Determination of glyphosate and AMPA in indoor settled dust by hydrophilic interaction liquid chromatography with tandem mass spectrometry and implications for human exposure

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Title

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Highlights

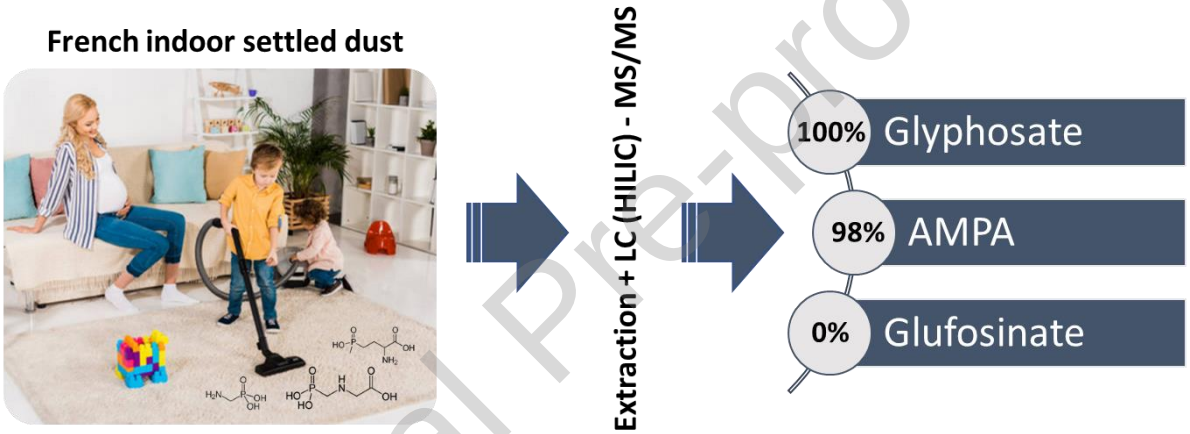
- Analysis of glyphosate, AMPA, and glufosinate in dust in 60 French households
- Glyphosate was found in all samples (median of 814 ng/g and maximum of 51 µg/g).
- No exceedance of European regulatory standards for daily intake

Abstract

The widespread application of glyphosate leads to significant contamination of outdoor environmental compartments, notably air and soil, which can contaminate indoor air and dust. This study assessed the contamination of indoor household dust for the first time in France and potential exposure to glyphosate through the inadvertent ingestion of dust. A specific and new analytical method was

developed using HILIC MS/MS (hydrophilic interaction liquid chromatography with tandem mass spectrometry) to measure polar pesticides, such as glyphosate, aminomethylphosphonic acid, and glufosinate, in indoor dust, with a low quantification limit (25 ng/g). The dust from vacuum cleaner bags of 60 rural and urban households (Brittany, France) was analyzed. All samples contained glyphosate (median 1,675 ng/g for rural dwellings (n = 29), 457 ng/g for urban dwellings (n = 31)), more than 90% contained aminomethylphosphonic acid, and none contained glufosinate. Concentrations were influenced by the rural or urban setting, the proximity of crops, and the use of weed killers on driveways or lawns. Glyphosate exposure via indoor dust ingestion was < 1% of both acceptable daily intake and dietary intake. However, the high quantification limit of the glyphosate concentration in the food analysis method probably leads to overestimation of the dose from food.

Graphical abstract



Keywords

pesticide, AMPA, glufosinate, HILIC, environmental, vacuum bag, contamination, children's dwellings.

Abbreviations

ADI: acceptable daily intake
AMPA: aminomethylphosphonic acid
APP: anionic polar pesticide
IS: internal standard
HILIC: hydrophilic interaction liquid chromatography
LC-MS: liquid chromatography with mass spectrometry

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1. Introduction

Glyphosate is a broad-spectrum, post-emergent, non-selective, synthetic universal herbicide that has been widely used around the world since 1970. Its use increased significantly in the 1990s due to its effectiveness for both domestic and agricultural applications (Myers et al., 2016). In the environment, glyphosate is degraded to aminomethylphosphonic acid (AMPA), its main metabolite (Rueppel et al., 1977).

These molecules contaminate various environmental compartments, such as water (Botta et al., 2009; Geng et al., 2021), soil (Silva et al., 2018), the breathable dust of soil, air (ANSES, 2020; Ramirez Haberkon et al., 2021), and plants used as food (Xu et al., 2019). Wind erosion of topsoil contaminated by glyphosate and AMPA (Bento et al., 2017; Ramirez Haberkon et al., 2021; Silva et al., 2018) leads to its being carried over varying distances by the wind (Aparicio et al., 2018; Mu et al., 2022; Ravier et al., 2019), thus contaminating not only outdoor air; as it was observed in France with 50% of air samples contaminated with glyphosate (ANSES, 2020) but also indoor dust as it was observed in rural dwellings in Iowa (USA) with > 85% of contaminated dust samples(Curwin et al., 2005).

Exposure to glyphosate and its metabolite AMPA in the general population in Europe has been shown by their detection in urine (Connolly et al., 2020a; Lemke et al., 2021). The identified exposure routes are food ingestion (Xu et al., 2019) and the inhalation of outdoor dust (Silva et al., 2018). However, exposure through the inadvertent ingestion of dust in closed environments has, thus far, not been well quantified. Indeed, children are the most concerned by this exposure route through repeated hand-to-mouth contact (Curwin et al., 2007; Le Cann et al., 2011).

Concerning the toxicity of glyphosate, work is still in progress to evaluate the impact of this molecule on health, including transgenerational effects (Milesi et al., 2021; Pham et al., 2019) and the toxicity of mixtures, for example, with pesticide additives (co-formulants or adjuvants), which are used to increase the performance or stability of active ingredients with possible additional toxic effects between mixtures of additives and pesticides. In the article of Nagy et al, 2019, examples of increased toxicity of glyphosate are identified with co-formulants or additives in commercial products (Dechartres et al., 2019; Lindberg et al., 2020; Nagy et al., 2019; Ren et al., 2019; Vandenberg et al., 2017).

