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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

Table 2
Optimised LC–MS parameters.

	Transition	Declustering potential (V)	Entrance potential (V)	Cell exit potential (V)	Collision energy
Methamidophos	142/94	46	10	4	21
d_6-Methamidophos	148/97	46	10	4	21

Table 3
Source/gas parameters.

Curtain gas (psi)	25
Collision cell gas (psi)	Medium
Ion spray voltage (V)	5500
Temperature (°C)	400
Source gas 1 (psi)	40
Source gas 2 (psi)	40

4. Discussion

Since this study was conducted methamidophos has been banned in Europe and the U.S (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 has 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

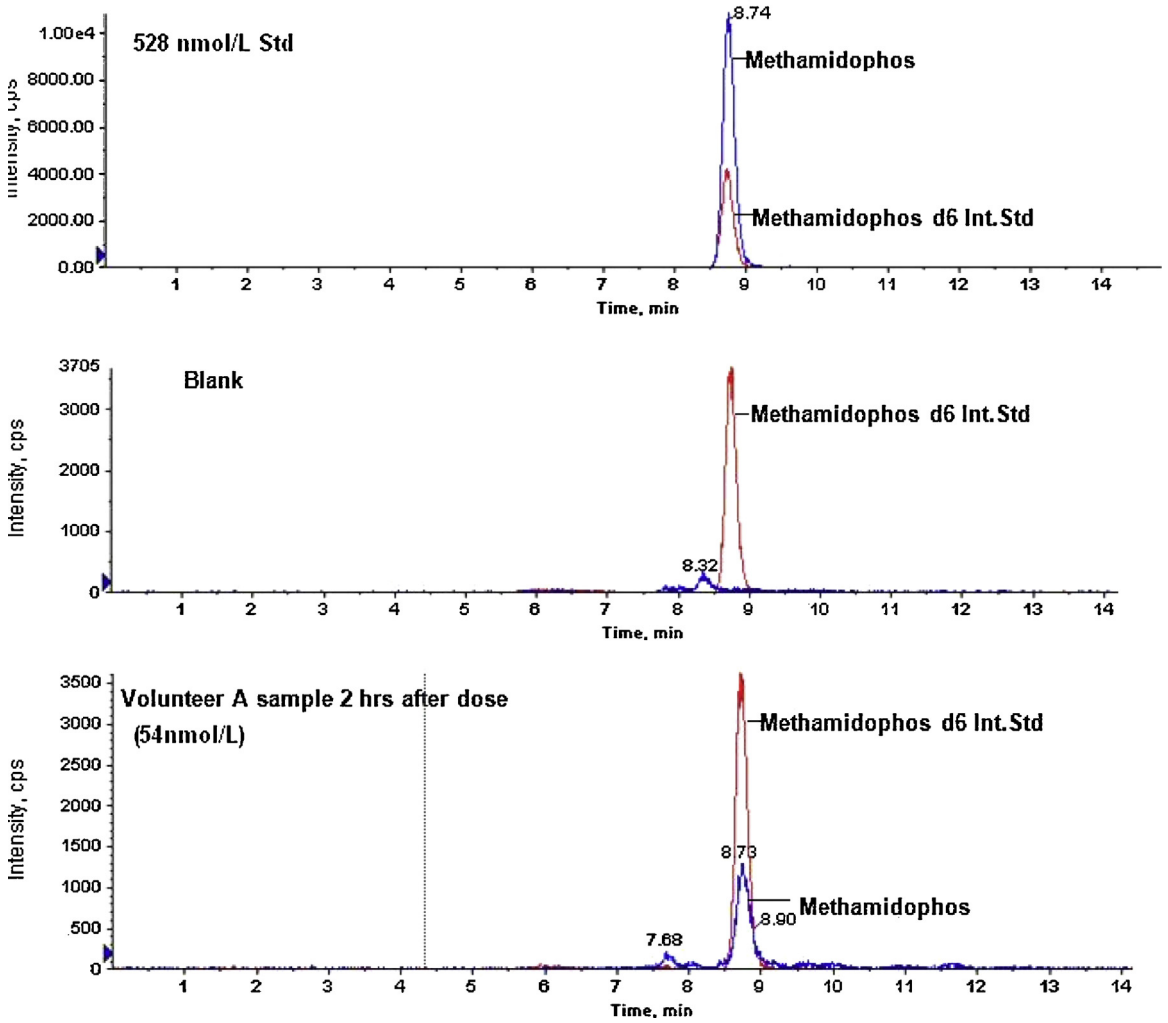

Fig. 1. Example chromatograms – top:- 528 nmol/L standard in urine, response of ~10,000. Middle:- blank urine (contains internal standard only). Bottom:- volunteer A urine sample containing 54 nmol/L, 2 h after dosing, response ~1200.

