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Quantification of Glyphosate and Other Organophosphorus Compounds in Human Urine via Ion Chromatography Isotope Dilution Tandem Mass Spectrometry

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Abstract

Organophosphorus pesticides are the most used pesticides in the United States. Most organophosphorus pesticides are composed of a phosphate (or phosphorothioate or phosphorodithioate) moiety and a variable organic group. Organophosphorus pesticides are scrutinized by regulatory bodies and agencies because of their toxicity or suspected carcinogenicity. Upon exposure, organophosphorus pesticides and their metabolites eliminate in urine; these urinary biomarkers are useful to evaluate human exposure. We developed a method using stable isotope dilution, ion chromatography tandem mass spectrometry for quantification in urine of 6 O,O-dialkylphosphates, metabolites of organophosphorus insecticides, and glyphosate, the most used herbicide in the United States. With simple and minimal sample preparation, the analytical method is selective and sensitive with limits of detection between 0.2 and 0.8 μg/L. Accuracy and precision are both >85%. To assess the suitability of the method in real exposure scenarios, we analyzed samples collected anonymously from subjects with suspected exposure to pesticides (n=40) or who had been on an organic diet (n=50). We detected glyphosate in 80% of subjects reporting an organic diet and in 78% samples from those with suspected glyphosate exposure; concentrations ranged from <0.2 to 28.6 μg/L. Median concentrations were 0.39 μg/L for the organic diet group and 0.40 μg/L for individuals with suspected exposure. Interestingly, interquartile ranges were considerably higher among those reporting pesticide exposure (0.63 μg/L) than those consuming organic diets (0.42 μg/L). These data suggest that the method meets typical validation benchmark values and is sensitive to investigate background exposures in the general population.

Keywords

Glyphosate; DAPs; biomonitoring; IC-IC-MS/MS; herbicides

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Conflicts of interests

The authors declare they have no competing financial or other conflicts of interests.

Disclaimer

The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the CDC. The use of trade names is for identification purposes only and does not constitute endorsement by the U.S. Department of Health and Human Services or the CDC.

1. Introduction

Pesticides are used to kill, repel, or control certain forms of plant or animal life considered to be pests. Pesticides include fungicides, herbicides and insecticides, among others, with diverse functional chemistries. Of these, organophosphorus (OP) compounds are by far the most used pesticides in the United States in both agricultural and residential settings. For example, as of 2012, glyphosate, one of these OP compounds, was the most used herbicide in the United States in the agricultural sector and the second most used in the home and garden market sector (EPA 2017). Other OP compounds are mostly used as insecticides and account for up to 33% of the market share in the United States (EPA 2017). In 2013, 36 OP insecticides, including chlorpyrifos and diazinon, were registered with the U.S. Environmental Protection Agency for use in the United States (U.S. EPA 2013). OP pesticides have been popular because of their broad spectrum of applications and their relatively inexpensive cost (Karalliedde et al. 2001).

Because of increasing concern about the safety of OP pesticides, many are highly scrutinized by regulatory bodies and agencies. For example, in the USA, parathion is no longer registered for any use and chlorpyrifos is no longer registered for home use (U.S. EPA, 2000, 2006). Also, in March 2015 the International Agency for Research on Cancer (IARC) classified glyphosate as "probably carcinogenic in humans" (category 2A) based on epidemiological studies, animal studies, and in vitro studies (IARC 2015). In November 2015, the European Food Safety Authority (EFSA) updated risk assessment report on glyphosate concluded that the substance is unlikely to be genotoxic or carcinogenic to humans (EFSA 2015). In May 2016, the Joint FAO/WHO Meeting on Pesticide Residues concluded that "glyphosate is unlikely to pose a carcinogenic risk to humans from exposure through the diet," even at oral doses as high as 2,000 mg/kg body weight (FAO/WHO 2016). The US EPA concluded that the “available data and weight-of-evidence clearly do not support the descriptors “carcinogenic to humans,” “likely to be carcinogenic to humans,” or “inadequate information to assess carcinogenic potential” “(US EPA 2016).

After entering the body and to facilitate excretion in urine, some OP pesticides are enzymatically converted to their oxon form, which is rapidly hydrolyzed by phosphotriesterase paraoxonase I to form dialkylphosphate (DAP) metabolites and/or a hydroxylated organic moiety specific for each pesticide (Bar et al. 2003, Kavvalakis and Tsatsakis 2012). Glyphosate, on the other hand, follows a different pathway. Recent estimates suggest 1% to 30% of orally ingested glyphosate in humans is eliminated as the unchanged compound in urine (European Commission 2002, Faniband 2020, Zoller et al. 2020). Similarly, among male and female Sprague-Dawley rats administered ^{14}C-glyphosate (99% purity) via single oral dose at 10 mg/kg, 7 days post-treatment, radioactivity accounting for 28.6 and 22.5% of the administered dose (males and females, respectively) was recovered in urine (IPCS 1994). Neither the DAPs nor glyphosate undergo phase II conjugation (Brewster at al 1991, ATSDR 2019, Sudakin and Stone 2011).

