sample was subjected to derivatization with FMOC-Cl. The derivatized sample was analyzed after on-line solid-phase extraction by HPLC-MS/MS. Activated sludge samples were first brought to pH≈12 by addition of 1 M aqueous NaOH (and left for ≈1.5 h to release the fraction of glyphosate and AMPA adsorbed to the suspended solids), centrifuged, and the supernatant was diluted 1:10 with 'fossil' groundwater (Aqui, Zurich) and then processed in the same way as the water samples (to yield the sum of the dissolved and the adsorbed fraction). More details of the analytical method for glyphosate and AMPA in activated sludge are provided in the Supplementary Information.

2.3 *Adsorption Experiments*

Adsorption of glyphosate and AMPA was studied in freshly collected activated sludge from WWTP Wädenswil (pH = 7.2; suspended solids content, 2.7 g/L). Aliquots of 20 mL were spiked with 10 µg/L glyphosate or AMPA and the pH was adjusted in the range of 5.0–11.7 by addition of 0.1 M aqueous HCl or NaOH, respectively. The samples were left for 1.5 h with occasional shaking to allow for equilibration. The adequacy of this equilibration time was verified in preliminary experiments (Supplementary Information, Fig. S4). Then, the samples were centrifuged at an RCF of 405 g for 5 min, and an aliquot of the clear supernatant was diluted 1:10 with 'fossil' groundwater, spiked with internal standard, derivatized, and analyzed by LC-MS/MS. The measured concentration was considered to correspond to the dissolved fraction and was expressed in % of the total concentration.

2.4 *Degradation Experiment in a Simulated Activated Sludge Treatment*

Biodegradability of glyphosate and AMPA was investigated under laboratory conditions in a simulated activated sludge treatment system. Primary effluent (0.5 L) from the WWTP in Wädenswil was mixed with recycled sludge (0.5 L; suspended solids content, 3.62 g/L), thus simulating typical operating conditions. The suspension was spiked with glyphosate and AMPA corresponding to 20 µg/L each. A sterile control (autoclaved at 120 °C during 20 min before incubation) was run in the same way.

The suspensions were kept at ambient temperature (≈20 °C) and aerated with water-saturated, compressed air through a glass frit at a flow rate of ≈100 mL/min. Periodically, duplicate 10 mL-samples were removed and analyzed for 'dissolved' and 'total' concentrations as described above.

2.5 *Simulation of Glyphosate Behavior During Activated Sludge Treatment*

A basic model of an activated sludge system was set up to simulate the behavior of glyphosate and the removal efficiency under varying conditions. The simulation tool AQUASIM Ver. 2.1g was used.[9] The system was defined with a small set of adjustable parameters including the hydraulic residence times of wastewater and activated sludge, the volumetric mixing ratio of recycled sludge and primary effluent at the inlet of the activated sludge basin, the concentration pattern in primary effluent, and the adsorption coefficient of glyphosate to sludge. A more detailed description of the model setup is provided in the Supplementary Information.

3. Results & Discussion

3.1 *Seasonal Occurrence of Glyphosate and AMPA in Wastewater*

Concentrations of glyphosate in primary effluent, secondary effluent, and final effluent of WWTP Horgen over the course of 8 months are shown in Fig. 1. During this period, glyphosate was always detectable (limit of quantification, 0.005 µg/L). Concentrations in primary effluent showed maxima in May, June, and August (up to 8.3 µg/L) and were comparatively low at the beginning of the campaign in April and towards the end, in October and November. Concentrations in final effluent followed the same qualitative trend, but were clearly lower (range, 0.047–0.58 µg/L; median, 0.16 µg/L). Overall, concentrations in treated wastewater were within the range measured at other WWTPs and at the upper end of those measured in Swiss streams.[3] Urban use of glyphosate thus appears to lead to significant discharge *via* wastewater over prolonged periods of time.

