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BRIEF REPORT

Development of a method to determine workers' personal exposure levels to glyphosate

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Abstract

Objectives: We aimed to develop a method to determine workers' personal exposure levels to *N*-(phosphonomethyl)glycine (glyphosate) for their risk assessments.

Methods: The proposed method was assessed as follows: recovery, stability of samples on storage, method limit of quantification, and reproducibility. Glyphosate in air was sampled using an air-sampling cassette containing a glass fiber filter. Ultrapure water was used to extract glyphosate from sampler filters. After derivation with 9-fluorenylmethyloxycarbonyl chloride, samples were analyzed by high-performance liquid chromatography using a fluorescence detector.

Results: Spiked samples indicated an overall recovery of 101%. After 7 days of storage at 4°C, recoveries were approximately 100%. The method limit of quantification was 0.060 μg/sample. Relative standard deviations representing overall reproducibility, defined as precision, were 1.4%–1.8%.

Conclusions: The method developed in this study allows 4-h personal exposure monitoring of glyphosate at 0.250–500 μg/m^3. Thus, this method can be used to estimate worker exposure to glyphosate.

KEYWORDS

Fmoc, glyphosate, high-performance liquid chromatography, personal exposure monitoring, worker, workplace air

1 | INTRODUCTION

N-(phosphonomethyl)glycine (glyphosate: CAS number: 1071-83-6), a colorless, odorless, and crystalline solid, is a non-selective herbicide. Its melting point and saturation vapor pressure are 184.5°C and 1.31×10^{-5} Pa (25°C), respectively. Occupational glyphosate exposure may occur during the manufacturing process or during spraying in agriculture and horticulture.[1,2] The Ministry of Health, Labour and Welfare (MHLW) of Japan selected glyphosate as a target substance in a project on workplace risk assessments from 2019 to 2020[3] because it has been classified as a Group 2A (probably carcinogenic to humans) compound by the International Agency for Research on Cancer[4] and a Group 2B (possibly carcinogenic to humans) compound by the Japan Society for Occupational Health (JSOH).[1,2] In 2021, the JSOH proposed an occupational exposure limit (OEL) of 1.5 mg/m^3 (provisional values) for glyphosate.[1,2]

Several methods have been described to monitor personal exposure to glyphosate in workplace air.[5–7] However, the sampling capacity and sensitivity of these methods did not meet the specified criteria of MHLW guidelines.[8]

We aimed to develop and validate a monitoring method for personal exposure to glyphosate for quantitative risk assessments. The method is based on the U.S. Department of Labor, Occupational Safety and Health Administration (OSHA) method No. PV2067.[9]

2 | MATERIALS AND METHODS

2.1 | Materials

Glyphosate solution (1000 μg/ml in H_2O) and glyphosate were purchased from Sigma–Aldrich, and 9-fluorenylmethyloxycarbonyl chloride (Fmoc-Cl) was bought from Tokyo Chemical Industry. Analytical grade acetone, sodium tetraborate decahydrate, phosphoric acid, hydrochloric acid, ethyl acetate, and disodium hydrogen phosphate were used. Acetonitrile and methanol were also used and were of high-performance liquid chromatography (HPLC) grade. A 0.1 M borate buffer was prepared by dissolving sodium tetraborate decahydrate in ultrapure water and adjusting the pH to 8.5 with 2 M hydrochloric acid. Phosphate buffer (10 mM) was made from disodium hydrogen phosphate dissolved in ultrapure water, and the pH was adjusted to 2.5 with phosphoric acid. The sampler used consisted of an air-sampling cassette (catalog no. 225-3LF; SKC Inc.) with a glass fiber filter (catalog no. AP2004200; Merck Millipore Ltd). An SKC AirChek 2000 (SKC Inc.) sampling pump was used to draw air through the sampler.