Various methods have been developed for the simultaneous analysis of glyphosate, AMPA, and, sometimes glufosinate, which is a broad-spectrum, non-selective contact herbicide with similar analytical properties. Measurements have been deployed on a large scale for food, water, air, and soil (ANSES, 2020; Silva et al., 2018). Due to the zwitterionic characteristics of these molecules, most

methods include a derivatization step. For example, a gas chromatography-tandem mass spectrometry method, using 2,2,2-trifluoroethanol and trifluoroacetic anhydride for the derivatization of glyphosate and AMPA, was applied to urine samples (Connolly et al., 2020b) Derivatization was also necessary with liquid chromatography methods, with for example, post-column derivatization using OPA (orthophthaldehyde) for the analysis of air (Marliere et al., 2012) or water (Hanke et al., 2008). Pre-column derivatization with 9-Fluorenylmethoxycarbonyl chloride (Fmoc-Cl) has also been used and coupled with liquid chromatography and fluorescence or mass spectrometry detection, for example, for the analysis of water (Le Bot et al., 2002) or soil (Delhomme et al., 2021). In order to avoid the derivatization process, new chromatographic columns, such as porous graphic carbon, have been used and applied to soil or food (Zhang et al., 2019), or ion chromatography mass spectrometry has been employed (Pareja et al., 2019). Hydrophilic interaction liquid chromatography (HILIC) coupled to mass spectrometry has more recently been used with two main benefits: (i) there is no need for derivatization which is time saving, and (ii) very low limits of detection (LODs) are obtained (Guo et al., 2019).

However, to date, there is very little data concerning the amount of glyphosate in house dust. Only Curwin et al. carried out measurements of glyphosate in house dust, in particular on farms (Curwin et al., 2005). There are, thus far, no French data or analytical methods that have been deployed for the study of indoor dust.

The objectives of our study were to (i) develop a sensitive, robust, and specific analytical method for the analysis of glyphosate, glufosinate, and AMPA in household dust samples; (ii) measure the concentration of these compounds in dust from vacuum bags collected from children's dwellings in urban or rural areas of the French region Brittany, which exhibits significant agricultural activity (Chevrier et al., 2014); and (iii) evaluate exposure by ingestion associated with the presence of glyphosate in indoor dust.

2. Materials and Methods

2.1. Chemicals (standards, solvents, and reagents)

Glyphosate (CAS n°1071-83-6, 99% pure), glyphosate 1,2-$^{13}C_2$,^{15}N (CAS n° 1185107-63-4, 100 µg/mL in water), AMPA (CAS n°1066-51-9, 98% pure), AMPA 1,2-^{13}C,^{15}N (no CAS number, 100 µg/mL in water), and ammonium glufosinate (CAS n°77182-82-2, 97% pure) were supplied by Dr. Ehrenstorfer (Wesel, Nordrhein-Westfalen, Germany). Glufosinate-d_3 hydrochloride (CAS n° 1323254-05-2, 98% pure) was obtained from Toronto Research Chemicals (Toronto, Ontario, Canada). A 10 mg/L mix of

glyphosate, AMPA, and glufosinate in water was purchased from LGC (Augsburg, Germany) and used as an alternative source to validate our calibration standards.

Methanol and acetonitrile (mass-spectrometry grade) were supplied by Biosolve (Dieuze, France) and formic acid (purity 99%) by Carlo Erba (Val-de-Reuil, France). Disodium EDTA dihydrate (EDTA diNa, 2 aq; CAS n°6381-92-6) was purchased from Chemlab (Zedelgem, Belgique). Ultrapure water was generated using a Millipore system.

Stock solutions of AMPA, glufosinate, glyphosate, and glufosinate-d_3 at 1 g/L in ultrapure water were prepared and stocked at +4°C for a maximum of six months.

The stock solutions of standards were diluted with ultrapure water to generate working solutions (50 µg/L and 500 µg/L of each target analyte).

Stock solutions of glufosinate-d_3 and commercial solutions of glyphosate 1,2-$^{13}C_2$,^{15}N (100 mg/L) and AMPA 1,2-^{13}C,^{15}N (100 mg/L) were used to prepare a working solution of isotope-labeled internal standards (IS) at 250 µg/L for each.

Finally, the working standard solutions were diluted with 50/50 ultrapure water/acetonitrile + 0.9% formic acid + 0.02 mM EDTA to prepare calibration samples in the range 0.125 to 10 µg/L (0.125, 0.250, 0.500, 1.25, 2.5, 5.0, and 10 µg/L) with 1.25 µg/L IS. One calibration sample was prepared at 0.5 µg/L (with 1.25 µg/L of IS) from the LGC mix to validate the preparation of the calibration solutions.

2.2. Dust sample collection

2.2.1. Standard reference material and working dust

The standard reference material (SRM) 2585 (organic contaminants in house dust), prepared by the National Institute of Standards & Technology (NIST), was supplied by Analab (Hœnheim, France). It was taken from vacuum cleaner bags collected from homes, cleaning services, motels, and hotels in the states of North Carolina, Maryland, Ohio, New Jersey, Montana, and Wisconsin in 1993 and 1994. The dust was sterilized by gamma irradiation and sieved to 90-100 µm. It was stocked at room temperature between 18 and 25°C. No certified or information values are provided for the concentrations of AMPA, glyphosate, or glufosinate in SRM 2585.

Dust samples, namely Dust A, B, C, and D, consist of the dust from French dwellings and were available in the laboratory collection, stocked at -18°C. They were used for analytical method development.

