Environmental Sciences Europe

RESEARCH

Open Access

Comparative analysis of detection techniques for glyphosate in urine and in water

Christian Vélot[1,2*], Frédéric Poitou[3] and Joël Spiroux de Vendômois[2]

Abstract

Background: Glyphosate is the declared active component of the most extensively used herbicides in the world, and is therefore widely present in the environment. Glyphosate urinary levels represent a relevant biomarker for each individual's exposure to glyphosate-based herbicides. However, GLY urine level measurement is controversial because different detection methods have led to contradictory results, especially in the cases of enzyme-linked immunosorbent assay (ELISA) *versus* liquid chromatography coupled to tandem mass spectrometry (LC/MS–MS) for urine, and ELISA *versus* high-performance liquid chromatography coupled to fluorescence detection (HPLC/Fluo) for water.

Methods: We compared the ELISA method to the LC/MS–MS or HPLC/Fluo one by submitting to two laboratories (Biocheck, Germany and Labocéa, France, respectively) identical urine and water samples, spiked or not with precise concentrations of glyphosate, but also with two chemically similar molecules: glycine and aminomethylphosphonic acid, GLY's analogue and primary metabolite, respectively.

Results: Both laboratories claimed similar glyphosate quantification threshold (LOQ): 0.08 and 0.05 ng/mL, respectively. Each one of the tested methods proved to be specific for glyphosate and therefore did not result in any cross-detection with glycine and aminomethylphosphonic acid. However, these methods showed differences both in reproducibility and reliability depending on the matrix used (water or urine).

Conclusion: While the ELISA method gave less accurate results than the HPLC/Fluo technique when applied to water samples, the glyphosate concentrations measured in urine were much more reliable and reproducible with the ELISA technology than those obtained with the LC/MS–MS one.

Keywords: ELISA, Glyphosate, LC/MS–MS, Limit of quantification (LOQ), Urine

Introduction

Glyphosate-based herbicides (GBHs) are non-selective, broad-spectrum herbicides and the most widely used pesticides in the world [1]. Glyphosate (GLY), N-(phosphonomethyl) glycine, is—as its name suggests—an amino-phosphonic analogue to the natural amino acid glycine. The main biodegradation product of GLY is (aminomethyl) phosphonic acid (AMPA). GLY was patented as a herbicide active ingredient in 1971 (U.S Patent No 3,799,758) by the US firm Monsanto (later acquired by the German company Bayer in 2018). The first GBHs were introduced in the pesticides market in 1974 under the popular trade name Roundup®, and others followed: Glyphogan®, Touchdown®, Glifoglex®. All are mixtures of GLY with other chemicals used to facilitate penetration of GLY through plant epidermis, and increase its stability and activity [2, 3]. GBHs, which represent more than 750 formulations [4], are extensively used in intensive farming, home gardens, forestry, landscaping and wetlands management. Their use has increased dramatically since 1996, especially with the development of Roundup®-tolerant crops [1], which include the vast majority of the genetically modified (GM) plants

*Correspondence: christian.velot@universite-paris-saclay.fr

[1] Laboratory VEAC, University Paris-Saclay, Faculty of Sciences, Bât. 350, Avenue Jean Perrin, 91405 Orsay, France
Full list of author information is available at the end of the article

commercially grown around the world [5]. GBHs' usage keeps increasing with the emergence of tolerant weeds induced by the widespread application of these herbicides: the proliferation of such weeds requires more frequent spraying and at higher concentrations [1].

Such extensive use of GBHs raises the question of population exposure, both in occupational settings and via drinking water and food. GLY has been detected in the dust of non-agricultural homes [6], and both GLY and AMPA have been found in soils, water, plants, animals and food [7–9]. In particular, GLY has been shown to accumulate in Roundup-tolerant GM soybeans [10]. This is more relevant as most animals raised in intensive farming are fed with soycakes made from such GM soybeans [11, 12] mainly grown in the United States, Brazil and Argentina [5]. Moreover, GLY and AMPA may also be dispersed by wind and water erosion [13].

