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 2020³ because it has been classified as a Group 2A (probably carcinogenic to humans) compound by the International Agency for Research on Cancer⁴ 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/m³ (provisional values) for glyphosate.¹ ²

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/m³. Thus, this method can be used to estimate worker exposure to glyphosate.
Several methods have been described to monitor personal exposure to glyphosate in workplace air.\(^{\text{5-7}}\) However, the sampling capacity and sensitivity of these methods did not meet the specified criteria of MHLW guidelines.\(^{\text{8}}\)

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.\(^{\text{9}}\)

## 2 | MATERIALS AND METHODS

### 2.1 | Materials

Glyphosate solution (1000 \(\mu g/ml\) 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 (HPLC grade). A 0.1 M borate buffer (1000 \(\mu l\)) was then added to 2 M hydrochloric acid, ethyl acetate, and disodium hydrogen phosphate. The addition of phosphoric acid, 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 \(\times\) 4.6 mm I.D., 5 \(\mu m\); 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 \(\times\) g. Extraction solution (100 \(\mu l\)) was transferred to another polypropylene test tube. After the addition of 0.1 M borate buffer (1000 \(\mu l\)) and 0.1% Fmoc-Cl in acetone (1000 \(\mu l\)), 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 \(\mu l\)). The aqueous layer (200 \(\mu l\) 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 \(\mu l\)). A sample solution (10 \(\mu l\)) was then injected into the HPLC-FLD.

### 2.4 | Method validation

The proposed method was validated according to the guidelines of MHLW.\(^{\text{8}}\) A standard solution (25 \(\mu l\)) 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 \(\mu g\) in a sampling volume of 240 L, which corresponded to air concentrations of approximately 0.25–500 \(\mu g/m^3\). Tests of storage stability involved the use of three different spiked amounts of glyphosate (0.06, 60, and 120 \(\mu g\)) on each filter in a 240 L sampling volume, which corresponded to air concentrations of about 0.25, 250, and 500 \(\mu g/m^3\). 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,\(^{\text{9}}\) midget impinger,\(^{\text{5}}\) ORBO 1000,\(^{\text{7}}\) and a sampler combining a glass fiber filter and Tenax tube.\(^{\text{10}}\) Of these, we chose a glass fiber filter because glyphosate is presumed to be present as a particulate in workplace air 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 \(\mu g\); 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 anhydride5 or a mixture of trifluoroacetic anhydride and 2,2,3,3,4,4,4-heptafluoro-1-butanol10 because the direct analysis is generally difficult. We used the HPLC method7,9,12,13 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 method9 using Fmoc-Cl. Fmoc-derivation7,9 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

![Chromatograms](image-url)

**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).
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/m³ 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/m³) reported in previous studies.²⁻⁷ 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/m³ 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.

### ACKNOWLEDGMENTS

This study was commissioned by the MHLW. We thank the staff of the Osaka Occupational Health Service Center, Japan Industrial Safety and Health Association, and the Occupational Health Research and Development Center, Japan Industrial Safety, and Health Association, without whose support the development of this method would not have been possible. The funding source had no role in the design, practice, or analysis of this study.

### 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.

### ORCID

Kenta Ishii  https://orcid.org/0000-0002-1525-7058
Akito Takeuchi  https://orcid.org/0000-0003-1782-6565

### REFERENCES

1. The Committee for Recommendation of occupational exposure limits, Japan Society for Occupational Health. Occupational exposure limits for acetaldehyde, 2-bromopropane, glyphosate, manganese and inorganic manganese compounds, and zinc oxide nanoparticle, and the biological exposure indices for cadmium and cadmium compounds and ethylbenzene, and carcinogenicity. J Occup Health. 2021;63(1):e12294.