2.2.2. French sample collection

In the PELAGIE mother-child cohort, set up in Brittany (France) (Chevrier et al., 2011), 289 homes participated in an environmental survey conducted from 2009 to 2012. At this time, a dust sample from household vacuum cleaners was collected from each dwelling. The characteristics of the homes were: a rural/semi-rural (< 20,000 inhabitants) or urban (≥ 20,000 inhabitants) location, GIS-based 500-m proximity to crops, and self-reported household use of pesticide products outdoors or indoors during the previous 12 months (Glorennec 2017). Sixty dust samples were randomly selected in 2020 from among the 289 samples stored at -18°C since 2012. These 60 samples were collected between 2010 and 2012.

2.3. Sample preparation

Dust samples were sieved to < 100 µm through a stainless steel sieve precleaned with dichoromethane, using an AS 200 vibratory sieve shaker (Retsch, Haan, Germany) and stored at -18°C in 20-mL amber glass vials until analysis.

For the analysis, 25 mg of settled dust was weighed in a 1.5 mL polypropylene microcentrifuge tube using a Cubis Semi-Micro Balance MSE225P-100-DA (Sartorius, Göttingen, Germany), to which 25 µL of the internal standard (IS) solution, at 250 µg/L, was added. After adding 1.25 mL of an acidic (pH2) extraction solvent (ultrapure water + 0.9% formic acid + 2 mM EDTA), it was vortexed until all the dust was suspended in solution. It was then shaken for 60 min at 50°C in a shaker-incubator system (Edmund Bühler, Bodelshausen, Germany) and centrifuged at 3,000 x g for 5 min at 4°C using a refrigerated microcentrifuge 17R (Fisher Scientific, Illkirch, France). After centrifugation, 1 mL of the supernatant was transferred to a polypropylene tube, and the pH was controlled to ensure it was between 2 and 3.

An aliquot (800 µL) of the supernatant was placed in a Strata X 33-µm polymeric reverse-phase 200 mg/6 mL cartridge (Phenomenex, Torrance, California, USA) conditioned with 3 mL ultrapure water + 0.9% formic acid. This cartridge was not use for its intended purpose of solid phase extraction, but rather as a means of filtering the supernatant and removing any dust particles left in suspension. The filtrate, followed by an 800 µL rinse of ultrapure water + 0.9% formic acid, was collected in a polypropylene tube, resulting in a two-fold dilution.. Then, 500 µL of each extract was transferred into a polypropylene LC vial and 500 µL acetonitrile + 0.9% formic acid was added, resulting in four-fold extract ready for analysis by LC-MS/MS. If a precipitate was present, centrifugation was performed and the supernatant collected for analysis.

2.4. LC-MS/MS analysis

An ACQUITY UPLC coupled to a Xevo-TQ-XS (Waters, Milford, Massachusetts, USA) was used for analysis. The sample manager temperature was set to 4°C and 20 µL was injected. Chromatographic separation was performed on an Anionic Polar Pesticides (APP) column (130Å, 5 µm, 100 mm x 2.1 mm i.d., Waters) and precolumn (130Å, 5 µm, 5 mm x 2.1 mm i.d., Waters). Ultrapure water + 0.9% formic acid was used as mobile phase A and acetonitrile + 0.9% formic acid as mobile phase B. The 20 min gradient elution program was: 0 to 3 min, 90% B; 3 to 7 min, from 90% B to 10% B (curve 2, convex); 7 to 14 min, 10% B; and 14 to 20 min, 90% B. The flow rate was 0.5 mL/min and the temperature of the column 50°C.

The mass spectrometer was operated using electrospray ionization (ESI) in the negative mode. The three most intense and specific multiple reaction monitoring (MRM) transitions for each compound (quantifier and qualifier transitions) were monitored. Optimized ESI-MS/MS parameters used in the acquisition method are presented in Table 1.

Table 1: Optimized ESI-MS/MS parameters

Analyte[a]	Time segment	ESI mode	Precursor ion (m/z)	Quantifying (#) or qualitative ion (m/z)	Cone voltage (V)	Collision energy (eV)	Dwell time (s)
AMPA	2.5 to 5 min	negative	109.7	62.9	26	18	0.06
				79.0	26	15	0.06
				80.8#	26	12	0.06
AMPA 1,2-^{13}C, ^{15}N			111.7	62.9	26	18	0.06
				79.0	26	15	0.06
				80.8#	26	12	0.06
Glufosinate	4.0 to 5 min	negative	179.9	84.9	20	18	0.06
				94.9#	20	14	0.06
				118.9	20	16	0.06
Glufosinate-d_3			182.9	62.9	20	28	0.06
				97.8#	20	16	0.06
				121.9	20	18	0.06
Glyphosate	5.0 to 8.5 min	negative	167.8	62.9	15	16	0.2
				80.8#	15	14	0.2
				150.0	15	10	0.2
Glyphosate 1,2-$^{13}C_2$, ^{15}N			170.8	62.9	15	16	0.2
				80.8#	15	14	0.2
				153.0	15	10	0.2

[a] Compounds listed in order of retention times.

MassLynx v4.2 (2017, Waters) was used to control the LC-MS instrument and identify and quantify the compounds present in the samples.

Chromatograms of all the compounds (targets and IS) are available in Supplemental Information (**Error! Reference source not found.** for a calibration solution and **Error! Reference source not found.** for the SRM 2585).

2.5. Method validation

The performance of the method was evaluated in terms of calibration, extraction recovery, precision (repeatability and reproducibility), limits of quantification, carryover, interferences ionization suppression/enhancement, and extract stability, following the NF T90-210 and SWGTOX guidelines ((AFNOR, 2010a; SWGTOX, 2013). The extraction kinetics were also evaluated.

Seven-point calibration curves were constructed by plotting the ratio of the base peak area of the compound (AMPA, glufosinate, or glyphosate) to the base peak area of the corresponding IS (AMPA 1,2- ^{13}C, ^{15}N, glufosinate-d_3, or glyphosate 1,2-$^{13}C_2$,^{15}N, respectively). A linear regression plot weighted by 1/x was generated.