Elimination of glyphosate during activated sludge treatment, as calculated from the concentrations in primary and secondary effluent, ranged from 33–93% (median, 67%). An additional 16–79% (relative to secondary effluent; median, 65%) were eliminated during sand filtration, highlighting the importance of this treatment step. Overall elimination of glyphosate ranged from 71 to 96% (median, 87%). Note that water samples from the respective sampling points were always taken during the same 24 h period. As the residence time of the water in the activated sludge basin, secondary clarifier, and sand filter is in the range of 5–10 h, the sampled water is thus not from the exact same water package. Nevertheless, while single values of the calculated elimination rates may contain some degree of uncertainty, the dataset as a whole should be indicative of the elimination behavior of glyphosate. For AMPA (Supplementary Information, Fig. S1), similar removal rates were observed during activated sludge

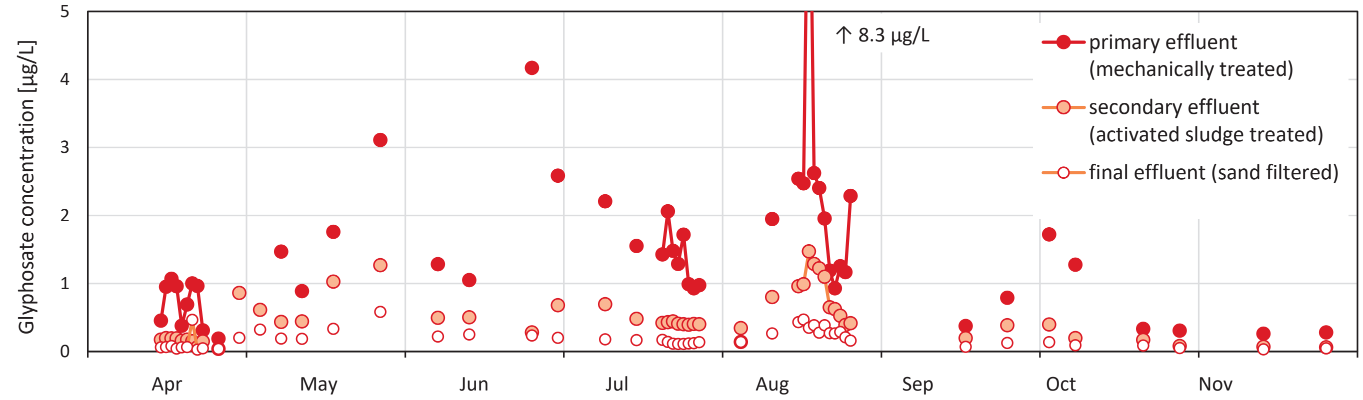


Fig. 1. Seasonal variation of glyphosate concentrations in wastewater at different treatment stages at the WWTP Horgen, Switzerland, from April to November 2009. Symbols represent data from 24 h flow-proportional composite samples; lines connect points where samples were taken on consecutive days.

treatment (36–89%; median, 64%), but elimination during sand filtration was lower (3 - 51%; median, 31%). Overall elimination was 51–94% (median, 74%).

On three occasions (April, July, and August), consecutive samples were analyzed over 8-10 days. In all three cases, day-to-day fluctuations were more pronounced in primary effluent. Particularly in August, fluctuations were evident with a factor of 8 between highest and lowest concentration. In comparison, concentrations fluctuated much less in secondary effluent and hardly at all in final effluent. The two treatment stages thus appear to act as a buffer that smooths out short fluctuations in primary effluent.

3.2 *Strong, pH-Dependent Adsorption to Activated Sludge*

Adsorption of glyphosate and AMPA to activated sludge was strong at pH <8, but weak at pH >10 (Fig. 2). Glyphosate has 3 ionizable protons (2 at the phosphonic acid group and 1 at the carboxylic acid group). Furthermore, the secondary amino group can be protonated (Scheme 1). AMPA has 2 ionizable protons and a primary amino group that can be protonated. In the pH range 1–10, glyphosate is zwitterionic, thus carrying a positive charge at the (protonated) amino group and 1–3 negative charges.[10] Above pH≈10, glyphosate is anionic (*e.g.* the amino group is uncharged). If the adsorption curve in Fig. 2 is interpreted as a titration curve, fitting yields a pK_a value of 9.8 (for AMPA, 8.9). This is somewhat lower than the range of pK_3 values for glyphosate compiled by Franz *et al.*,[10] which are in the range of 10.0 to 10.9. However, iron oxides seem to be decisive for the adsorption of glyphosate (and AMPA) to activated sludge.[11] These iron oxides are formed from iron salts added to the activated sludge basin to remove phosphate *via* co-precipitation. The adsorption of glyphosate and AMPA to these iron oxides not only depends on the speciation of glyphosate but also on the changes in the surface properties of the iron oxides with changing pH.