2.2 | Instruments

The HPLC system used was a Chromaster (Hitachi) with a 5440 fluorescence detector (FLD). The separation column used was an Inertsil ODS-2 (150 mm × 4.6 mm I.D., 5 μm; GL Sciences Inc.); a flow rate of 1.0 ml/min at 40°C was used. The mobile phase consisted of Eluent (A), a 10 mM phosphate buffer, and Eluent (B), acetonitrile. Gradient elution was as follows: 0.0–8.0 min, 30% (B); 8.1–15.0 min, 90% (B), and 15.1–20.0 min, 30% (B). The excitation and fluorescence wavelengths of the FLD were set to 265 and 315 nm, respectively.

2.3 | Sample preparation

After sampling was completed, each filter was put into a polypropylene test tube with 12 ml of ultrapure water. Each tube was shaken for 1 min, sonicated for 5 min, followed by centrifugation for 10 min at 1870 × g. Extraction solution (100 μl) was transferred to another polypropylene test tube. After the addition of 0.1 M borate buffer (1000 μl) and 0.1% Fmoc-Cl in acetone (1000 μl), the tube was vortexed for 10 s before sitting for 10 min at room temperature. The tube was then vortexed for 10 s again after the addition of ethyl acetate (1000 μl). The aqueous layer (200 μl of the lower layer) was transferred to another polypropylene test tube and then vortexed for 10 s after adding 0.1 M borate buffer (1000 μl). A sample solution (10 μl) was then injected into the HPLC-FLD.

2.4 | Method validation

The proposed method was validated according to the guidelines of MHLW.[8] A standard solution (25 μl) at a certain concentration was spiked onto the filter of a sampler. At the same time, room air (temperature, 22.6–23.2°C; relative humidity, 30%–32%) was drawn through the sampler at a flow rate of 1 L/min for 4 h.

A recovery test used spiked amounts from 0.06 to 120 μg in a sampling volume of 240 L, which corresponded to air concentrations of approximately 0.25–500 μg/m^3. Tests of storage stability involved the use of three different spiked amounts of glyphosate (0.06, 60, and 120 μg) on each filter in a 240 L sampling volume, which corresponded to air concentrations of about 0.25, 250, and 500 μg/m^3. After the drawing of air through the spiked sampling filters, these were then sealed and stored for a week at 4°C in the dark.

3 | RESULTS AND DISCUSSION

3.1 | Sampler selection

Several types of samplers have been used in previous studies for sampling glyphosate in air, including a glass fiber filter,[9] midget impinger,[5] ORBO 1000,[7] and a sampler combining a glass fiber filter and Tenax tube.[10] Of these, we chose a glass fiber filter because glyphosate is presumed to be present as a particulate in workplace air[11] due to its very low vapor pressure at ambient temperature. Ultrapure water was used as the solution to extract glyphosate from the glass fiber filter after sampling because glyphosate is highly soluble in water. We evaluated three types of glass fiber filters (AP20, GB-100R, and T60A20) by an extraction test using ultrapure water (spiked amount, 0.06 or 60 μg; sampling volume, 10 L; $n = 2$). The extraction efficiencies from the spiked samplers for each type of filter were more than 90% (AP20, 96%–100%; GB-100R, 92%–99%; T60A20,

94%–101%). From these results, we adopted AP20 as a sampling filter because it yielded the best results.

3.2 | Modification of sample preparation and optimization of HPLC analysis

Many of the analytical methods for glyphosate described in previous studies require derivatization procedures using Fmoc-Cl,[7,9] o-phthalaldehyde,[12,13] trifluoroacetic anhydride[5] or a mixture of trifluoroacetic anhydride and 2,2,3,3,4,4,4-heptafluoro-1-butanol,[10] because the direct analysis is generally difficult. We used the HPLC method[7,9,12,13] because glyphosate is heat-labile and, therefore, not suitable for the GC method.[5,10]