In the absence of an SRM dust sample with reference concentration of AMPA, glyphosate, and glufosinate, extraction recoveries were evaluated using labelled internal standards for three dust samples (Dust C, SRM 2585, and Dust D) at three concentrations (50, 250, and 500 ng/g). Internal standards were added by liquid spiking before extraction or just before analysis. The extraction recovery is the ratio of the area of the internal standard of the sample spiked before extraction to the area of the internal standard of the sample spiked just before analysis.

The LOQ was defined as the lowest concentration for which a signal-to-noise (S/N) ratio was > 10 and the bias was within ±25% for at least five different samples. Repeatability and reproducibility were evaluated by analysis of an internal laboratory dust sample (Dust D) containing glyphosate and AMPA from six series (different days) of three extractions (same day). The stability of the polar pesticides post-extraction was evaluated for extracts stored at +4°C for up to two weeks.

Extraction kinetics were evaluated for two dust samples containing AMPA and glyphosate (Dust A and Dust B) with various agitation times (30 min and 1, 2, 4, and 8 h). Internal standards were added to the supernatant after the first extraction step. Three samples of the same dust sample were analyzed per condition.

2.6. Quantitative analysis and quality assurance

Each analytical sequence included: (i) seven calibration samples to generate calibration curves intended for quantification and one calibration blank sample, (ii) one calibration sample prepared from the alternative mix to validate the preparation of the calibration solutions, (iii) one calibration sample analyzed at the end of the batch to check for the stability of the detector response, (iv) up to 20 four-fold diluted samples, (v) blanks (50/50 ultrapure water/acetonitrile + 0.9% formic acid) analyzed between each sample to avoid carry-over between two samples, and (vi) three four-fold

diluted samples of Dust D, analyzed as a regular sample to check for intermediate fidelity of the method.

Positive values for each substance were confirmed by comparing the retention times and MRM transition ratios between the calibration samples and dust samples. The data validation protocol of the proposed method included several criteria: (i) the response of a substance (area of the chromatographic peak) in the calibration blank sample had to be lower than 50% of that in the calibration sample at the LOQ, (ii) the concentration measured in the calibration sample prepared from the LGC mix had to be within ±25% of its nominal concentration value, (iii) the concentration measured in the calibration sample analyzed at the beginning and end of the batch had to be within ±25% of its nominal concentration value, and (iv) the concentration measured in the procedural blank samples had to be lower than half the LOQ. If all these conditions were not met, the samples were re-analyzed.

If the concentration of the compound of interest was within the calibration range with the four-fold dilution, the result was reported. If the concentration exceeded the range, the result of a further diluted extract was reported.

2.7. Statistical data analysis

GraphPad (version 8.3.0 (538)) was used for all statistical data analysis. A Mann-Whitney nonparametric test was performed to compare ranks for the sampling-site characteristics and a two-way analysis of variance (ANOVA), which tests for differences in the effects of independent variables on a dependent variable, was performed for the kinetic study.

2.8. Calculation of “median case”/ “worst case” glyphosate exposure due to indoor dust ingestion

The calculation was performed for glyphosate and AMPA only, as glufosinate was not detected in the dust extracts. As AMPA is the main metabolite of glyphosate, calculations of glyphosate exposure were carried out for the sum of the glyphosate dose and AMPA dose, expressed as glyphosate, as suggested by the EFSA (EFSA, 2012). The underlying default assumptions are, knowing that AMPA exposure is very small compared to the glyphosate’ s one: i) toxicity of AMPA and glyphosate are similar ii) a molecule of glyphosate decay in one of AMPA iii) environmental exposure to AMPA is little compared to the amount decayed from glyphosate. To perform this calculation, the following formula was used:

$$E_{Gly+AMPA/dust} = \frac{([Gly]_{dust} + [AMPA]_{dust} * \frac{M_{Gly}}{M_{AMPA}}) * Q_{ing\ dust}}{BW} * 10^{-6}$$

With [Gly or AMPA]$_{dust}$, the concentration of glyphosate or AMPA in indoor dust, in ng/g; $M_{Gly\ or\ AMPA}$, the molar mass of glyphosate or AMPA; $Q_{ing\ dust}$, the mass of dust ingested, in mg/day (US EPA 2017); BW, the body weight, in kg (Anses 2017); and $E_{Gly+AMPA/dust}$, the dose of glyphosate and AMPA (expressed as glyphosate) due to indoor dust ingestion, in µg/kg of bw/day. The parameters used are summarized in Table 2.

Table 2. Parameters used for the calculation of « worst case » and « median case » glyphosate exposure

	Median cases			Worst cases		
Age	6 months	1 year	30 years (adult)	6 months	1 year	30 years (adult)
$Q_{ing\ dust}$ (mg/day)	40	50	20	100	100	60
BW (kg of bw)	6.6	12.9	72.2	6.6	12.9	72.2
[Gly or AMPA]$_{dust}$ (ng/g)	Median glyphosate or AMPA concentration of this study			Maximum glyphosate or AMPA concentration of this study		

3. Results and discussion

3.1. Method validation

3.1.1 Calibration

Calibration curves with $r^2 > 0.99$ were obtained for glyphosate, AMPA and glufosinate (**Error! Reference source not found.** in Supplemental Information).

3.1.2 Extraction recoveries

The extraction recoveries for the internal standards are available in SI Table 1 in Supplemental Information. The average recoveries were > 80% with inter-dust RSD < 25%, regardless of the concentration studied. These yields take into account the first solid/liquid extraction step, the centrifugation, and the passage on the Strata X column (purification step).

3.1.3 Precision (repeatability and reproducibility)

Repeatability and reproducibility were evaluated on Dust D, with six series of three extractions. The results for AMPA and glyphosate are presented in **Error! Reference source not found.** and **Error! Reference source not found.** and **Error! Reference source not found.**, respectively, in Supplemental Information. The mean AMPA concentrations was 207 and that of glyphosate 2,019 ng/g. According to the standard NF V03-110 (AFNOR, 2010b), the intra-day relative standard deviation (RSD) was 14% for AMPA and 10% for glyphosate and the inter-day RSD was 8% for both, giving an intermediate fidelity RSD of 16% for AMPA and 13% for glyphosate. The test was not performed for glufosinate, as it was not present in Dust D.