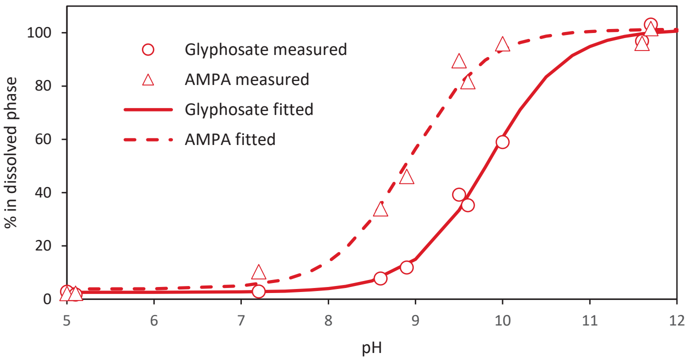


Fig. 2. Sorption of glyphosate and AMPA to activated sludge (2.7 g suspended solids/L) as a function of pH.

At ambient pH (7.2) glyphosate was mostly adsorbed to sludge particles with only ≈2.9% remaining in solution. Considering the suspended solids concentration in the sludge (2.7 g dry weight/L), this leads to an apparent adsorption K_d value of 12'400 mL/g which is much higher than the range of adsorption values for glyphosate observed in soils (K_f 33–700 mL/g[5]). This stronger sorption is probably due to the high proportion of amorphic iron oxides in sludge. Adsorption K_d values determined in a total of 29 activated sludge samples taken at the WWTP Horgen over the course of this study ranged from 6'800 to 33'000 mL/g (median, 19'700 mL/g), those for AMPA from 3'900 to 17'000 mL/g (median, 8'400 mL/g).

Concentrations in activated sludge samples observed during the monitoring campaign at WWTP Horgen thus always were 1–2 orders of magnitude higher than corresponding concentrations in secondary effluent (Supplementary Information, Fig. S2). The sludge, which is recycled in the system, can be considered as a reservoir that accumulates or releases glyphosate, depending on the concentration in the primary effluent.

3.3 *Negligible Degradation During Activated Sludge Treatment*

In soil, glyphosate is fairly rapidly degraded (median half-life in soil, 5.8 d), but often with a considerable deceleration over time (time to 90% degradation up to 1600 d).[5] However, in our laboratory-scale activated sludge system, glyphosate was not degraded during an incubation time of 4.5 h (Fig. 3). Likewise, no degradation was observed for AMPA (Fig. 3) and no degradation was observed in the sterile controls (Supplementary Information, Fig. S5).

Further indication that glyphosate is not degraded in the activated sludge system comes from the fact that the herbicide is strongly enriched in the sludge compared to the concentration in the input water. In fact, concentrations in activated sludge, on average, were ≈30 times higher than in primary effluent. Indeed, for strongly adsorbing compounds, not only the hydraulic residence time of the wastewater in the activated sludge system (a few hours) is relevant, but also the average residence time of the sludge, which is in the order of days. Substantial enrichment in activated sludge, however, means that degradation of glyphosate not only is negligible during a few hours of water treatment but also during the residence time of the sludge.

3.4 *Simulation of Glyphosate Behavior in Activated Sludge Treatment*

To better understand the behavior of glyphosate in the activated sludge system (including enrichment of the compound in

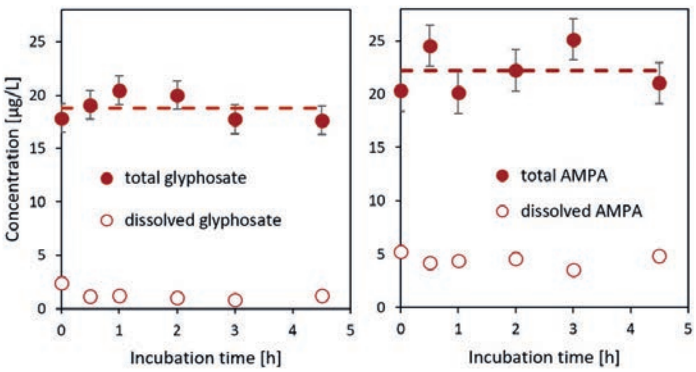


Fig. 3. Incubation experiment with glyphosate (left) and AMPA (right) in a simulated activated sludge treatment system. The dashed line indicates the mean of all measured total (dissolved + adsorbed) concentrations.