Despite an abundant scientific literature reporting adverse environmental and health outcomes of GLY and GBH (for reviews: [14–16]), the adverse impacts of GBHs continue to trigger controversial debates in public, scientific and political spheres [17–19]. Ongoing debate has led to scrutiny of evaluation procedures and analytical methodologies [20].

The routes by which GLY enters the body are dermal, oral and inhalation [2, 21, 22]. Even if the dermal route allows a poor absorption (about 2%), it is the main reported route of entry for exposed farmers [23]. Once in the body, GLY accumulates mainly in the detoxification organs (kidneys and liver), colon, small intestine, as well as in bone and bone marrow, and is eliminated mostly via the feces (90%) but also in urine [2]. The detection of GLY in urine is therefore an easy way to assess the exposure and contamination of individuals with GBHs [24, 25]. However, GLY in urine is also controversial since contradictory results have been obtained depending on the detection methods used. A vast campaign was launched in France to detect GLY in urine, and all tests conducted in a laboratory (Biocheck, Germany) using the enzyme-linked immunosorbent assay (ELISA) method were found to be positive [26]. Other studies commissioned by other individuals and carried out in other laboratories (for instance, Labocéa, France) using liquid chromatography coupled with tandem mass spectrometry (LC/MS–MS) gave systematically lower rates or even negative results.

The aim of this study was to carry out a comparative analysis of these two detection techniques for GLY in urine by subjecting identical samples to the two laboratories, supplemented or not with accurate known concentrations of GLY, glycine or AMPA. Supplementation with glycine or AMPA was intended to test a possible cross-detection of chemically similar molecules. Samples of tap water were also submitted to the two laboratories, in order to know if the accuracy of the measured glyphosate levels could be matrix-dependent.

Materials and methods

Collection of original samples

A sample of tap water of at least 400 mL was collected in a sterile 500 mL vial. Two samples of morning fasting urine (at least 400 mL each) from two individuals were collected in sterile 500 mL vials.

Chemicals

N-(phosphomethyl) glycine (GLY; CAS: 1071–83-6, also known as "technical glyphosate", Glycine (CAS: 56–40-6), and (Aminomethyl) phosphonic acid (AMPA; CAS: 1066-51-9) were purchased from Sigma-Aldrich. For each one of these three chemicals, a 1 mg/mL stock solution was obtained by dissolving 10 mg in 10 mL of ultrapure water (Millipore). Each stock solution was then serially diluted (two successive 10^{-2} dilutions) in ultrapure water to obtain final 0.1 µg/mL solutions.

Protocol design

The protocol design is summarized in Fig. 1. Each one of the three original samples (one of water and two of urine) was distributed evenly into 8 sterile medical grade polypropylene tubes of 50 mL (8 × 50 mL). For each set of 8 tubes, the first (A, I, Q) served as a control (no chemicals added). 50 µL (5 ng), 250 µL (25 ng) and 1000 µL (100 ng) of the final GLY solution was added to the 2nd (B, J, R), 3rd (C, K, S) and 4th (D, L, T) tubes, respectively. 250 µL (25 ng) and 1000 µL (100 ng) of the final glycine solution was added to the 5th (E, M, U) and 6th (F, N, V) tubes, respectively. 250 µL (25 ng) and 1000 µL (100 ng) of the final AMPA solution was added to the 7th (G, O, W) and 8th (H, P, X) tubes, respectively.

For each series, 40 mL of each one of the eight tubes was then evenly distributed in four tubes (4 × 10 mL): two tubes (identical) destined to the first analysis laboratory (Biockeck, Germany), and two others (identical) destined to the second analysis laboratory (Labocéa, France). All the tubes sent to the same laboratory (16 per series, i.e., per original sample) were randomly numbered so that the laboratory could neither identify them nor detect duplicates. The final tubes were packaged in dry ice and shipped by a transporter specialized in the transport of biological samples. Each package contained a single reading temperature probe.