3.1.4 Limits of quantification

The LOQ was 0.125 µg/L for AMPA, glyphosate, and glufosinate, which corresponds to 25 ng/g of dust (considering the four-fold dilution) in the absence of a matrix effect (variable depending on the dust sample) (Figure 1). This method is highly sensitive relative to other methods, with LOQs between 4 and 700 times lower than methods using derivatization and reverse phase (C18) liquid chromatography (SI Table 4 in Supplemental Information) This comparison was undertaken with soil samples, in the absence of reported method for indoor dust in the literature.

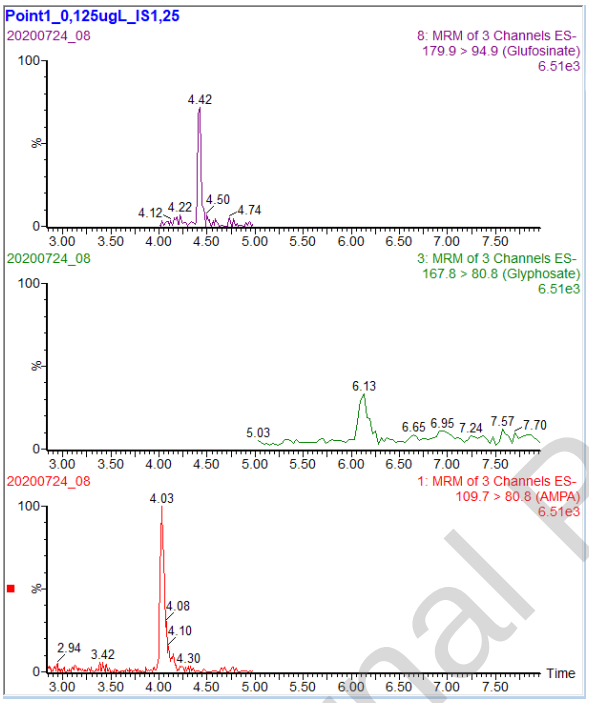

Figure 1. MRM chromatogram of a calibration solution containing AMPA, glufosinate, and glyphosate at the limit of quantification (LOQ) of 0.125 µg/L

3.1.5 Carryover

Some level of carryover was observed, particularly for samples loaded with high concentrations of glyphosate. A blank sample was injected between each dust sample to counteract this problem.

3.1.6 Interferences

The analysis of each individual substances at the maximum concentration of the calibration range did not show any signal on the MRM of the other substances. Likewise, the analysis of the metabolites n-acetyl glyphosate, n-acetyl glufosinate, and n-acetyl AMPA did not show any signal on the parent molecules.

3.1.7 Ionization suppression / enhancement

Dust is a complex matrix, which when extracted and analysed by LC/MS/MS, can be prone to ionization suppression or enhancement. To reduce these matrix effect, we adopted a double strategy: first to filter the supernatant on a Strata-X cartridge (in the same way that Guo et al did a filtration on Oasis PRIME HLB (Guo et al., 2019)) to remove some interferents and second to dilute to extracts for a further decrease of the matrix effects. This approach allowed to improve the quality of the ionization with a reasonable compromise on the sensitivity of the method.

3.1.8 Extract stability

The stability of the calibration standards was tested up to 14 days for AMPA, glufosinate, and glyphosate. The stability was acceptable for glyphosate, with a maximum deviation of 18% after 14 days, with the exception at the LOQ, which showed a deviation of 41%, still within the acceptable range for this concentration. For AMPA and glufosinate, the stability was also acceptable for the six highest concentrations, with a maximum deviation of 12% for AMPA and 20% for glufosinate. However, degradation at the LOQ was observed for AMPA (76% deviation), whereas an increase in the concentration was observed for glufosinate at the LOQ (+53%) and the following calibration point (+24%). Stability was then reassessed at seven days and showed a maximum deviation of 12% for AMPA, 22% for glufosinate, and 15% for glyphosate, except at the LOQ, at which degradation occurred, with deviations of 57% for AMPA, 98% for glufosinate, and 4% for glyphosate. Stability was also assessed at seven days for two dust sample extracts. The maximum deviation was 15% for the AMPA concentration and 9% for glyphosate concentration. The concentration of glufosinate was below the LOQ for all injections. Seven-day stability of extracts and calibration standards was therefore validated, subject to an increase of the LOQ to 50 ng/g.

3.1.9 Glyphosate and AMPA extraction kinetics

The results of the extraction kinetics for glyphosate and AMPA in Dust A and B are presented in Figure 2. The test was not performed for glufosinate, as it was not present in Dust A or B. Regardless of the extraction time, the repeatability study showed variability < 20%. The variability between the mean concentration of glyphosate of each extraction condition was 8% for Dust A (average to 513 ng/g) and 7% for Dust B (average to 217 ng/g). For AMPA, the intra-day repeatability study also showed variability < 20%. The observed greater variability (14% for Dust A and 13% for Dust B) was likely due to the lower concentrations (98.8 ng/g for Dust A and 108 ng/g for Dust B). A two-way ANOVA test performed on these data showed no statistical difference in the concentration of glyphosate over time for either dust sample. However, for AMPA, a statistical difference was observed over time (30 min to 8 h) for Dust B. The observed variability for AMPA was not attributed to the kinetics and, based on the glyphosate results only, a one-hour incubation was selected.