One common way to assess OP pesticide exposure is by quantifying six non-specific urinary DAP metabolites of many OP pesticides: dimethylphosphate (DMP), dimethylthiophosphate (DMTP), dimethyldithiophosphate (DMDTP), diethylphosphate

(DEP), diethylthiophosphate (DETP), and diethyldithiophosphate (DEDTP). On the analytical side, current human biomonitoring methods measure either DAPs or glyphosate. Common approaches involve offline solid phase extraction with or without derivatization and subsequent injection into high performance liquid chromatographs or gas chromatographs coupled with tandem mass spectrometry (MS/MS) detection for quantification (Petchuay et al. 2008, Oglobline 2001, Bravo et al. 2004, Ueyama et al. 2014). Methods using derivatization without liquid-liquid extraction or SPE have also been reported (Conrad et al. 2017, Connolly et al. 2020). Another analytical approach involves enzyme-linked immunosorbent assays (ELISA). ELISA is sensitive with reported glyphosate limits of detection (LOD), based on sample matrix and analytical detection, between 0.05 μg/L and 0.6 μg/L (Jayasumana et al. 2015, Rendon-van Osten et al. 2017). However, ELISA has lower selectivity compared to mass spectrometry. Only a relatively small number of analytical methods exist in the peer-reviewed literature to measure environmental levels of glyphosate in human urine (Zoller et al. 2018, Connolly et al. 2020, Conrad et al. 2017).

Ion chromatography (IC) methods have been in use for various analytes and matrices, most commonly, water analytics and inorganic anions (Edgell et al. 1994, West et al. 2015). Thanks to technological advances which allow the combination IC with MS/MS systems, IC is used routinely to quantify polar ionic compounds (e.g., perchlorate) in human biomonitoring (Valentín-Blasini et al. 2005).

In this work, we report the development of an on-line IC coupled with MS/MS detection method for human biomonitoring purposes to measure DMP, DMTP, DMDTP, DEP, DETP, DEDTP and glyphosate (Figure 1).

2. Method

2.1 Materials

We obtained labeled and native DAP metabolites form Cambridge Isotope Laboratories (Andover, MA, USA). including DMP, DEP, DMTP, DETP, DMDTP and DEDTP and the corresponding alkyl-D_6 and D_{10} labeled analogs for dimethylphosphates and diethylphosphates, respectively. Glyphosate was obtained from Supelco (Bellefonte, PA, USA), and ^{15}N, 2-^{13}C Glyphosate was purchased from Cambridge Isotope Laboratories. HPLC grade solvents used include isopropanol and methanol from Fisher Scientific (Pittsburg, PA, USA). Ultrapure water was generated in house (AQUA solutions, Lab water systems, Jasper, GA, USA).

2.2 Standard preparation and calibration

Glyphosate (10 mg/L) and DAPs stock solutions (1 mg/L) in water were stored at −20 °C in Teflon™ capped silanized glass vials until further use. Ten calibration standards containing known amounts of DAPs and glyphosate were prepared by serial dilution of the stock solutions in water to final concentrations ranging from 0.1 μg/L to 60 μg/L. The lowest concentration calibrator (0.1 μg/L) was used as an “instrument ready check” to confirm low baseline conductivity, good chromatographic resolution and high sensitivity before the start of an analytical run, and was excluded from the 1/x weighted calibrations curves, leaving

the calibration in a range between 0.2 μg/L – 60 μg/L. The internal standard stock solutions containing isotope-labeled DAPs and glyphosate were diluted with water in a volumetric flask to reach concentration levels of 75-100 μg/L, depending on the analyte. All involved glassware was silanized, to minimize adsorption of analytes onto the glass surface.

2.3 Sample preparation

200 μL of the urine sample or aqueous standard was transferred into a silanized vial and 50 μL of internal standard and 200 μL water was added. For matched matrix calibration, 200 μL of blank urine was added instead of 200 μL water. The diluted sample was vortexed for 30 sec before analysis.

2.4 Quality control (QC).

As of today, commercial quality control (QC) materials for human biomonitoring do not exist for OP metabolites and glyphosate. Therefore, for QC and method validation purposes, we collected human urine anonymously from male and female adults in Atlanta, GA. No personal information was obtained. We cannot rule out that multiple samples were obtained from the same individuals. Collected samples were stored at −70 °C before analysis. CDC's Institutional Review Board approved the urine collection and analysis. A waiver of informed consent was requested under 45 CFR 46.116(d).

After screening for endogenous DAP and glyphosate concentrations we pooled urine samples with the lowest concentrations (representing a "blank" urine). We adjusted the concentration of the target analytes in low concentration pool aliquots to 1.0 and 2.0 μg/L for the QC low and 10 to 21 μg/L for the QC high. Two replicates of each QC material were included in all runs to ensure high quality measurements. Each QC material was characterized by repeated measurements to define mean concentrations and 95% and 99% control limits of each target analyte. The calculated mean of the concentrations of the two QCH and two QCL samples analyzed with each analytical batch were evaluated by standard statistical probability rules (Caudill 2008). Furthermore, as part of an in-house proficiency testing (PT) program, we prepared PT samples at 3 concentration ranges. PT_{low} has concentrations between 0.6 to 1 μg/L, PT_{mid} 6-10 μg/L, and PT_{high} 10-20 μg/L. PT samples were prepared in duplicates and characterized by at least 20 repeat measurements and data were evaluated by a PT administrator to determine the mean and standard deviation for each analyte. PT samples were externally blinded and stored at −70 °C. Every six months, a PT administrator randomly selects 5 samples for analysis and evaluates results to ensure long term method ruggedness and accuracy.

In addition to the in-house QC and PT programs, we regularly participate in the German External Quality Assessment Scheme (GEQUAS) for glyphosate and DAP metabolites (see http://www.g-equas.de/) and the External Quality Assessment Scheme for Organic substances in Urine (OSEQAS) for glyphosate (see https://www.inspq.qc.ca/en/ctq/eqas/oqesas/description).