---

**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/m³ for glyphosate.

Abbreviations: RSD, relative standard deviation; SD, standard deviation.
2. The Committee for Recommendation of Occupational Exposure Limits, Japan Society for Occupational Health. Documentation of the proposed reasons for provisional occupational exposure limits (2021). *Sangyo Elseigaku Zasshi*. 2021;63(5):213-272. (in Japanese).

3. Japan Industrial Safety and Health Association. *Report on the Implementation Results of the Promotion Project for Risk Assessment of Chemical Substances in the Workplace (Survey of Actual Exposure), Part 1 and Part 2*. http://id.ndl.go.jp/bib/031378785 (in Japanese) Published March 2021; Accessed March 17, 2022.

4. International Agency for Research on Cancer. Some organophosphate insecticides and herbicides: IARC monographs on the evaluation of carcinogenic risks to humans. Vol 112. https://publications.iarc.fr/_publications/media/download/6083/ee47b45697ec10087638e430c5b573d462a32143.pdf. Published January 2017. Accessed May 21, 2022.

5. Jauhiainen A, Räsänen K, Sarantila R, Nuutinen J, Kangas J. Occupational exposure of forest workers to glyphosate during brush saw spraying work. *Am Ind Hyg Assoc J*. 1991;52(2):61-64.

6. Centre de Toxicologie du Québec. *Etude de l’exposition professionnelle des travailleurs forestiers exposés au glyphosate*. 1988 Le Centre Hospitalier de l’Université Laval. http://www.sante.com/Bibliothèquevirtuelle/sante.com/35567000039898.pdf. Accessed March 17, 2022.

7. Morshed MMO, Omar D, Mohamad RB, Wahed SBA. Determination of glyphosate through passive and active sampling methods in a treated field atmosphere. *African J Agric Res*. 2011;6(17):4010-4018.

8. Ministry of Health, Labour and Welfare (MHLW), Japan. Guidelines for exposure assessment of workers to hazardous substances. Accessed March 9, 2022. https://www.mhlw.go.jp/content/11201000/000854537.pdf http://www.mhlw.go.jp/shingi/2010/01/s0115-4.html (in Japanese). Published January 2009.

9. Occupational Safety and Health Administration. OSHA method. PV2067. Accessed January 26, 2022. http://www.osha.gov/sites/default/files/methods/pv2067.pdf. Published November 1989.

10. Johnson PD, Rimmer DA, Garrod ANI, Helps JE, Mawdsley C. Operator exposure when applying amenity herbicides by all-terrain vehicles and controlled droplet applicators. *Ann Occup Hyg*. 2005;49(1):25-32.

11. Humphries D, Byrtus G, Anderson A-M. Glyphosate residues in Alberta’s atmospheric deposition, soils and surface waters. Alberta Research Council; 2005.

12. Archer TE, Stokes JD. Residue analysis of glyphosate in blackberries by high-performance liquid chromatography and postcolumn reaction detection. *J Agric Food Chem*. 1984;32(3):586-588.

13. Moye HA, Scherer SJ, Miles CJ. A simplified high-performance liquid chromatographic residue procedure for the determination of glyphosate herbicide and (aminomethyl)phosphonic acid in fruits and vegetables employing postcolumn fluorogenic labeling. *J Agric Food Chem*. 1983;31(1):69-72.

14. Usui K, Minami E, Fujita Y, et al. Application of probe electrospray ionization-tandem mass spectrometry to ultra-rapid determination of glufosinate and glyphosate in human serum. *J Pharm Biomed Anal*. 2019;174:175-181.

15. Akuzawa H, Akaia H. Rapid determination of DL-homoalanine-4-yld(methyl)phosphinic acid by HPLC with a fluorescence detector. *Bunseki Kagaku*. 1997;46(1):69-74.

**How to cite this article:** Ishii K, Takeuchi A, Nishinoiri O, Endo G, Ono-Ogasawara M. Development of a method to determine workers’ personal exposure levels to glyphosate. *J Occup Health*. 2022;64:e12345. doi: 10.1002/1348-9585.12345