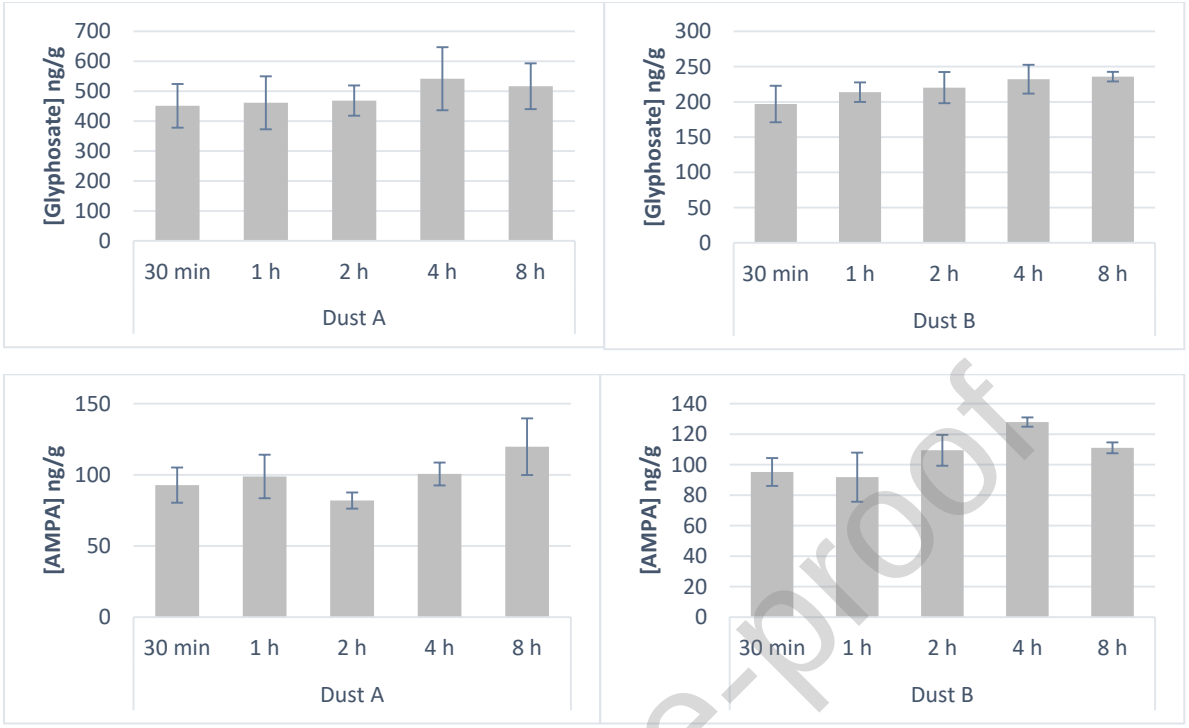

Figure 2. Extraction yields of glyphosate and AMPA in Dust A and B according to the time of incubation (n = 3).

3.1.5 Long term performances and maintenance

The HILIC/MS/MS method developed in this study is rapid and easy to implement, yet sufficiently sensitive for the concentrations of glyphosate and AMPA found in French dust samples. To maintain its performance in terms of sensitivity and robustness, a few limitations have to be overcome: (i) a blank sample needs to be injected between each dust sample to avoid carry over due to the potentially high concentrations present in dust, in particular for glyphosate, (ii) the chromatographic system and ion source have to be cleaned for each series of injections and the Stepwave on a regular basis to avoid the loss of internal standard due to matrix effects in dust samples, bearing in mind that dust is a complex matrix and that extracts undergo a purification step but no extraction, and (iii) the pH of dust extracts has to be maintained at approximately pH 2 to maintain the correct ionic form for glyphosate and AMPA (SI Figure 5).

3.2. Application to real samples

3.2.1. Results for the AMPA, glyphosate, and glufosinate concentrations in SRM 2585

Use of the described method on SRM 2585 gave concentrations of 63 ng/g (RSD of 14%, n = 14) for AMPA and 735 ng/g (RSD of 10%, n = 19) for glyphosate. No glufosinate was detected.

3.2.2. Results for the AMPA, glyphosate, and glufosinate concentrations from the French survey

The results for the AMPA, glyphosate, and glufosinate concentrations measured in the 60 dust samples from the PELAGIE mother-child cohort are presented in Table 3.

AMPA was quantified in 59 (98%) and glyphosate in 60 (100%) of the samples. For these two compounds, the distribution appears to be exponential, with very large differences between the minimum and maximum. This was even more pronounced for glyphosate, with a minimum of 94 ng/g and a maximum of 51,300 ng/g. No relationship could be established between the concentrations of glyphosate and its metabolite. The AMPA/glyphosate ratio ranged from 3 to 105%. Glufosinate was never detected.

Table 3. Concentrations of AMPA, glyphosate, and glufosinate (in ng/g) in 60 dust samples (France 2010-2012)

	n > LOQ	min	25th percentile	median	75th percentile	max	Mean ± SD	GM
AMPA	59	31	108	210	413	2,130	380 ± 440	229
Glyphosate	60	94	355	814	2,020	51,300	3,110 ± 7,630	958
Glufosinate	0	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	-

Glyphosate is widely detected, which is consistent with its use. For example in France, during the collection period of dust samples (2009-2012), the sale of glyphosate reached more than 8 000 tons each year, which represents approximately 10% of total pesticide sales (see the BNVD at https://geo.data.gouv.fr/fr/). The AMPA is also widely detected. It is expected because AMPA is the main environmental metabolite of glyphosate and is also a metabolite of amino-phosphonates ((Grandcoin et al., 2017)). In contrast, glufosinate was not detected. This molecule was approved in the European Union much later than glyphosate and only two years after the dust collection campaign (in 2007). Its sales were very limited compared to glyphosate (approximately 160 tons each year in France at this period). This may explain why it was not found in dust dwellings.

For glyphosate, concentrations are also presented by sampling-site characteristics in Table 4. The statistical tests carried out show different distributions, with higher median concentrations in rural than in urban settings, in households located < 500 m from crops, and in households reporting the use of weedkillers on driveways or lawns.