Experiment supervision and monitoring

This comparative analysis being likely to be cited in the context of legal proceedings, the complete experimental process (from the reception of chemical standards, and collection of the original samples to the shipment)

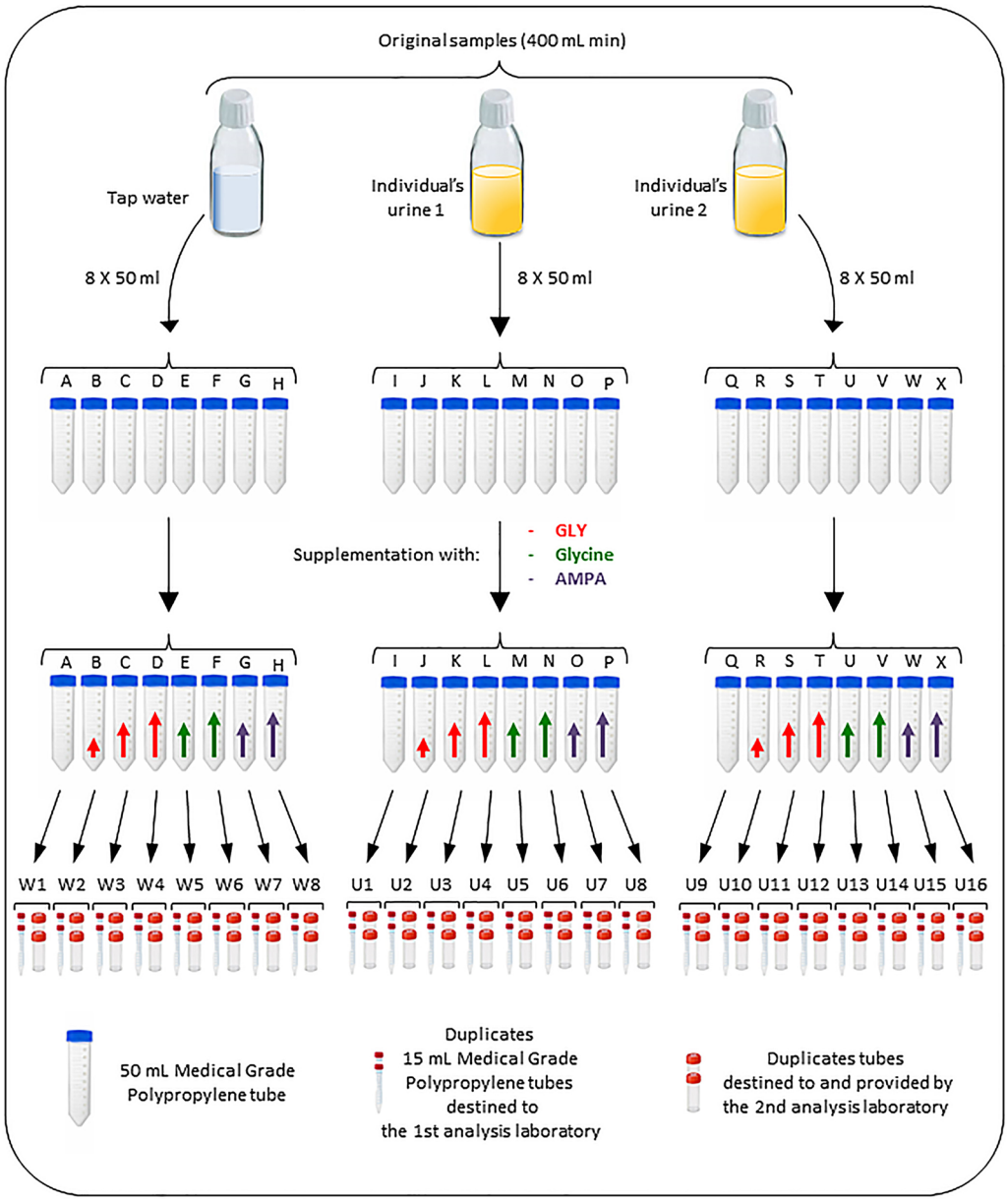

Fig. 1 Outline of the protocol design. Details for GLY, glycine and AMPA supplementation are described in the "Materials and methods" section

was supervised by a legal chemist expert to the French and European courts, and was followed and photographed by a judicial officer. In addition, the entire experience was filmed in a one-shot time-stamped take. Upon receipt of the packages, each of the two laboratories returned the above-mentioned temperature probe to the judicial officer who read it in order to ensure that there had been no break in the cold chain during transport.

