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Biological monitoring for exposure to methamidophos: A human oral dosing study

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HIGHLIGHTS

- An oral dose of methamidophos was administered to six volunteers at ADI level.
- The study has quantified methamidophos in timed urinary collections.
- Methamidophos exhibited a rapid elimination half-life of 1.1 h.
- Mean dose recovery excreted as unchanged methamidophos in urine is low – only 1.1%.
- Short half-life means estimates of exposure likely to be highly variable.

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ABSTRACT

An oral dose of the organophosphate insecticide methamidophos was administered to six volunteers at the acceptable daily intake (ADI, 0.004 mg/kg).

Urine was collected from the volunteers at timed intervals for 24 h post-exposure. Methamidophos itself was quantified in urine using liquid/liquid extraction and LC–MS-MS analysis (detection limit 7 nmol/L/1 μg/L).

Methamidophos exhibited a rapid elimination half-life of 1.1 h, (range 0.4–1.5 h). Mean metabolite levels found in 24 h total urine collections (normalised for a 70 kg volunteer) were 9.2 nmol/L (range 1.0–19.1). One volunteer was anomalous; excluding this result the range was 6.7–19.1 nmol/L, with a mean of 10.9 nmol/L. Individual urine samples collected during the first 24 h ranged from below the detection limit (ND) to 237 nmol/L. The mean dose recovery excreted as methamidophos in urine was 1.1% (range 0.04–1.71%).

Three environmental studies have been reported in the literature with levels ranging from ND to 66 nmol/L. The number of positive results in all three studies was low (<1.5% of total samples analyzed). When compared with our results (ND – 237 nmol/L), the studies suggest general population exposures are within the ADI. However, the very short half-life makes determining intermittent environmental exposures difficult.

1. Introduction

Methamidophos is an organophosphate pesticide, used widely in agriculture for the protection of a wide range of crops. It is also a metabolite of acephate, another widely used organophosphate pesticide. As organophosphate (OP) pesticides have been reported as the most commonly used insecticides in agriculture (Jaga and Dharmani, 2004; Kamanyire and Karalliedde, 2004) it is difficult to completely avoid exposure. Methamidophos is toxic via all routes of exposure and is a cholinesterase inhibitor, capable of over stimulating the central nervous system causing dizziness, confusion, and ultimately death at very high exposures (Christiansen et al., 2011; Mason, 2000). Consequently, it is important to control exposure. An acceptable daily intake (ADI) of 0.004 mg/kg of body weight per day has been established for methamidophos (JMPR, 2002).

Biological monitoring is a useful approach for determining systemic exposure to chemicals by all routes; it enables the quantification of a compound, or its metabolites, in non-invasive samples such as urine. This approach is suitable for monitoring

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environmental and occupational exposure, since it enables the determination of the actual absorbed amount of chemical in an individual. However, such an approach requires both a suitable analytical method and an appropriate reference range in order to interpret the data.

Once exposure occurs OP insecticides are usually metabolized via hydrolysis and the alkylphosphate or specific metabolite residue is analyzed (Montesano et al., 2007), but with methamidophos the intact parent pesticide can be measured, with several methods having been reported (Montesano et al., 2007; Olsson et al., 2003; Jayatilaka et al., 2010; Savieva et al., 2004).

There have been no published studies in the open literature describing human volunteer exposure to methamidophos. The Joint FAO/WHO Meeting on Pesticide Residues (JMPR, 2002) describes two unpublished reports – one looking at cholinesterase activity from multiple oral dosing (no urine sampling reported) and one looked at dermal exposure using radiolabelled methamidophos. The present study has quantified methamidophos excretion in timed urinary collections from six volunteers who received a single oral dose at the ADI. Data from three other studies is included (Montesano et al., 2007; Olsson et al., 2003; Centers for Disease Control and Prevention, National Biomonitoring Programme, 2013) for comparison of methamidophos levels in general population against that of urine levels after ADI exposure.

2. Methods

2.1. Chemicals

Certified methamidophos was purchased from QMX Laboratories (Thaxted, UK) and internal standard d_6-methamidophos (100 mg/L) from Dr. Ehrenstorfer (Augsburg, Germany). All solvents and reagents used were of analytical grade.