To the best of our knowledge, the only other available study showing glyphosate concentrations in indoor dust was carried out by Curwin et al. in the US (Curwin et al., 2005). This study compared glyphosate contamination (among other pesticides) inside 25 farm and 25 non-farm US homes. Dust was collected on two occasions, first, shortly after a spraying event and, second, approximately four

weeks later, using a high-volume small surface sampler (HVS3). Dust samples (0.5 g) were acidified with H_3PO_4 and extracted by sonication in deionized water. Extracts were than dried and derivatized with a 2:1 mixture of trifluoroacetic acid and trifluoroethanol before analysis by GC-MS/MS.

The present study shows that the concentrations found in French dust, collected from 2010 to 2012, were higher than the concentrations in dust collected in the US in 2001. US non-farm dwellings had a geometric mean (GM) of 140 ng/g, with a 21-610 ng/g 95% confidence interval (CI), whereas French dwellings with no proximity to fields had a GM of 558 ng/g, with a 200-1,580 ng/g 95% CI. Similarly, concentrations found in French dwellings close to fields, with a GM of 1,408 ng/g (574-8447 95% CI), were higher than concentrations found in dwellings on US Farms, whether spraying had recently occurred (GM: 920 ng/g , 95% CI: 140-7400 ng/g) or within seven days of spraying (GM: 1100 ng/g, 95% CI: 180-9700 ng/g). Despite these differences, which can be explained not only by the geographical difference but also the different period of sampling, it is reassuring that the concentrations are in the same range, regardless of the analytical method used.

*Table 4. Concentrations of glyphosate (ng/g) by sampling-site characteristics in 60 dust samples (France, 2010-2012), (ns: P > 0.05, *P ≤ 0.05, **P ≤ 0.01)*

	n	min	25th perc.	median	75th perc.	max	GM	95% CI	Mann-Whitney test
Type of location									
Rural / semi-rural	29	116	415	1,680	4,350	51,300	1,570		** (p = 0.0065)
Urban	31	94	254	457	1,380	16,000	602		
Residential proximity (< 500m) to crops									
Yes	35	116	395	1,410	2,290	51,300	1,410	574-8,447	*
No	25	94	246	457	873	6530	558	201-1,580	(p = 0.0267)
Parents working with pesticides									
Yes	7	381	1,320	1,860	4,340	51,300	2,640		ns
No	53	94	334	609	1,980	24,400	838		(p = 0.0759)
Household use of weed killer on driveways/lawns									
Yes	18	315	582	2,040	5,990	24,400	2,070		**
No	42	94	281	462	1,670	51,300	688		(p = 0.0055)
Household use of weed killer on outdoor plants									
Yes	4	1,310	1,810	2,040	2,140	2,260	1,870		ns
No	56	94	347	640	1,920	5,1300	913		(p = 0.1387)
Household use of weed killer on driveways/lawns or outdoor plants									
Yes	19	315	591	2,110	5,440	24,400	2,080		**
No	41	94	268	457	1,640	51,300	668		(p = 0.0024)

3.2.3. Glyphosate exposure via dust ingestion

As this study showed the presence of glyphosate and AMPA in French indoor dust, an exposure assessment by ingestion of this media was performed for these two compounds. The doses of glyphosate exposure (sum of glyphosate and AMPA expressed as glyphosate) from indoor dust ingestion are presented in Table 5.

According to French monitoring plans (2011), the mean and 95th percentile of the estimated intake of glyphosate from food are 0.03 and 0.27 µg/kg/bw/d, respectively, for adults (ANSES, 2014). For children, considering the last study of the total infant diet, the estimated dietary intake of glyphosate (lower-bound/upper-bound of the median/90th percentile) are 0/1.304/1.889 and 0/1.280/1.852 µg/kg/bw/d for infants aged 5 to 6 months and 7 to 12 months, respectively (ANSES, 2016). These values were calculated from data of glyphosate and AMPA concentrations in food and water (ANSES 2014 and 2016). Taking into account the available data of the French dietary intake ($E_{Gly + AMPA/diet}$), median cases were compared to the mean or upper-bound of the median and worst cases to the 90th or 95th percentile. Median and worst cases were also compared to the acceptable daily intake (ADI). The glyphosate ADI is currently 500 µg/kg bw/d (European Commission, 2017).

Table 5. Glyphosate exposure via indoor dust ingestion

	Median cases			Worst cases		
Age	**6 months**	**1 year**	**Adult (30 years)**	**6 months**	**1 year**	**Adult (30 years)**
ADI (µg/kg bw/d)	500					
$E_{Gly+AMPA/diet}$ (µg/kg bw/d) *median/mean or #P90/P95	1.304*	1.280*	0.03*	1.889#	1.852#	0.27#
$E_{Gly+AMPA/dust}$ (µg/kg bw/d)	0.006	0.004	0.000 3	0.81	0.41	0.04
% of $E_{Gly+AMPA/diet}$	0.53%	0.34%	1.05%	43.8%	22.8%	16.8%
% of ADI	< 0.002%	< 0.002%	< 0.002%	0.17%	0.08%	0.01%

The main route of exposure for this pesticide in the general population is by food. Regardless of age, for median cases, glyphosate exposure by indoor dust ingestion accounts for < 2% of dietary glyphosate intake (0.53% for a six-month-old child, 0.34% for a one-year-old child, and 1.05% for adults). Indoor dust ingestion appears to be a minor route of glyphosate exposure. For the worst cases tested, it represents between 16.8 and 43.8% of the French dietary glyphosate intake (P90 or P95) but < 0.2% of the current ADI of 500 µg/kg bw/d (0.17% for a six-month-old child, 0.08% for a one-year-old child, and 0.01% for adults) (European Commission, 2017).