Results and discussion

While Biocheck laboratory used ELISA technology for glyphosate detection both in water and urine, Labocéa laboratory used two different technologies depending on the matrix: high-performance liquid chromatography coupled to fluorescence detection (HPLC/Fluo) for water samples and LC/MS–MS for urine samples.

The results obtained for the water samples are presented in Table 1. Limit of quantification (LOQ) was 0.08

Table 1 Measured and expected GLY concentrations ([GLY]) in tap water samples

Sample	W1		W2		W3		W4		W5		W6		W7		W8	
Supplementation (ng/mL)	/		GLY 0.1		GLY 0.5		GLY 2.0		Glycine 0.5		Glycine 2.0		AMPA 0.5		AMPA 2.0	
Expected [GLY][a]	x		x + 0.1		x + 0.5		x + 2.0		x		x		x		x	
	b															
Measured [GLY][a] by ELISA[c]	<0.08	0.08	<0.08	<0.08	0.12	0.11	0.53	0.46	<0.08	<0.08	<0.08	<0.08	0.08	<0.08	<0.08	<0.08
Expected value[a]			*≤0.18* *>0.10*	*≤0.18* *>0.10*	*≤0.58* *>0.50*	*≤0.58* *>0.50*	*≤2.08* *>2.00*	*≤2.08* *>2.00*	*≤0.08*	*≤0.08*	*≤0.08*	*≤0.08*	*≤0.08*	*≤0.08*	*≤0.08*	*≤0.08*
Measured [GLY][a] by HPLC/Fluo[d]	<0.05	<0.05	0.06	0.07	0.36	0.38	1.41	1.51	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	0.08	<0.05
Expected value[a]			*<0.15* *≥0.10*	*<0.15* *≥0.10*	*<0.55* *≥0.50*	*<0.55* *≥0.50*	*<2.05* *≥2.00*	*<2.05* *≥2.00*	*<0.05*	*<0.05*	*<0.05*	*<0.05*	*<0,05*	*<0.05*	*<0.05*	*<0.05*

[a] All measured and expected GLY concentration values are expressed in ng/mL

[b] Braces indicate duplicates of the same sample

[c] Biocheck

[d] Labocéa

and 0.05–0.1 ng/mL for Biocheck and Labocéa, respectively. The reproducibility (i.e., the results obtained for each pair of identical samples) was very good for both laboratories. Two of the methods used (ELISA and HPLC/Fluo) gave results lower than expected values; the HPLC/Fluo method (Labocéa) produced more accurate results. Results obtained with samples W5 to W8 (supplemented with glycine or AMPA), when compared to those obtained with the control sample (W1), indicated that both detection methods were found to be perfectly GLY-specific, in that the values detected were not increased when the chemically similar molecules (glycine and AMPA) were added in the samples.

The results obtained for the urine samples are compiled in Tables 2 and 3. For Biocheck, the LOQ remained unchanged compared to the water matrix, namely 0.08 ng/mL. As for Labocéa, while the LOQ initially claimed was 0.05 ng/mL, it turned out to be 10 times higher (0.5 ng/mL). The rationale given by the laboratory was the need for them to dilute all urine samples 10 times due to a "*strong matrix effect*" (i.e., "*urine was rich in various compounds disturbing analysis by preventing detection of internal labeled standards*"). While it is completely understandable to have to dilute samples in order to be within a detection window compatible with the method (and in this specific case, to better detect chromatographic peaks), it is however difficult to understand why this impacts the quantification threshold, which depends on the method and on the target compound [27, 28], but not on the sample. It should therefore be the same, regardless of whether the urine is diluted or not. Why not simply then multiply by 10 the concentration measured in the 10× diluted sample to know the concentration in the original one? Labocéa nevertheless sometimes estimated values lower than the LOQ of 0.5 ng/mL (samples U2, U3, U11, and one of the samples U8, U10, U16) "*when a chromatographic peak significantly emerged from the background noise*", but "*without the precision of this estimate meeting the requirements of COFRAC*" (French accreditation committee).