2.5 Ion chromatography (IC)

Separation was accomplished with a Dionex ICS-5000+ ion chromatography system (Thermo Fisher Scientific, Sunnyvale, USA) consisting of a column and ion suppressor compartment, an autosampler, a dual pump system (1-channel and 3-channel pump) and an electrochemical KOH generator (Figure 2). In addition, we used three 1-channel auxiliary pumps, one attached to the suppressor, the second one for infusing isopropanol into the mass spectrometer after chromatographic separation and the third for infusing 50% isopropanol into the mass spectrometer electrospray ionization (ESI) source for cleaning purposes while chromatographic separation was carried out. All columns were obtained from Thermo Fisher Scientific. The two column forward-flush assembly consists of a Dionex UTAC-LP2 column for clean-up and enrichment followed by a Dionex AS24 (2 × 250 mm; 4 μm particles) ion exchange column for chromatographic separation. To increase the column durability, we added a guard column (AG24, 2 x 5mm, 4 um). The temperature in the column compartment was adjusted to 30 °C. 20 μL of the processed sample were injected with a constant flow of 1.5 mL/min H_2O. After 2 min, the analytes were transferred to the analytical column using 30 mM KOH. At 7 min, the concentration of KOH was increased to 100 mM and kept for 3 minutes (we also increased the flow rate between 8 and 9 min to 0.5 mL/min). The concentration of KOH was kept at 100 mM for 7 minutes. At 17.1 min the concentration was reduced to 30 mM KOH for 2.9 min (Table 2).

2.6 Mass spectrometry

We used an AB Sciex 5500 triple quadrupole mass spectrometer (Applied Biosystems, Foster City, CA, USA) equipped with a TurboIonSpray® source. The ESI source was operated in the negative ion mode at −4500 V, declustering potential (DP) of −80V and entrance potential of −10V. Nitrogen was used as collision gas (CAD cell pressure set to 9 (unitless)), curtain gas 20 psi, turbo heater and evaporator gas each at 60 psi. The ESI source temperature was optimized at 600 °C. The collision cell exit potential was set at −10V. The target scan time was set to 1 second. Resolution of Q1 and Q3 was set to “unit” and the time between transitions was 0.5 ms. Time-programmed multiple reaction monitoring was used, allowing peak monitoring for ±40 seconds from the analyte specific retention time. Specific ion transitions can be found in Table 1.

2.7 Urine samples for method validation

We purchased 90 urine samples from BioreclamationIVT (Hicksville, NY). The company had IRB approval to collect urine and obtained informed consent from donors who were verbally asked whether they had been in contact with glyphosate within the last 24 hrs of specimen donation (n=40) or whether they had been on an organic diet (n=50). No personal identifiers were provided to CDC.

3 Results and Discussion

3.1 General considerations

On-line extraction and liquid chromatography in back-flush mode combined with tandem mass spectrometry have become an integral part of human biomonitoring (Baker et al. 2019,

Kato et al. 2005, Kato et al. 2018, Schütze et al. 2016, Valentin-Blasini et al. 2005, Wang et al. 2017, Ye et al. 2005). We investigated several columns including reversed-phase, weak ion exchangers and HILIC columns and tested several combinations for a fast reliable and robust on-line HPLC method for extraction and separation of glyphosate and DAPs but lack of retention, low sensitivity, interferences and carry-over were extremely challenging and neither of those options satisfied the method objectives (data not shown).

Glyphosate is a small molecule, highly polar and forms metal complexes, which complicates its quantification (Subramaniam and Hoggard 1988, Sundaram and Sundaram 1997). The IC system we chose uses polyether ether ketone (PEEK) only materials to prevent carry-over and interaction with metal surfaces. Here we describe an IC extraction/separation using a forward-flush elution mode with a highly corrosive eluent and not volatile, KOH, with mass spectrometry detection, which under regular circumstances would not be compatible with such mobile phase.

Two key components of the system are the hydroxide eluent generator and the 500e eluent suppressor. To generate KOH, deionized water is pumped through the eluent chamber and a DC current is applied between the anode and cathode. This leads to the electrolysis of water at the Pt anode and Pt cathode. While water is oxidized at the anode, O_2 and H^+ are generated in the reservoir. H^+ displaces the K^+ in the reservoir, after which K^+ migrates across the cation exchange connector into the eluent generation chamber. At the cathode, water is reduced to form hydrogen gas and OH^-, which combines with the displaced K^+ ion. Formed hydrogen gas is taken out of the mobile phase via a degasser module. The 500e eluent suppressor applies DC current to cleave water into H^+ and oxygen at the anode and reduces water to OH^- and hydrogen gas at the cathode. The H^+ ions are then transported across a membrane into the eluent to neutralize the highly basic eluent, while K^+ is driven towards the cathode and transported into the waste, thus preventing K^+ build-up and corrosive damage to the mass spectrometry ESI source.

3.2 Ion Chromatography

The ion chromatography system consists of a two-column assembly, a UTAC LP2 column used for analyte extraction and clean up and an AG24/AS24 guard/column combination for analytical separation. Sample clean-up was achieved within the first minute of the method runtime. All analytes were well retained and longer washing times had no significant impact on matrix effects (data not shown) but led to slight peak distortion and loss of chromatographic resolution on the analytical column. 30 mM KOH proved to be useful to transfer the analytes from the extraction column forward onto the analytical column. The elution order was DEP, DMP, DETP, DMTP, glyphosate, DEDTP followed by DMDTP (Figure 3). DMP and DEP coeluted, however the analytes do not share the same ion transitions and unlikely interfered with each other. A closer look at the suppressor gradient reveals some “over-suppression.” For example, at 7 min, the KOH concentration is 30 mM at a flow rate of 0.4 mL/min and corresponds to 30 mA for full suppression. However, at 8 min we increase the flow rate to 0.5 mL/min and the current at the suppressor to 124 mA which can suppress 100 mM, however this concentration is generated later in the gradient at 10 min and takes approximately another 120 seconds to reach the suppressor (Table

2). Noteworthy, the suppressor current does not increase linearly, but rather jumps to the next current level at any given time a command is set. Therefore, the suppressor current command needs to be issued before linearly increasing the KOH concentrations to protect the ESI source from KOH influx at any given time, which is the case within this method.