Nevertheless, several limitations are worth mentioning. First, the dose from food is probably over estimated because the detection limits of glyphosate in food matrices are higher than in indoor settled dust. In contrast to dust, in which 100% of the samples of this study showed a glyphosate concentration above the LOD, most food products show results under the LOD. Second, this study concerned only a limited sampling of indoor dust in France that is not representative of French contamination. Therefore, there could be cases with lower or higher concentrations of glyphosate. Third, new toxicological studies on endocrine disrupting effects or co-exposition (with other pesticides or adjuvants) should lead to a change in the current ADI in the coming months. Indeed, the ADI of glyphosate was set to 0.5 mg/kg/d by the European Commission in 2017 based on a teratogenesis study (NOAEL at 50 mg/kg/d). Glyphosate is currently undergoing a re-approval process and in the meantime, the European Commission decision has renewed the approval until 15 December 2023.. Toxicological data from the scientific literature should also be taken into account in the decision because the standardized tests required by European regulations do not cover all the effects potentially involved in diseases and health disorders. The latest collective expertise on this issue concluded that there was an increased risk of non-Hodgkin lymphoma with “at least one good quality study that shows a statistically significant association”, and suggested a non-mutagen mechanism of action, inducing oxidative stress and genotoxicity (chromosomal aberrations have been demonstrated in rodents exposed to 25 and 50 mg/kg, intraperitoneally) (INSERM, 2021). Oxidative stress and genotoxicity were also observed for in vitro AMPA exposure (Woźniak et al., 2018). However, glyphosate is currently mostly studied for other types of effects, in particular, reprotoxicity (transgenerational and epigenetic effects) and neurotoxicity (microbiota perturbations), due to possible mechanisms of endocrine disruption at lower doses. Thus, Pham et al. (Pham et al., 2019) showed that perinatal exposure to glyphosate may affect spermatogenesis in offspring male mice exposed in utero at 0.5 mg/kg/day. Exposure to 1.75 mg/kg/d resulted in an increase in anogenital distance in males, delayed age at first estrus and increased testosterone concentration in females, and increase plasma TSH levels in males (Manservisi et al., 2019). These observations are consistent with mechanistic hypotheses concerning the inhibition of aromatase and activation of the ERα receptor signaling pathway. In addition, another study has shown that glyphosate may alter behavior of the mother (licking behavior toward pups) for exposure levels similar to those previously described (0.5 mg/kg/d). This study also showed that glyphosate exposure at 0.5 mg/kg/d during pregnancy modulates neuroplasticity (increase in immature neurons) and affects the gut microbiota (significant alteration of the phyla Bacteroidetes and Firmicutes) in the mother (Dechartres et al., 2019). The impact of glyphosate on the gut microbiota was further studied using a metabolomics approach. Hu et al. (Hu et al., 2021) found that urinary metabolite profiles of rodent male pups exposed to 1.75 mg/kg/d were significantly altered, with an increase in homocysteine, and the

relative abundance of Prevotella to negatively correlate with the level of homocysteine. Homocysteine is a metabolite that may be dysregulated in cardiovascular disease and inflammation through the commensal microbiome, opening up new perspectives for studies of other types of adverse effects. To date, further studies are still needed to fully assess the toxicity of glyphosate for humans.

4. Conclusion

In this study, we developed a new analytical method that is rapid, sensitive, and robust to measure the contamination of indoor dust and provide the first French data for glyphosate and AMPA concentrations in settled dust samples from children's dwellings. AMPA was quantified in 98% of dust samples, with concentrations ranging from 31 to 2,130 ng/g and glyphosate was quantified in 100% of samples, with concentrations ranging from 94 to 51,300 ng/g, which is slightly higher than glyphosate concentrations measured in the US (Curwin, 2006). Human exposure to glyphosate via dust ingestion is much lower than the ADI and this exposure pathway should therefore not be of concern relative to that from food ingestion, except for the worst-case scenario, in which it can reach up to 44% of the dietary intake (6-month-old child). However, recent toxicity data concerning the potential reprotoxic effects of glyphosate may lead to a new evaluation towards a lower ADI. In addition, more studies need to be performed on the use of adjuvants and co-formulants, which could also have an impact on the overall toxicity associated with glyphosate use.

CRediT author statement

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CRediT author statement

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Declaration of interests

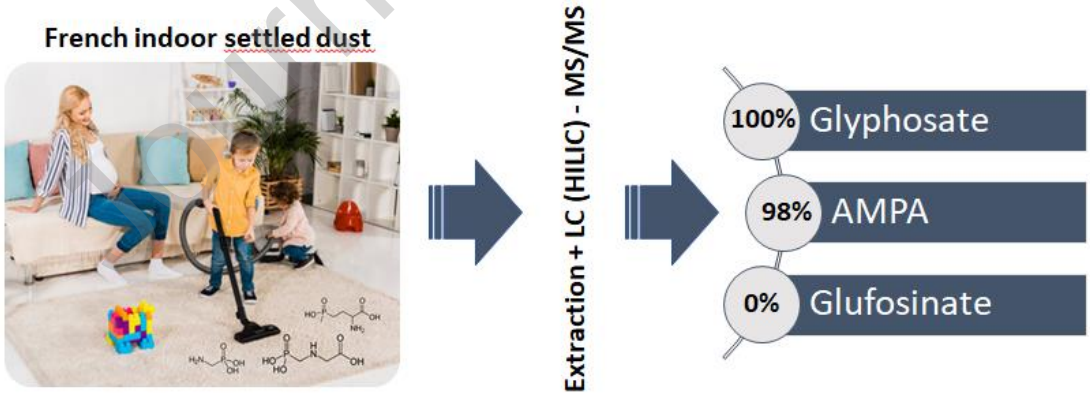
☒The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Environmental implication

Glyphosate is the most widely used herbicide in the world in terms of volume, and should be considered “hazardous materials” to public health due to an increased risk of non-Hodgkin lymphoma, as well as reprotoxicity and neurotoxicity effects due to possible mechanisms of endocrine disruption, as shown in animal studies. This works aims to characterize the presence of glyphosate in indoor dust, thus contributing to address a lack of knowledge regarding human exposure to glyphosate via non-dietary ingestion of indoor dust.

Graphical abstract



Highlights

- Analysis of glyphosate, AMPA, and glufosinate in dust in 60 French households
- Glyphosate was found in all samples (median of 814 ng/g and maximum of 51,300 ng/g).
- No exceedance of European regulatory standards for daily intake