Besides this LOQ issue, making it difficult to interpret some of Labocéa's values, it seems that the Biocheck's method (ELISA) resulted in an overall better reproducibility, with the exception of a single pair of identical samples (U4). In contrast, several duplicate samples returned relatively distant values in the case of Labocéa (U4, U7, U10, U12). Moreover, the Biocheck's measured values were much closer to the expected concentrations than those of Labocéa's, even when the measured values were greater than 0.5 ng/mL (Labocéa's LOQ)—and therefore met the COFRAC requirements—(samples U4 and U12). Finally, as in the case of water samples, it is clear from our results that neither of the two methods (ELISA and LC/MS–MS) showed the slightest cross-reaction or

Table 2 Measured and expected GLY concentrations ([GLY]) in samples of individual's urine 1

Sample	U1		U2		U3		U4		U5		U6		U7		U8	
Supplementation (ng/mL)	/		GLY 0.1		GLY 0.5		GLY 2.0		Glycine 0.5		Glycine 2.0		AMPA 0.5		AMPA 2.0	
Expected [GLY][a]	y[b]		y + 0.1		y + 0.5		y + 2.0		y		y		y		y	
Measured [GLY][a] by ELISA[c]	1.11	1.12	1.19	1.14	1.04	1.42	3.46	1.88	0.91	0.96	1.14	1.21	1.14	1.11	0.98	1.13
Expected value[a]			*1.21*	*1.21*	*1.51*	*1.51*	*3.11*	*3.11*	*1.11*	*1.11*	*1.11*	*1.11*	*1.11*	*1.11*	*1.11*	*1.11*
Measured [GLY][a] by LC/MS-MS[d]	<0.50	<0.50	0.10	0.10	0.36	0.42	1.81	1.20	<0.50	<0.50	<0.50	<0.50	<0.50	N/A	0.10	<0.50
Expected value[a]			*≥0.10*	*≥0.10*	*≥0.50*	*≥0.50*	*≥2.00*	*≥2.00*	*<0.50*	*<0.50*	*<0.50*	*<0.50*	*<0.50*	*<0.50*	*<0.50*	*<0.50*

[a] All measured and expected GLY concentration values are expressed in ng/mL

[b] Braces indicate duplicates of the same sample

[c] Biocheck

[d] Labocéa

Table 3 Measured and expected GLY concentrations ([GLY]) in samples of individual's urine 2

Sample	U9		U10		U11		U12		U13		U14		U15		U16	
Supplementation (ng/mL)	/		GLY 0.1		GLY 0.5		GLY 2.0		Glycine 0.5		Glycine 2.0		AMPA 0.5		AMPA 2.0	
Expected [GLY][a]	z[b]		z + 0.1		z + 0.5		z + 2.0		z		z		z		z	
Measured [GLY][a] by ELISA[c]	0.89	0.93	0.65	0.77	1.08	1.02	2.95	2.93	0.89	0.85	1.02	0.90	1.01	1.29	0.91	0.83
Expected value[a]			*1.00*	*1.00*	*1.40*	*1.40*	*2.90*	*2.90*	*0.90*	*0.90*	*0.90*	*0.90*	*0.90*	*0.90*	*0.90*	*0.90*
Measured [GLY][a] by LC/MS-MS[d]	<0.50	<0.50	0.92	0.10	0.43	0.34	1.00	1.90	<0.50	<0.50	<0,50	<0.50	<0.50	<0.50	0.10	<0.50
Expected value[a]			*≥0.10*	*≥0.10*	*≥0.50*	*≥0.50*	*≥2.00*	*≥2.00*	*<0.05*	*<0.50*	*<0.50*	*<0.50*	*<0.50*	*<0.50*	*<0.50*	*<0.50*

[a] All measured and expected GLY concentration values are expressed in ng/mL

[b] Braces indicate duplicates of the same sample

[c] Biocheck

[d] Labocéa

cross-detection with glycine or AMPA. However, Labocéa also measured AMPA concentration in all samples. Surprisingly, the concentration of AMPA was overestimated in the samples supplemented with GLY (when compared with the control samples), indicating that even though the LC/MS–MS method allowed a specific detection of GLY, the latter still interfered with AMPA detection (data not shown).

