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.

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**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 Harman, 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...
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 methami-
dophos 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 meth-
amidophos. 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 Laborato-
ries (Thaxted, UK) and internal standard d9-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 °C). Samples were also analyzed for creatinine concentra-
tion 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 (d9-methamidophos, 1 mg/L). Calibration stan-
ards (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 electro-
spray 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 (d9-methamidophos), see Table 2.

2.2.3. Method characteristics

The assay was linear up to at least 282 nmol/L (least squares
regression coefficient >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 picate 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 concen-
trations 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>
<thead>
<tr>
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<th>Sex</th>
<th>Age (y)</th>
<th>Height (m)</th>
<th>Weight (kg)</th>
<th>BMI</th>
</tr>
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<tbody>
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<td>A</td>
<td>F</td>
<td>35</td>
<td>1.715</td>
<td>77</td>
<td>26.2</td>
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<tr>
<td>B</td>
<td>M</td>
<td>55</td>
<td>1.71</td>
<td>94</td>
<td>32.1</td>
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<tr>
<td>C</td>
<td>F</td>
<td>23</td>
<td>1.75</td>
<td>107</td>
<td>34.9</td>
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<tr>
<td>D</td>
<td>M</td>
<td>26</td>
<td>1.76</td>
<td>102</td>
<td>32.9</td>
</tr>
<tr>
<td>E</td>
<td>M</td>
<td>54</td>
<td>1.895</td>
<td>96</td>
<td>26.7</td>
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<tr>
<td>F</td>
<td>F</td>
<td>41</td>
<td>1.75</td>
<td>78</td>
<td>25.5</td>
</tr>
</tbody>
</table>
4. Discussion

Since this study was conducted methamidophos has been banned in Europe and the USA (The Pesticide Manual, 2012; US EPA, 2009), and is being phased out of use. It is still used throughout the rest of the world, e.g., South Africa (Quinn et al., 2011). The present study has quantified urinary metabolite levels in volunteers exposed to a single oral dose at the ADI (0.004 mg/kg). Our data shows that methamidophos is rapidly excreted in urine (mean half-life 1.1 h) compared to some other organophosphate pesticides such as chlorpyrifos-methyl, which has a half-life of 16 h (Sams and Jones, 2011). However, it does have similar characteristics of other organophosphate pesticides investigated by this laboratory, such as diazinon with a half-life of 2 h (Garfitt et al., 2002).

The dose recovery of methamidophos was low in our study with a mean recovery of only 1.1% (range 0.04–1.71%) of the dose being excreted as methamidophos in urine. One report that has been published (Salama et al., 1992) compares well with our findings and shows that methamidophos undergoes extensive metabolism in rats, only 23% of the methamidophos dose was excreted in urine, and only a small percentage of this was actually excreted as unchanged methamidophos. Another, study in rats (Fakhr et al., 1982) found only 1.4% of the methamidophos was recovered unchanged in urine. This suggests that low dose recovery may be

<table>
<thead>
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<th>Table 2</th>
<th>Optimised LC–MS parameters.</th>
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<tbody>
<tr>
<td></td>
<td>Transition</td>
</tr>
<tr>
<td>Methamidophos</td>
<td>142/94</td>
</tr>
<tr>
<td>d6-Methamidophos</td>
<td>148/97</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Source/gas parameters.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Curtain gas (psi)</td>
</tr>
<tr>
<td></td>
<td>Collision cell gas (psi)</td>
</tr>
<tr>
<td></td>
<td>Ion spray voltage (V)</td>
</tr>
<tr>
<td></td>
<td>Temperature (°C)</td>
</tr>
<tr>
<td></td>
<td>Source gas 1 (psi)</td>
</tr>
<tr>
<td></td>
<td>Source gas 2 (psi)</td>
</tr>
</tbody>
</table>

![Figure 1](image-url)  
**Fig. 1.** Example chromatograms – top: 528 nmol/L standard in urine, response of ~ 10,000. Middle: blank urine (contains internal standard only). Bottom: volunteer A urine sample containing 54 nmol/L, 2 h after dosing, response ~1200.
due to the measurement of the parent compound only, and better
dose recovery results in urine may be obtained by measuring
methamidophos metabolites such as 0,5-dimethyl hydrogen
phosphorothioate (Tomaszewska and Hebert, 2003). This would
require further investigation. However, methamidophos was
chosen as the biomarker in this study to reflect the risk of
exposure to methamidophos, rather than a detoxification metab-
olite. Diet is likely to be a source of exposure to the general
population and it has been shown that metabolites can be present
as residues, therefore measuring methamidophos itself better
reflects the risk from food.