One major concern for ion chromatography is retention time stability. We found no significant retention time changes within the same column regardless of the type and number of urine samples analyzed. To investigate the potential impact of retention time changes between columns for larger human biomonitoring studies such-as the National Health and Nutrition Examination Survey (NHANES) we rotated 6 analytical columns (3 different column lots) and 2 extraction columns in a total of 24 batches (2 runs per combination) and found that retention time differences among columns did not exceed the column specifications provided by the manufacturer.

An integral part of the system is the conductivity detector whose response is affected by all anions and allows us to determine when inorganic matrix components are eluted or whether OH^- is not fully suppressed. Certain interferences can cause ion defocusing in the ESI source that can considerably decrease system sensitivity over time. As can be seen in Figure 3, the analytes (Figure 3a) are separated from most of those anions (Figure 3b), which allowed us to divert these matrix ions into the waste during that time. Furthermore, to increase system stability and sensitivity we used two additional auxiliary pumps (AXP2 and 3). AXP2 pumped 100% isopropanol at 0.1 mL/min to enhance ionization efficiency and AXP3 pumped 50% isopropanol at the same flow rate for additional cleaning while the chromatographic separation was performed (divert position).

3.3 Validation parameters: Limit of Detection, Precision, Accuracy and Stability

The limit of detection (LOD) was established using the Taylor et al. (1987) method, which assesses the relative standard variation at each analyte calibration level. LODs are 0.2 μg/L for all analytes except DEDTP which was set to 0.8 μg/L.

The total precision was estimated as percent relative standard deviation (%RSD) by preparing and analyzing duplicate QClow and QChigh samples in 10 consecutive days using 3 different AS24 columns. Precision ranged from 3.4 to 18.4 %RSD. All values, except those of DMDTP and DEDTP in the QClow (16.6 % and 18.4 %, respectively) were within the 15 %RSD recommended by U.S. FDA guidance document (US-FDA, 2018). A detailed breakdown including intra and inter-day precision can be found in table 3

Because there are no commercial standard reference materials for glyphosate or DAPs in urine, the accuracy of the method was established by spiking two urine specimens at native (zero), low, mid and high concentrations and analyzing them in triplicate in two consecutive days (n=6 per sample and spike level, Table 4). After subtracting native concentrations, the calculated mean relative recovery was between 85-115% for all analytes at all spike levels in both investigated samples. One additional spike level was tested in a blank urine sample and showed an average of 102% (range 92 – 112%) relative recovery for glyphosate at 1 μg/L. Sample and analyte stability were investigated using QC materials under 3 different conditions. First, bench-top stability, which assessed short-term stability for length of time

needed to handle study samples (typically at room temperature). Samples were left outside at room temperature overnight and processed for injection the next day. Second, freeze-thaw stability, in which samples were frozen at −80°C and thawed 3 times and later prepared and analyzed. Third, processed sample stability, in which samples were analyzed after remaining in the autosampler for 2 days. We found no significant impact for any of the analytes, all analytes remained stable.

3.4 Study population

We analyzed 90 samples from subjects with either suspected exposure to pesticides (n=40) or who reported being on an organic diet (n=50). as shown in table 5, glyphosate concentrations varied from <0.2 to 28.6 μg/L, with an 80% detection frequency in the organic diet group and 78% in the glyphosate usage group. Median concentrations (calculated after replacing concentrations <LOD by LOD/SQRT(2) (Hornung and Reed 1990)) are comparable in both subsets, 0.40 μg/L for those potentially exposed vs 0.39 μg/L for those on an organic diet. However, a comparison of the interquartile range shows the influence from usage (0.63 μg/L vs 0.42 μg/L), suggesting exposure to glyphosate in both groups. Our glyphosate results compare well to published data here in the United States. Curwin et al. (2007) reported values for fathers (n=47) and mothers (n=48) from farm and non-farm families. Concentrations of glyphosate ranged between 0.02 μg/L and 18 μg/L, with geometric means for fathers from farm families of 1.9 μg/L and non-farm, 1.4 μg/L. Mothers' concentrations were 1.2 μg/L for non-farm and 1.5 μg/L for farm families, respectively. Parvez et al. (2018), reported concentrations in the range of 0.5-7.2 μg/L with a mean of 3.4 μg/L in pregnant women (n=71). Internationally, data from German university students showed relatively overall low concentrations of glyphosate ranging from 0.11 μg/L to 0.6 μg/L in 2012 (Conrad et al. 2017).

Similarly, for the DAP metabolites, the organic group had comparatively lower median concentrations for DMP (organic diet 0.27 μg/L vs suspected exposure 0.90 μg/L), DMTP (0.41 μg/L organic diet vs suspected exposure 0.97 μg/L) and DEP (0.86 μg/L organic diet vs suspected exposure 1.86 μg/L). Detection frequency ranged from 54 to 82%, depending on the analyte and group, except for DMDTP and DETP that were detected in only about a quarter of the samples. DEDTP was only detected in one sample, just above the LOD of 0.8 μg/L. Overall, concentrations in the samples analyzed for this study are slightly higher than those reported for NHANES (CDC 2019). Reported 2007-2008 NHANES median and 95^{th} percentile concentrations were <LOD (0.47 μg/L) and 35.6 μg/L (DMP), 2.10 and 36.8 μg/L (DMTP), <LOD (0.51 μg/L) and 5.60 μg/L (DMDTP), <LOD (0.37 μg/L) and 15.3 μg/L (DEP), <LOD (0.56 μg/L) and 4.35 μg/L (DETP), and both <LOD (0.39 μg/L) (DEDTP). However, factors that may contribute to variations in the urinary concentrations of DAPs include different sampling procedures (e.g., spot urine vs first morning void), differences in year(s) of urine collection, or analytical method differences. Of note, results of accredited external quality assessment schemes (e.g. GEQUAS) showing relatively wide tolerance ranges when determining DAP metabolite concentrations highlight the technical challenges encountered in the quantification of DAPs.