The ELISA method for the detection of GLY was developed on water samples [29]. The GLY concentrations

determined by ELISA in water samples then correlated perfectly (correlation coefficient of 0.99) with those determined by HPLC [29]. Such a similar performance between the two techniques for water samples was later confirmed by another comparison analysis [30]. This slight discrepancy with our results could be explained by the different GLY concentration ranges between their samples and ours. Indeed, the main differences that we obtained between the two techniques for the water samples concerned those which had been spiked with GLY. Therefore, ELISA technique would prove to be less efficient than the HPLC/Fluo for GLY detection in water only for the most concentrated samples. Another difference with the original work of Clegg et al. [29] concerns the specificity of the ELISA method towards GLY: unlike us, they observed that the GLY polyclonal antisera did cross-react with AMPA. This indicates that the ELISA kits used by Biocheck [31] were more specific than the original one.

Urine is a much more complex matrix than water. In particular, urine can contain a large number of metabolites such as amino acids including glycine [32]. Therefore, the transposition of the ELISA method for the detection of GLY in urine could have been limited by possible cross-reactions with molecules structurally similar to GLY, and especially glycine. Such a cross-reaction would lead to a general overestimation of the GLY urine levels, and even to false positives. Our data clearly showed that this is not the case.

Conclusions

For the determination of the GLY levels in water, the HPLC/Fluo technique used by Labocéa gave more precise results than the ELISA technique used by Biocheck.

Conversely, for the determination of the GLY urine levels, the Biocheck ELISA technology gave much more reliable and reproducible results than the LC/MS–MS method used by Labocéa.

None of the three techniques applied for the detection of GLY, whether in water or in urine, showed a cross-reaction or cross-detection with the chemically similar molecules glycine and AMPA.

Labocéa's LC/MS–MS method required dilution of the first morning urines due to a "*strong matrix effect*", implying—according to Labocéa—an increase in their quantification threshold (LOQ) which had to be multiplied by the dilution factor of the samples. Therefore, when urinary GLY levels are given by Labocéa with a LOQ of 0.05 ng/mL (the originally stated LOQ), this implies that the urine samples analyzed had not required dilution, and therefore that these urines were probably not first morning urines, as recommended by the laboratory, but later and clearer urines which, of course, were not representative of the real GLY levels to which the body has been exposed.

Abbreviations

AMPA: (Aminomethyl) phosphonic acid; ELISA: Enzyme-linked immunosorbent assay; GLY: Glyphosate; GBHs: Glyphosate-based herbicides; HPLC/Fluo: High-performance liquid chromatography coupled to fluorescence detection; LC/MS–MS: Liquid chromatography coupled to tandem mass spectrometry.

Acknowledgements

We thank Guillaume de Crop for technical assistance and the production of the experiment movie. We are grateful to Marcel Lamour, Morgane Michel and Dominique Berlet-Noel for proofreading the English.

Author contributions

CV and JSdV designed the protocol. CV conducted the experiment. FP, as a legal chemist expert, supervised procedures followed during the experiment and ensured that they complied perfectly with the pre-established protocol. CV wrote the first version, and revised the manuscript based on feedback from the co-authors. All authors read and approved the manuscript.

Funding

This work was supported by the Foundation *Un Monde par Tous* (Grant n°. 00081254).

Availability of data and materials

Please contact the authors for data requests.

Declarations

Ethics approval and consent to participate

Non applicable (urine samples were provided by two of the co-authors: CV and JSdV).

Consent for publication

Not applicable.

Competing interests

The authors declare they have no competing interests.

Author details

[1]Laboratory VEAC, University Paris-Saclay, Faculty of Sciences, Bât. 350, Avenue Jean Perrin, 91405 Orsay, France. [2]Committee for Independent Research and Information on Genetic Engineering (CRIIGEN), 42 rue de Lisbonne, 75008 Paris, France. [3]Legal Expertise Laboratory Signatures, BP 800-23, 13791 Aix-en-Provence CEDEX 3, France.