In a preliminary experiment, we investigated the derivatization procedure for the OSHA method[9] using Fmoc-Cl. Fmoc-derivation[7,9] is easier to perform and is more useful as a common system than o-phthalaldehyde-derivation,[12,13] so we used the former. Fmoc-Cl reacted with the amino group of glyphosate to produce a highly fluorescent derivative (Fmoc-glyphosate). Under our HPLC-FLD conditions, although excess underivatized Fmoc-Cl was eluted after Fmoc-glyphosate and Fmoc-OH without interfering with these peaks, complete elution was largely time-consuming. Therefore, it was necessary to remove excess underivatized Fmoc-Cl to reduce analysis time. To that end, we adopted the analytical methods of previous studies by Usui et al. and Akuzawa et al.[14,15] with some modifications. These methods involve the removal of excess underivatized Fmoc-Cl by extraction with ethyl acetate. This procedure properly removed excess underivatized Fmoc-Cl and reduced the analysis time to 20 min, including the time to clean the column (Figure 1A). Fmoc-glyphosate was not detected on chromatograms of a solution extracted from a blank AP20 sampling filter (Figure 1B).

3.3 | Recovery of glyphosate from AP20 sampling filters after sampling

According to MHLW guidelines, the minimum sampling capacity required to monitor personal exposure to chemical substances is 240 L (1 L/min, 4 h). Therefore, recovery and storage stability tests were conducted with a sampling volume of 240 L. Overall recoveries from spiked AP20 were 101% (Table 1). Therefore, using AP20