3.5 Strengths and Limitations

Overall, this method offers high selectivity, excellent precision (<5% RSD) and accuracy (mean relative recovery 99%, range 97-103%) for glyphosate. A fast and simple sample preparation (dilute and shoot) using 200 μL of urine, combined with a run-time of 20 min makes it an excellent choice for large human biomonitoring studies to investigate glyphosate exposure in the general population.

After optimizing every instrumental parameter (e.g. injection volume) and testing several types of columns for robustness, we concluded that only extensive sample preparation, out of scope for this study, is likely to improve sensitivity, which our data showed is enough to quantify glyphosate concentrations in urine samples with environmental exposure levels from the United States. Further investigation showed that the quantification of DAPs in large number of samples for population-based studies (e.g. NHANES) was not as rugged as desired despite successful participation in external performance assessment schemes (i.e., GEQUAS, OSEQAS) and solid results during method validation. The used QC material drifted between 60-140% from the average, first only for DMTP, then DEP followed by DMDTP, and then the rest of the DAP metabolites. Unsurprisingly, the deuterated internal standards were almost baseline separated for some DAP analytes, which can be explained by physicochemical properties of the deuterated analytes compared to their native analogs, and therefore can cause these retention time differences. In combination with a highly variable composition of the urine matrix, the retention time differences between internal and native standard might negatively impact method performance. This effect seems to be enhanced with the batch size and QC placement in the measured batch which is randomized for NHANES. Typical batches during the method validation had a total of 25 samples (including calibrators and QCs), batches containing NHANES samples, however, had 62 samples total, increasing the matrix load dramatically.

It is unclear what matrix components might have caused these problems because the columns were thoroughly flushed. We ruled out the presence of ghost peaks or analyte carry-over by extending the method runtime. Instead, we found the matrix related effect to be reversible by the introduction of methanol. However, this approach would decrease life expectancy of the suppressor while increasing backpressure, and total run time (+60 min). This runtime would far exceed the desired run-time for large human biomonitoring studies significantly. Therefore, we determined that the current method with deuterated internal standards is only suitable for human biomonitoring studies of relatively small sample sizes and is not applicable for NHANES.

4 Conclusions

This is the first multi-analyte method for dialkylphosphate metabolites and glyphosate. We demonstrate the reliability, robustness and applicability of the IC-IC-M S/MS system for glyphosate which requires no sample preparation besides aliquoting and diluting the urine and adding a defined amount of internal standard. Although adequate for glyphosate, DAP metabolites, however, cannot be quantified in large population-based biomonitoring studies (e.g., NHANES) with this method. The developed method is also suitable to investigate glyphosate exposure in the general population even among people consuming organic diets