Received: 8 April 2022 Accepted: 12 June 2022
Published online: 02 July 2022

References

1. Benbrook CM (2016) Trends in glyphosate herbicide use in the United States and globally. Environ Sci Eur 28:3. https://doi.org/10.1186/s12302-016-0070-0
2. Williams GM, Kroes R, Munro IC (2000) Safety evaluation and risk assessment of the herbicide Roundup and its active ingredient, glyphosate, for humans. Regul Toxicol Pharmacol 31:117–165. https://doi.org/10.1006/rtph.1999.1371
3. Cox C (2004) Herbicide factsheet—glyphosate. J Pesticide Reform 24:10–15
4. Connolly A, Coggins MA, Koch HM (2020) Human biomonitoring of glyphosate exposures: state-of-the-art and future research challenges. Toxics 8:60. https://doi.org/10.3390/toxics8030060
5. ISAAA (2018) Brief 54: Global status of commercialized biotech/GM crop in 2018. https://www.isaaa.org/resources/publications/briefs/54/

6. Curwin BD, Hein MJ, Sanderson WT, Nishioka MG, Reynolds SJ, Ward EM, Alavanja MC (2005) Pesticide contamination inside farm and nonfarm homes. J Occup Environ Hyg 2:357–367. https://doi.org/10.1080/15459620591001606
7. Alferness PL, Iwata Y (1994) Determination of glyphosate and (aminomethy1) phosphonic acid in soil, plant and animal matrices, and water by capillary gas chromatography with mass-selective detection. J Agric Food Chem 42:2751–2759
8. Caloni F, Cortinovis C, Rivolta M, Davanzo F (2016) Suspected poisoning of domestic animals by pesticides. Sci Total Environ 539:331–336. https://doi.org/10.1016/j.scitotenv.2015.09.005
9. El-Gendy K, Mosallam E, Ahmed N, Aly N (2018) Determination of glyphosate residues in Egyptian soil samples. Anal Biochem 557:1–6. https://doi.org/10.1016/j.ab.2018.07.004
10. Bøhn T, Cuhra M, Traavik T, Sanden M, Fagan J, Primicerio R (2014) Compositional differences in soybeans on the market: glyphosate accumulates in Roundup Ready GM soybeans. Food Chem 153:207–215. https://doi.org/10.1016/j.foodchem.2013.12.054
11. Van Eenennaam AL, Young AE (2014) Prevalence and impacts of genetically engineered feedstuffs on livestock populations. J Anim Sci 92:4255–4278. https://doi.org/10.2527/jas.2014-8124
12. Weiss WP, Simons CT, Ekmay RD (2015) Effects of feeding diets based on transgenic soybean meal and soybean hulls to dairy cows on production measures and sensory quality of milk. J Dairy Sci 98:8986–8993. https://doi.org/10.3168/jds.2015-9955
13. Silva V, Montanarella L, Jones A, Fernández-Ugalde O, Mol HGJ, Ritsema CJ, Geissen V (2018) Distribution of glyphosate and aminomethylphosphonic acid (AMPA) in agricultural topsoils of the European Union. Sci Total Environ 621:1352–1359. https://doi.org/10.1016/j.scitotenv.2017.10.093
14. de Araujo JSA, Delgado IF, Paumgartten FJR (2016) Glyphosate and adverse pregnancy outcomes, a systematic review of observational studies. BMC Public Health 16:472. https://doi.org/10.1186/s12889-016-3153-3
15. Davoren MJ, Schiestl RH (2018) Glyphosate-based herbicides and cancer risk: a post-IARC decision review of potential mechanisms, policy and avenues of research. Carcinogenesis 39:1207–1215. https://doi.org/10.1093/carcin/bgy105
16. Van Bruggen AHC, He MM, Shin K, Mai V, Jeong KC, Finckh MR, Morris JG Jr (2018) Environmental and health effects of the herbicide glyphosate. Sci Total Environ 616–617:255–268. https://doi.org/10.1016/j.scitotenv.2017.10.309
17. Valavanidis A (2018) Glyphosate, the Most Widely Used Herbicide. Health and safety issues. Why scientists differ in their evaluation of its adverse health effects. https://www.researchgate.net/publication/323727351