2.2. Volunteer study

The protocol used in this study was approved by the HSE Research Ethics Committee (ETHCOM/REG/06/03). After giving informed written consent, six volunteers were given a single oral dose (based on body weight) of methamidophos at the ADI (0.004 mg/kg) dissolved in ethanol and diluted with a soft drink. Volunteer details are shown in Table 1. Total urine excreted was collected at timed intervals up to 24 h post-exposure. The volume of each sample was recorded and an aliquot retained for analysis ($<-15\,^{\circ}C$). Samples were also analyzed for creatinine concentration to account for dilution. Samples for five of the six volunteers were stored frozen for five years prior to analysis.

2.2.1. Sample preparation

We investigated previously reported methods (Montesano et al., 2007; Olsson et al., 2003; Jayatilaka et al., 2011; Savieva et al., 2004) and found problems with recovery when freeze drying. Liquid/liquid extraction also gave some problems, but these were overcome with the use of a higher volume of solvent (10 mL). This was found to give fewer interferences in the chromatography and increased sensitivity, enabling a detection limit of 7 nmol/L, although this is higher than reported for some other methods (Montesano et al., 2007 – 1.1 nmol/L; Centers for Disease Control and Prevention, National Biomonitoring Programme, 2013 – 0.7 and 2.6 nmol/L).

All samples were analyzed in duplicate. Aliquots of urine (10 mL) were added to a sterilin tube and spiked with 50 μL internal standard (d_6-methamidophos, 1 mg/L). Calibration standards (0–282 nmol/L were prepared in urine and quality control samples (prepared by spiking urine with methamidophos at a concentration of 70 nmol/L) were also analyzed throughout the analytical run. Liquid/liquid extraction was carried out by adding 10 mL of dichloromethane to all tubes and rolling for 20 min. The samples were then centrifuged and the solvent layer was removed and evaporated to dryness under nitrogen. Samples were reconstituted in 50 μL methanol and transferred to vials for analysis.

2.2.2. Sample analysis

LC–MS/MS analysis was performed on a Shimadzu SPD-M20A HPLC coupled to a 3200 Q-Trap AB Sciex tandem mass spectrometer with compounds optimised in positive ion electrospray MRM (Tables 2 and 3). An isocratic HPLC method (70% A:30% B) was set up using a ZORBAX SB-C3 Agilent column (4.6 × 150 mm – 5 μm), with mobile phase A (0.1% formic acid in water) and B (0.1% formic acid in methanol) run at a total flow rate of 0.2 mL/min with an overall run time of 15 min. The injection volume was 2 μL. Selected transitions monitored were *m/z* 142/94 (methamidophos) and 148/97 (d_6-methamidophos), see Table 2.

2.2.3. Method characteristics

The assay was linear up to at least 282 nmol/L (least squares regression coefficient of >0.99). Analysis of quality control samples gave an inter-assay variation of 4% ($N = 5$, at a concentration of 70 nmol/L). The method had a detection limit of 7 nmol/L (three times signal:noise ratio).

2.2.4. Creatinine analysis

Creatinine was determined in all urine samples by an automated alkaline picrate method (Jaffé reaction) using a Pentra 400 (ABX, France) (Cocker et al., 2011). The coefficient of variation for within-day analysis was 1.5% and for between-day analysis was 3% at 6 mmol/L.

3. Results

Example chromatograms for a calibration standard, blank urine, and positive urine after dosing are shown in Fig. 1. Fig. 2 shows the time course of urinary excretion of methamidophos (normalised for a 70 kg volunteer). Elimination was rapid, with the majority of the recovered dose (range 0.04–1.71%) being excreted within 8 h of dosing, and a mean half-life of 1.1 h (range 0.4–1.5 h, Fig. 3). Peak urinary concentrations were found at 2 h post-dose (except for volunteer C, 6 h post-dose). Table 4 shows individual concentrations of methamidophos in each volunteer sample, up to 24 h after dosing (not normalised), and the total percentage of dose recovered for each volunteer.

Mean methamidophos levels found in the 24 h total urine collections (normalised for a 70 kg volunteer) were found to be 9.2 nmol/L (range 1.0–19.1). One volunteer excreted exceptionally low levels of methamidophos following dosing (volunteer C); excluding this result the range was 6.7–19.1 nmol/L, with a mean of 10.9 nmol/L.

There was little difference in inter-individual variability, whether creatinine correction was used or not. As a consequence (and as other researchers have not used creatinine correction), all results are discussed here without creatinine correction.

Table 1
Details of the volunteers.

Code	Sex	Age	Height (m)	Weight (kg)	BMI
A	F	35	1.715	77	26.2
B	M	55	1.71	94	32.1
C	F	23	1.75	107	34.9
D	M	26	1.76	102	32.9
E	M	54	1.895	96	26.7
F	F	41	1.75	78	25.5