and to assess occupational or home usage of glyphosate; however, preliminary results show relatively low concentrations of glyphosate in the analyzed samples. We are using the current method for the analysis of NHANES samples to investigate the extent of glyphosate exposure among the U.S. general population.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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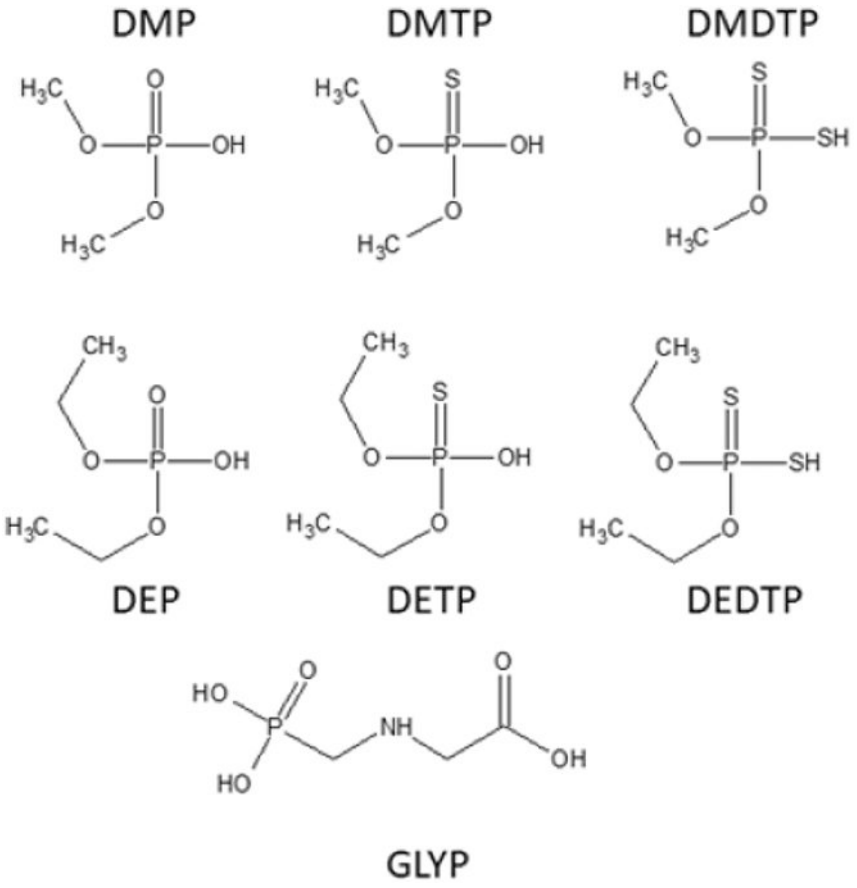
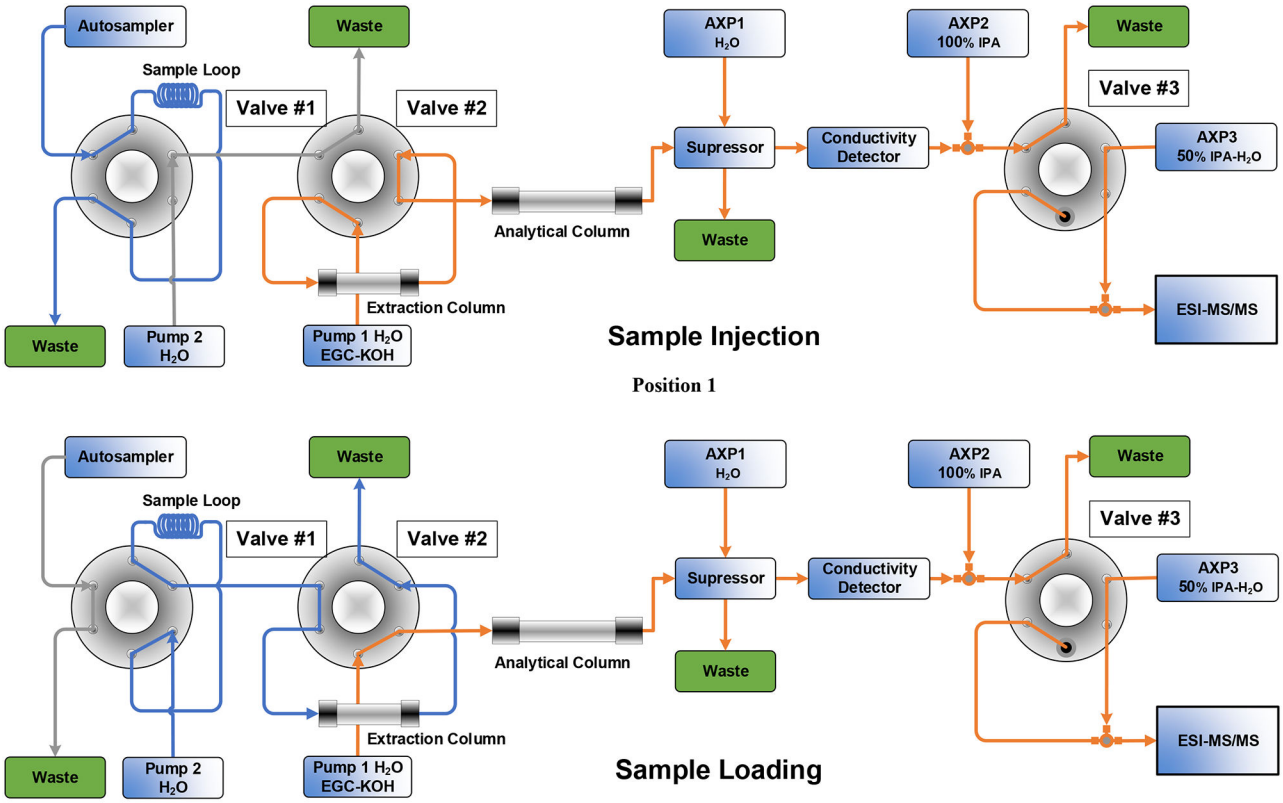