One of our volunteers showed exceptionally low excretion of
methamidophos following dosing; this may be due to differences
in metabolism but this has not been investigated further.
Alternatively, methamidophos may be hydrolyzed to its metab-
olites in the acidity of the stomach and then absorbed into the
body, although available data suggests that methamidophos is
stable under acidic conditions (IPCS, 2014).

Due to research priorities, samples for five of the six volunteers
were stored frozen for five years prior to analysis. In order to check
stability, samples from a further volunteer were collected prior to
analysis. Volunteers A–E (except C) showed comparable excretion
to volunteer F (analyzed immediately after collection) indicating
that the earlier samples were stable. This supports data from
Montesano et al. (2007) showing methamidophos stability at
−20 °C. It is therefore unlikely that the results from the anomalous
volunteer C are due to degradation.

With such rapid elimination it would be appropriate to collect
samples soon after exposure or at the end of each shift for
occupational studies. For environmental studies, the short half-life
means that estimates of exposure using biomonitoring are likely to
be highly variable (Aylward et al., 2012). Significant inter-
individual variability in excretion is also likely, judging by
volunteer C in our cohort. Three environmental studies have been
reported in the literature (Table 5). The number of positive samples
in all three of the studies was low (<1.5% in all three studies),
probably reflecting the rapid excretion and intermittent exposures

Table 4
Showing concentration (nmol/L) of methamidophos in volunteer urine samples up to 24 h after dosing (not normalised), and total percentage of dose recovered.

<table>
<thead>
<tr>
<th>Concentration in nmol/L</th>
<th>Volunteer</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>C</td>
<td>D</td>
<td>E</td>
<td>F</td>
</tr>
<tr>
<td>Pre sample</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2 h after dose</td>
<td>54</td>
<td>218</td>
<td>19</td>
<td>237</td>
<td>59</td>
<td>88</td>
</tr>
<tr>
<td>4 h after dose</td>
<td>31</td>
<td>92</td>
<td>12</td>
<td>28</td>
<td>45</td>
<td>53</td>
</tr>
<tr>
<td>6 h after dose</td>
<td>17</td>
<td>24</td>
<td>22</td>
<td>0</td>
<td>38</td>
<td>9</td>
</tr>
<tr>
<td>8 h after dose</td>
<td>9</td>
<td>21</td>
<td>0</td>
<td>0</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>12 h after dose</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>20 h after dose</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>24 h after dose</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total % of dose recovered</td>
<td>0.82</td>
<td>1.71</td>
<td>0.04</td>
<td>1.20</td>
<td>1.28</td>
<td>1.62</td>
</tr>
</tbody>
</table>

Table 5
Comparison data with other studies (nmol/L).

<table>
<thead>
<tr>
<th>Study</th>
<th>LoD</th>
<th>Geometric mean</th>
<th>Range</th>
<th>%&gt; LoD</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Montesano et al. (2007)</td>
<td>1.3</td>
<td>13.9</td>
<td>&lt;LoD-66</td>
<td>1.3</td>
<td>499 General population (California, USA)</td>
</tr>
<tr>
<td>Olsen et al. (2003)</td>
<td>5.6</td>
<td>10.56</td>
<td>&lt;LoD-16.3 [over 24 h]</td>
<td>1.4</td>
<td>140 General population(2 sites, USA)</td>
</tr>
<tr>
<td>This study (2014)</td>
<td>7</td>
<td>–</td>
<td>&lt;LoD-237</td>
<td>–</td>
<td>6 Volunteers at the ADI</td>
</tr>
<tr>
<td>CDC (2013)</td>
<td>0.7</td>
<td>–</td>
<td>All&lt;LoD</td>
<td>0</td>
<td>1401 Adults(across USA)</td>
</tr>
<tr>
<td></td>
<td>2.6</td>
<td>–</td>
<td>All&lt;LoD</td>
<td>0</td>
<td>1535 Adults(across USA)</td>
</tr>
</tbody>
</table>

LoD – Limit of detection.

*a* 2003/2004 survey.

of methamidophos. When compared with our own results (particularly taking into account the extent of negative results in the environmental surveys) it shows that general population exposure in countries where methamidophos is still in use is likely to be well within the ADI.

Conflict of interest

The authors declare that there are no conflicts of interest.

Transparency document

The Transparency document associated with this article can be found in the online version.

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