18. Benbrook CM (2019) How did the US EPA and IARC reach diametrically opposed conclusions on the genotoxicity of glyphosate-based herbicides? Environ Sci Eur 31:2. https://doi.org/10.1186/s12302-018-0184-7
19. Tosun J, Lelieveldt H, Wing TS (2019) A case of 'muddling through'? The politics of renewing glyphosate authorization in the European Union. Sustainability 11:440. https://doi.org/10.3390/su11020440
20. Spiroux de Vendômois J, Bourdineaud J-P, Apoteker A, Defarge N, Gaillard M, Lepage C, Testart J, Vélot C (2021) Trans-disciplinary diagnosis for an in-depth reform of regulatory expertise in the field of environmental toxicology and security. Toxicol Res 37(4):405–419. https://doi.org/10.1007/s43188-020-00075-w
21. Martinez TT, Long WC, Hiller R (1990) Comparison of the toxicology of the herbicide roundup by oral and pulmonary routes of exposure. Proc West Pharmacol Soc 33:193–197
22. Peillex C, Pelletier M (2020) The impact and toxicity of glyphosate and glyphosate-based herbicides on health and immunity. J Immunotoxicol 17:163–174. https://doi.org/10.1080/1547691X.2020.1804492
23. Connolly A, Coggins MA, Galea KS, Jones K, Kenny L, McGowan P, Basinas I (2019) Evaluating glyphosate exposure routes and their contribution to total body burden: a study among amenity horticulturalists. Ann Work Expo Health 63:133–147. https://doi.org/10.1093/annweh/wxy104
24. Gillezeau C, van Gerwen M, Shaffer RM, Rana I, Zhang L, Sheppard L, Taioli E (2019) The evidence of human exposure to glyphosate: a review. Environ Health 18:2. https://doi.org/10.1186/s12940-018-0435-5
25. Zoller O, Rhyn P, Zarn JA, Dudler V (2020) Urine glyphosate level as a quantitative biomarker of oral exposure. Int J Hyg Environ Health 228:113526. https://doi.org/10.1016/j.ijheh.2020.113526
26. Grau D, Grau N, Gascuel Q, Paroissin C, Stratonovich C, Lairon D, Devault DA, Di Cristofaro J (2022) Quantifiable urine glyphosate levels detected in 99% of the French population, with higher values in men, in younger people, and in farmers. Environ Sci Pollut Res Int 29:32882–32893
27. Kim J, Park H, Kang HS, Cho BH, Oh JH (2020) Comparison of sample preparation and determination of 60 veterinary drug residues in flatfish using liquid chromatography-tandem mass spectrometry. Molecules 25:1206. https://doi.org/10.3390/molecules25051206
28. Millet A, Khoudour N, Bros P, Lebert D, Picard G, Machon C, Goldwasser F, Blanchet B, Guitton J (2021) Quantification of nivolumab in human plasma by LC-MS/HRMS and LC-MS/MS, comparison with ELISA. Talanta 224:121889. https://doi.org/10.1016/j.talanta.2020.121889
29. Clegg BS, Stephenson GR, Hall JC (1999) Development of an enzyme-linked immunosorbent assay for the detection of glyphosate. J Agric Food Chem 47:5031–5037
30. Rubio F, Veldhuis LJ, Clegg BS, Fleeker JR, Hall JC (2003) Comparison of a direct ELISA and an HPLC Method for glyphosate determinations in water. J Agric Food Chem 51:691–696
31. Krüger M, Scheldorn P, Schrödl W, Hoppe HW, Lutz W, Shehata AA (2014) Detection of glyphosate residues in animals and humans. J Envoron Anal Toxicol. 4:2. https://doi.org/10.4172/2161-0525.1000210
32. Öztürk Er E, Özbek B, Bakirdere S (2021) Determination of seventeen free amino acids in human urine and plasma samples using quadruple isotope dilution mass spectrometry combined with hydrophilic interaction liquid chromatography - Tandem mass spectrometry. J Chromatogr A 1641:461970. https://doi.org/10.1016/j.chroma.2021.461970

Publisher's Note