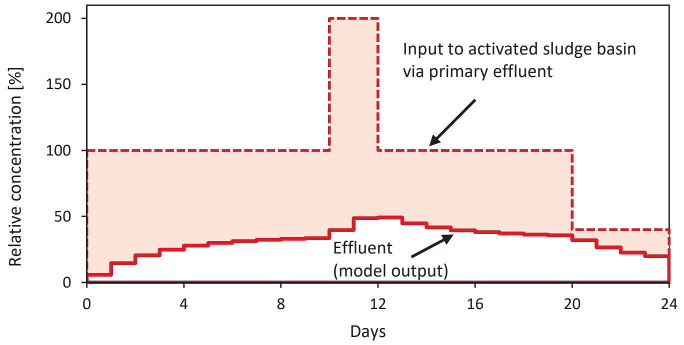


Fig. 5. Simulated glyphosate concentrations in secondary effluent assuming different glyphosate input regimes (dashed line). The shaded area corresponds to the glyphosate mass that is eliminated during treatment.

sludge and recycling of the sludge in the system), a basic model of an activated sludge system was set up. In this model, elimination was assumed to occur solely due to adsorption to activated sludge while degradation of glyphosate was not included. The mass flows of glyphosate calculated with the model are qualitatively illustrated in Fig. 4. In a steady-state situation, the input mass flow from primary effluent is at least one order of magnitude smaller than the mass flow entering the basin with recycled sludge (not drawn to scale). Export of glyphosate from the system occurs at two points, *via* treated water and *via* excess sludge (primarily adsorbed fraction).

The dynamic behavior simulated with this model is illustrated in Fig. 5 based on an assumed input pattern with constant input during 10 d, followed by a peak input during 2 d, then another 8 d of constant input followed by a period where the input is 60% lower. At the beginning, effluent concentrations increase slowly until a steady state is reached after several days of continuous loading. During this time, glyphosate is accumulated in the sludge, which remains in the system much longer than the wastewater.

The peak input results in only a small increase in effluent concentrations. Likewise, the effluent response to reduced input loading is slow so that, for a short period, concentrations in in- and effluent are actually equal. Qualitatively the model thus describes the observed behavior of glyphosate well, both in terms of removal efficiency and in terms of smoothening of fluctuating influent concentrations.

4. Conclusions

Glyphosate is present in municipal wastewater almost year round, pointing to considerable urban use in the catchment. The compound was removed at WWTP Horgen to varying extents (overall elimination was in the range of 71-96%). Based on our findings, in WWTPs employing activated sludge treatment as means of biological treatment, biodegradation of glyphosate is not expected to contribute significantly to the removal. This may, however, be different in WWTPs employing other biological treatment processes. Adsorption to sludge was demonstrated to be the primary removal process in activated sludge treatment (median elimination, 67%). Given the strong adsorption of glyphosate to activated sludge, much higher elimination may be expected. However, due to the continuous input, glyphosate accumulates in sludge, which in turn limits the removal efficiency. The observed, strong adsorption to activated sludge most likely is due to the presence of iron oxides which are formed from iron(II) salts added to chemically remove phosphate *via* co-precipitation. Even though this is the most common type of treatment, other systems, such as biological phosphate removal are also employed and it is conceivable that in such systems, elimination of glyphosate *via* adsorption may be far less important.

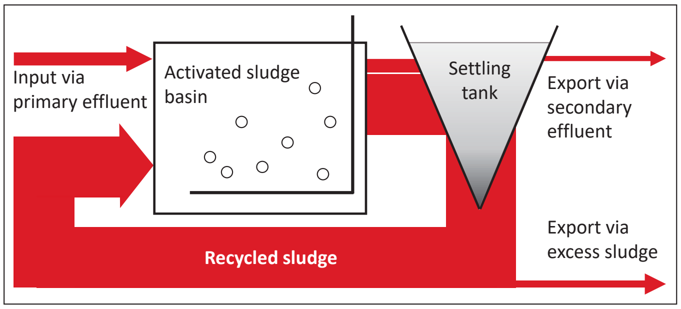


Fig. 4. Model setup of an activated sludge system. The arrows qualitatively indicate the glyphosate mass associated with different water masses. The model assumes sorption to sludge but no degradation. Consequently, the only two export routes are secondary effluent (high volume/low concentration) and excess sludge (low volume/high concentration). Note the large glyphosate mass that is recycled within the system (not to scale, mass flow of glyphosate in recycled sludge is ≈30 times that in primary effluent).