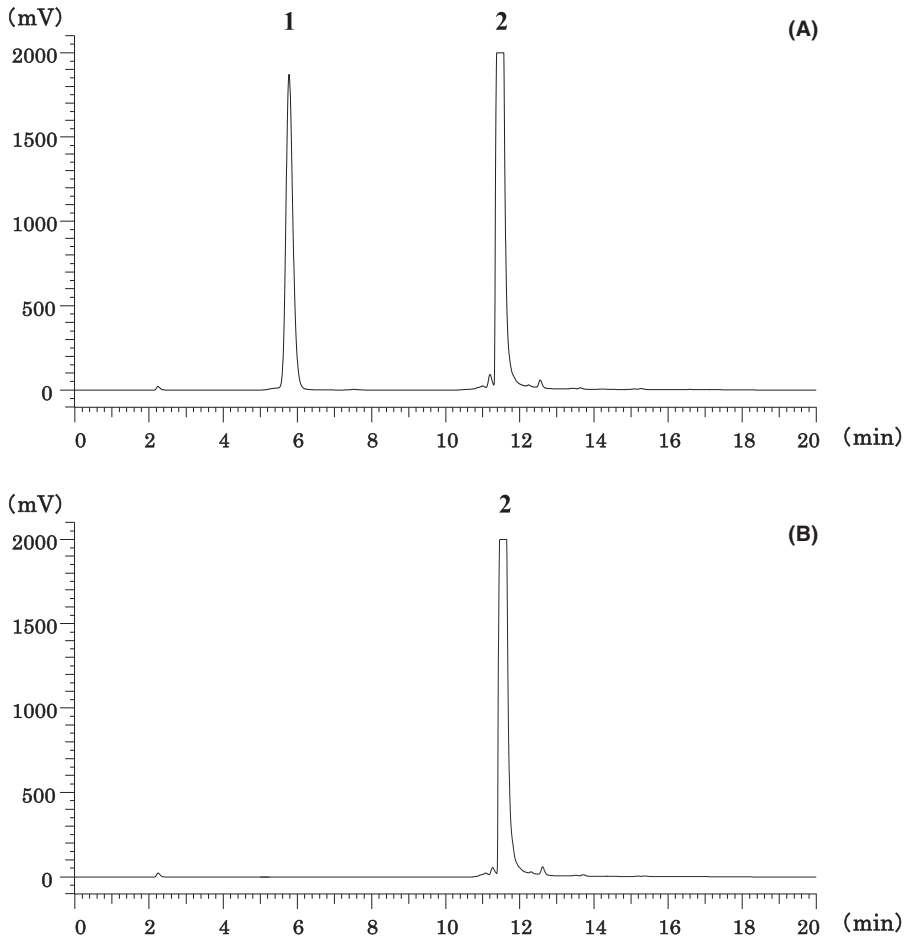

FIGURE 1 Chromatograms of (A) a solution extracted from a glass fiber filter spiked with a standard solution containing 120 μg of glyphosate; and (B) a solution extracted from a blank glass fiber filter. Peak 1, 9-fluorenylmethyl (Fmoc) derivative of glyphosate; peak 2, 9-fluorenylmethanol (Fmoc-OH).

TABLE 1 Recovery tests ($n = 5$)

Spiked amount (μg)	Mean ± SD (%)	RSD (%)
0.06	101.0 ± 1.8	1.7
60	101.2 ± 1.8	1.8
120	100.8 ± 1.4	1.4

Note: Solutions with a given amount of glyphosate were spiked onto the filter of a sampler. Simultaneously, room air was drawn through the sampler at a flow volume of 1 L/min for 4 h. The spiked amounts correspond to air concentrations of approximately 0.25–500 μg/m^3 for glyphosate.

Abbreviations: RSD, relative standard deviation; SD, standard deviation.

as a sampler is appropriate for monitoring personal exposure to glyphosate.

3.4 | Storage stability of glyphosate on AP20 sampling filters

Storage stabilities were evaluated by comparing the amounts of glyphosate determined in stored AP20 filters after sampling with those in samples analyzed immediately after preparation. Recoveries from all spiked samplers were almost 100% after 7 days of storage. This indicates storage of glyphosate on a glass fiber filter for at least 7 days at 4°C is acceptable.

3.5 | Method limit of quantification and reproducibility

Calibration curves were linear in the range of 0.0050–10 μg/ml, and correlation coefficients were greater than 0.999. The instrumental limit of quantification (ILOQ) was defined as 10 times the standard deviation ($n = 5$) of the peak area of the lowest standard and determined from the calibration curves. The ILOQ was assessed as being 0.040 μg/sample. The method limit of quantification (MLOQ) was defined as the smallest amount of glyphosate resulting in a >90% recovery within a range of recovery test and was found to be 0.060 μg/sample. As a result, the range of measurable air concentrations for the proposed method was from 0.250 to 500 μg/m^3 with a 4 h sample. Although this concentration range corresponds from 1/6000 to 1/3 times the OEL proposed by the JSOH, this covers glyphosate concentrations (0.63–43 μg/m^3) reported in previous studies.[5–7] If the glyphosate concentration exceeds the calibration range, the extracted sample solution should be reanalyzed after an appropriate dilution. Through sampling and analysis, relative standard deviations (RSD) relating to the overall reproducibility of the proposed method were determined to be from 1.4% to 1.8% (Table 1). Such a range of RSD values highlights the good reproducibility of the proposed method.

4 | CONCLUSIONS

The proposed method enables the monitoring of personal exposure to glyphosate in a concentration range of between 0.250 and 500 μg/m^3 in a 4 h period; this corresponds to between 1/6000 and 1/3 times the OEL proposed by the JSOH. Thus, this highlights the usefulness of the proposed method for estimating workers' exposure to glyphosate.

AUTHOR CONTRIBUTIONS

K.I. conceived the idea and drafted the manuscript. K.I and O.N. collected the data. K.I., A.T., and O.N. analyzed the data. M.O. provided technical expertise. A.T. and O.N. provided theoretical expertise. A.T., M.O., and G.E. provided critical feedback and contributed to the preparation of the manuscript.

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DISCLOSURE

Approval of the research protocol: N/A. *Informed Consent*: N/A. *Registry and the Registration No. of the study/trial*: N/A. *Animal Studies*: N/A. *Conflict of interest*: N/A.

DATA AVAILABILITY STATEMENT

Data openly available in a public repository that issues datasets with DOIs.

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