Figure 1:
Chemical structure of dialkylphosphate metabolites and glyphosate



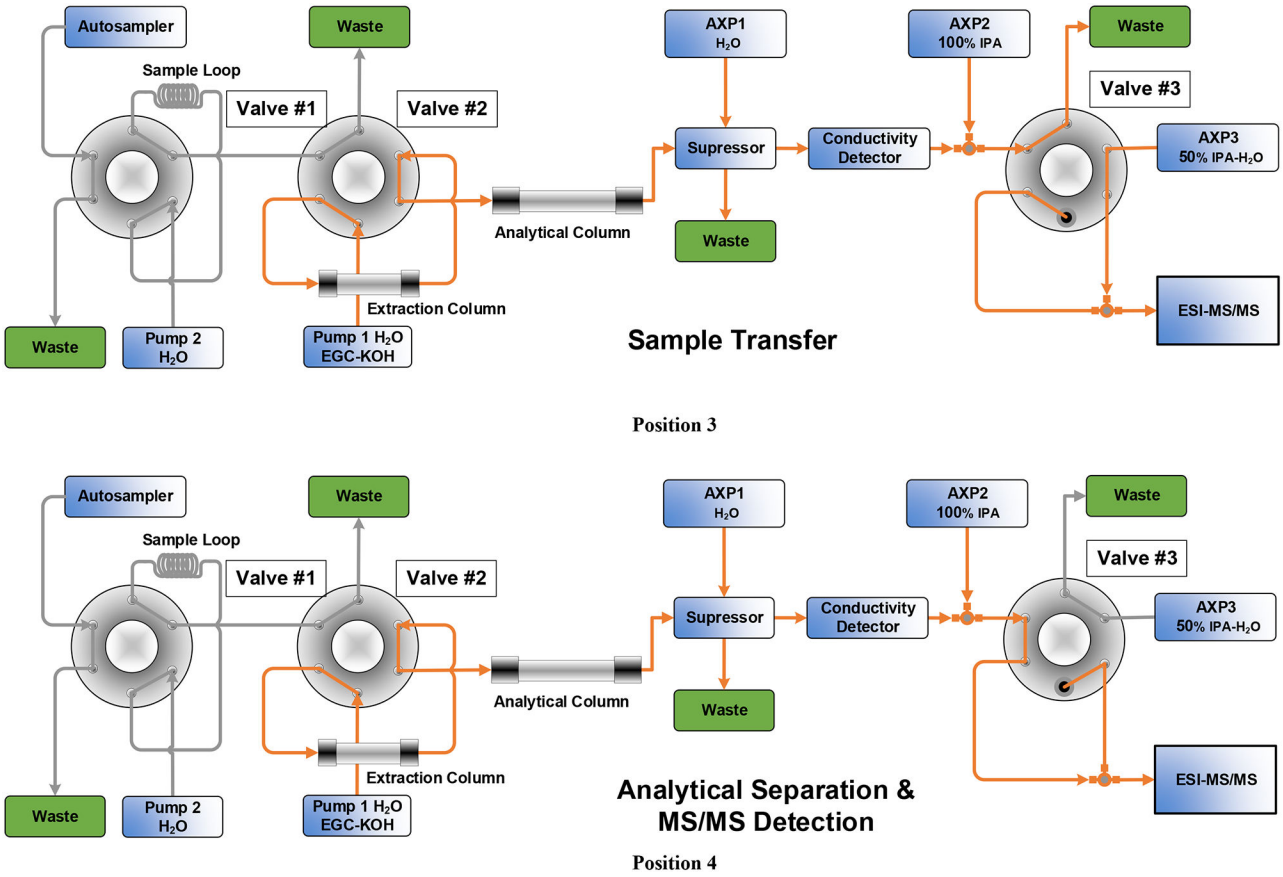


Figure 2:

Two column switching assembly of the IC-IC-MS/MS method. A) Sample injection. B) Sample loading and retention of analytes on the UTAC column. C) forward flush of analytes from UTAC to the AS-24 where the analytes are separated from the remaining matrix. D) MS/MS for detection. AXP: auxiliary pump, EGC: Eluent generator cartridge

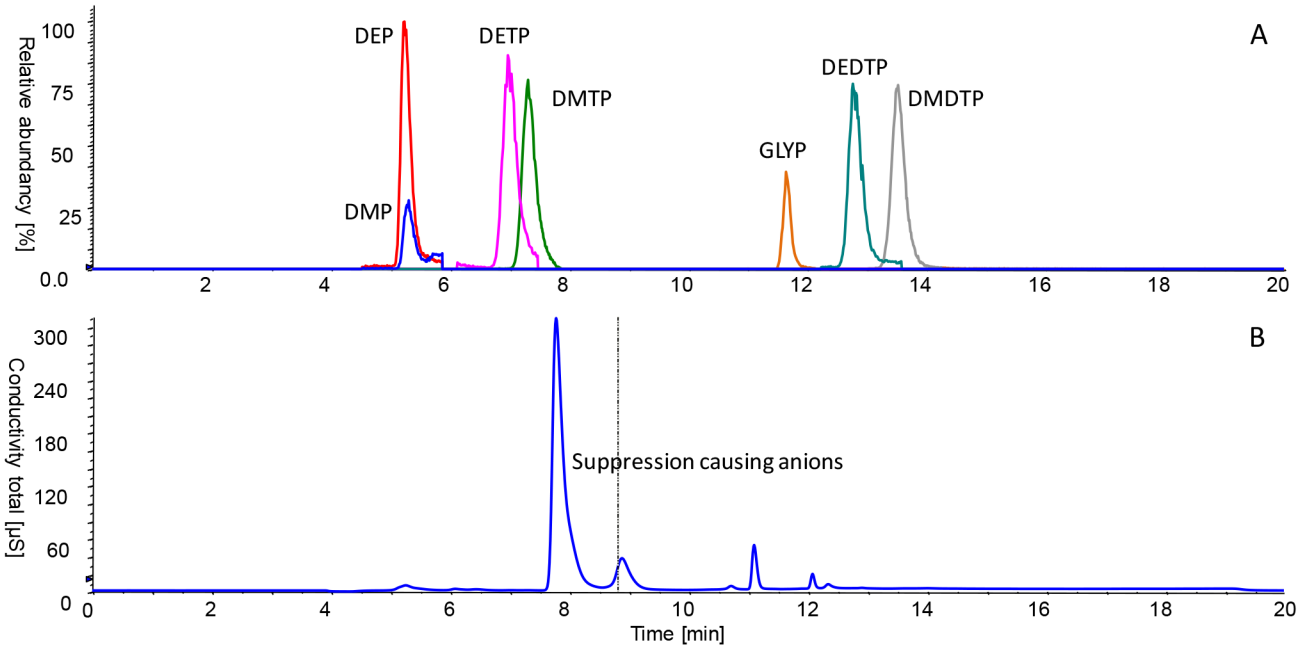


Figure 3:

Chromatogram of the low concentration quality control material with approximate concentrations of 1-2 µg/L native analytes depicting the detector response as relative abundancy in percent (A), and total conductivity in µS (B). The main suppression causing anions are diverted to waste to enhance longevity of the instrument.