Supplementary Information

Supplementary information is available on *https://www.ingentaconnect.com/content/scs/chimia*

Acknowledgements

The authors gratefully acknowledge the assistance of the staff at the WWTPs Horgen and Wädenswil with sampling of wastewater and sludge. The project was funded in part by the Swiss Federal Office of the Environment FOEN.

Received: November 15, 2019

[1] C. M. Benbrook, *Environ. Sci. Europe* **2016**, *28*, 1, DOI: 10.1186/s12302-016-0070-0.

[2] Bundesamt für Landwirtschaft (BLW), ‘Verkaufsstatistik von Pflanzenschutzmitteln in der Schweiz, Verkaufszahlen der 10 meistverkauften Pflanzenschutzmittel-Wirkstoffe’, **2019**.

[3] a) I. Hanke, I. Wittmer, S. Bischofberger, C. Stamm, H. Singer, *Chemosphere* **2010**, *81*, 422, DOI: 10.1016/j.chemosphere.2010.06.067; b) T. Poiger, I. J. Buerge, A. Bächli, M. D. Müller, M. E. Balmer, *Environ. Sci. Poll. Res.* **2017**, *24*, 1588, DOI: 10.1007/s11356-016-7835-2.

[4] a) F. Botta, G. Lavison, G. Couturier, F. Alliot, E. Moreau-Guigon, N. Fauchon, B. Guery, M. Chevreuil, H. Blanchoud, *Chemosphere* **2009**, *77*, 133, DOI: 10.1016/j.chemosphere.2009.05.008; b) S. Huntscha, M. A. Stravs, A. Bühlmann, C. H. Ahrens, J. E. Frey, F. Pomati, J. Hollender, I. J. Buerge, M. E. Balmer, T. Poiger, *Environ. Sci. Technol.* **2018**, *52*, 4641, DOI: 10.1021/acs.est.8b00314; c) D. W. Kolpin, E. M. Thurman, E. A. Lee, M. T. Meyer, E. T. Furlong, S. T. Glassmeyer, *Sci. Total Environ.* **2006**, *354*, 191, DOI: 10.1016/j.scitotenv.2005.01.028.

[5] European Food Safety Authority (EFSA), *EFSA Journal* **2015**, *13*, 4302, DOI: 10.2903/j.efsa.2015.4302.

[6] B. Nowack, *Water Res.* **2003**, *37*, 2533, DOI: 10.1016/s0043-1354(03)00079-4.

[7] Tiefbauamt, 'Broschüre zur Abwasserreinigungsanlage Horgen - Oberrieden', 2011, available at *https://www.horgen.ch/_docn/168439/1104_ARA_Broschuere_2011.pdf*, accessed 14. Nov. 2019.

[8] Amt für Abfall, Wasser, Energie und Luft (AWEL), Informationsblatt zur ARA Wädenswil Rietliau, available at *https://www.hw.zh.ch/ara/Waedenswil.pdf*, accessed 14. Nov. 2019.

[9] P. Reichert, *Water Sci. Technol.* **1994**, *30*, 21 DOI: 10.2166/wst.1994.0025.

[10] J. E. Franz, M. K. Mao, J. E. Sikorski, 'Glyphosate: A Unique Global Herbicide', American Chemical Society, **1997**.

[11] a) A. L. Gimsing, O. K. Borggaard, *Clays and Clay Minerals* **2001**, *49*, 270, DOI: 10.1346/CCMN.2001.0490310; b) A. L. Gimsing, O. K. Borggaard, *Clays and Clay Minerals* **2007**, *55*, 108, DOI: 10.1346/CCMN.2007.0550109; c) J. Sheals, S. Sjöberg, P. Persson, *Environ. Sci. Technol.* **2002**, *36*, 3090, DOI: 10.1021/es010295w.

License and Terms