Table 1:

Quantitation and confirmation MS/MS transitions for native and labeled internal standards

Analyte	Precursor Ion	Product Ion	CE [eV]	RT [min]
DEP	153	124.9	−14	5.35
DEP_C	153	78.9	−26	5.35
DMP	125	109.9	−22	5.4
DMP_C	125	62.9	−24	5.4
DMP_L	130.9	62.9	−24	5.4
DETP	168.9	141	−16	6.85
DETP_C	168.9	63	−40	6.85
DETP_L	179.3	94.8	−26	6.75
DMTP	140.9	95.9	−26	7.2
DMTP_C	140.9	63	−45	7.2
DMTP_L	146.7	95.1	−20	7.1
DEDTP	184.9	110.9	−24	12.57
DEDTP_C	184.9	157	−18	12.57
DMDTP	157	142.1	−22	12.5
DMDTP_C	157	111.7	−28	12.5
DMDTP_L	163	113	−31	13.1
GLYP	168	63	−30	11.5
GLYP_L	170	63	−25	11.5

RT: retention time, CE: collision energy, _C: confirmation ion for native standard, _L labeled analog quantitation ion. As labeled internal standard for DEP and DEDTP, we used D6_DMP and D6_DMDTP respectively.

Table 2:

Ion chromatographic parameters

Time (min)	Pump1[mL/min]	Pump2 [ml/min]	KOH [mM]	Suppressor [mA]	Valve #2
0	0.4	2	30	30	A
2		2			B
2.1		0.1			
7		0.1	30	30	
8	0.4			124	
9	0.5				
10			100		
10.1		1.5			B
16.9					A
17			100		
17.1			30	124	
20	0.5	1.5	30	38	A

Table 3:

Precision of the pool urine quality control material QClow and QChigh concentrations and their respective relative standard deviations (RSD).

N=20	QClow				QChigh			
	Conc [µg/L]	Intra-day RSD [%]	Inter-day RSD [%]	Total RSD [%]	Conc [µg/L]	Intra-day RSD [%]	Inter-day RSD [%]	Total RSD [%]
DMP	1.50	4.25	5.17	6.69	16.0	3.91	3.53	5.27
DEP	1.50	4.04	6.88	7.98	16.7	2.92	3.47	4.53
DMTP	1.64	3.48	6.85	7.68	17.2	3.26	4.69	5.71
DETP	1.65	6.65	4.55	8.06	17.4	7.43	1.48	7.58
DMDTP	1.27	2.32	16.4	16.6	16.8	3.42	6.42	7.28
DEDTP	1.13	2.83	18.2	18.4	16.8	2.71	4.32	5.10
GLYP	1.96	1.77	2.88	3.38	20.7	3.00	2.01	3.62

N=number of measurements per concentration; conc = concentration, Total precision reflects both intra-batch and inter-batch measures

Table 4:

Accuracy testing of the method, using three different spike levels. Analysis was performed in triplicate on two consecutive days.

			Sample 1		Sample 2			
Analyte	**Level**	**Spike [µg/L]**	**Mean [µg/L]**	**Recovery [%]**	**Mean [µg/L]**	**Recovery [%]**	**Mean Recovery [%]**	**RSD* [%]**
DMP	Native	0	0.4		0.5			
	Low	3.81	4.2	100	4.0	93.4		
	Mid	7.62	8.1	101	7.9	96.9	98.4	2.9
	High	15.2	15.7	100	15.5	98.5		
DEP	Native	0	0.4		1.0			
	Low	3.98	4.8	111	5.7	117		
	Mid	7.97	9.2	110	10.3	116	113	3.4
	High	15.9	17.7	109	19.0	113		
DMTP	Native	0	0.0		0.9			
	Low	3.92	4.3	110	5.0	105		
	Mid	7.83	8.6	109	9.1	105	107	2.7
	High	15.7	17.1	109	17.3	104		
DETP	Native	0	0.0		0.0			
	Low	4.06	3.6	88.8	3.5	86.6		
	Mid	8.13	7.0	86.3	6.9	85.2	85.8	1.9
	High	16.3	13.8	84.6	13.6	83.4		
DMDTP	Native	0	0.1		0.1			
	Low	4.01	4.3	106	4.6	111		
	Mid	8.01	8.6	106	9.4	115	109	4.1
	High	16.0	16.9	105	18.1	112		
DEDTP	Native	0	0.0		0.0			
	Low	4.13	3.7	90.7	3.8	92.4		
	Mid	8.27	7.6	91.6	7.9	96.0	92.9	2.7
	High	16.5	14.9	90.2	16.0	96.5		
GLYP	Native	0	0.1		0.3			
	Low	5	5.1	99.8	5.3	99.9		
	Mid	10	10.1	99.6	10.3	101	99.2	1.3
	High	20	19.5	96.9	20.0	98.8		

* relative standard deviation

Table 5:

Urinary glyphosate and DAP concentrations (in µg/L)

Analyte	LOD	Organic diet					Potential exposure				
		%>LOD	Min	Max	Median	IQR	%>LOD	Min	Max	Median	IQR
DMP	0.2	54%	<LOD	14.7	0.27	1.07	73%	<LOD	39	0.90	2.24
DEP	0.2	82%	<LOD	83.2	0.86	3.93	78%	<LOD	58.0	1.86	3.50
DMTP	0.2	58%	<LOD	23.0	0.41	0.98	75%	<LOD	111	0.97	1.31
DETP	0.2	26%	<LOD	9.33	<LOD	0.074	25%	<LOD	2.6	<LOD	0.029
DMDTP	0.2	22%	<LOD	4.03	<LOD	*	35%	<LOD	2.67	<LOD	0.16
DEDTP	0.8	2%	<LOD	1.04	<LOD	*	10%	<LOD	17.6	<LOD	*
GLYP	0.2	80%	<LOD	4.09	0.39	0.42	78%	<LOD	28.6	0.40	0.63

IQR: Interquartile Range

*detection frequency <25%, IQR cannot be calculated

To calculate the median and IQR, concentrations <LOD were replaced by LOD/SQRT(2)