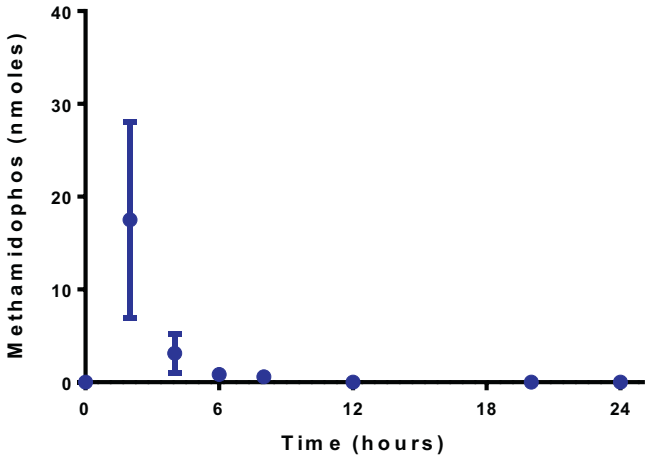

Fig. 2. Mean (±standard deviation, $n = 6$) excretion of methamidophos (nmol) in urine after an oral dose at the ADI (0.004 mg/kg), normalised to a 70 kg volunteer.

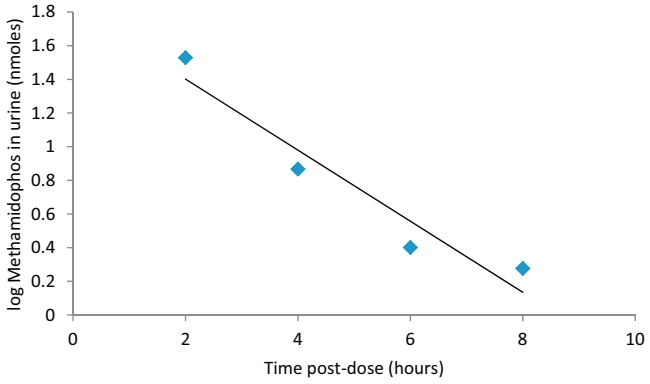

Fig. 3. Example excretion curve (volunteer B) showing a half-life of 1.4 h ($r = 0.96$, least squares regression).

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 *O*,*S*-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 metabolite. 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 metabolites 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.

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

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

Study	LoD	Geometric mean	Range	%> LoD	Details
Montesano et al. (2007)	1.1	13.9	<LoD-66	1.3	499 General population (California, USA)
Olsen et al. (2003)	5.6	10.56	<LoD-16.3 [over 24 h]	1.4	140 General population(2 sites, USA)
This study (2014)	7	–	<LoD-237	–	6 Volunteers at the ADI
CDC (2013)	0.7[a]	–	All <LoD	0	1401 Adults(across USA)
	2.6[b]	–	All <LoD	0	1535 Adults(across USA)

LoD – Limit of detection.
[a] 2003/2004 survey.
[b] 2005/2006 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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References

Aylward, L.L., Kirman, C.R., Adgate, J.L., McKenzie, L.M., Hays, S.M., 2012. Interpreting variability in population biomonitoring data: role of elimination kinetics. J. Expo. Sci. Environ. Epidemiol. 22 (4), 398–408. doi:http://dx.doi.org/10.1038/jes.2012.35 Epub 2012 May 16 July.

Centers for Disease Control and Prevention, National Biomonitoring Programme, (2013). http://www.cdc.gov/biomonitoring/Methamidophos_Biomonitoring-Summary.html (accessed 13.03.14.).

Cocker, J., Mason, H.J., Warren, N.D., Cotton, R.J., 2011. Creatinine adjustment of biological monitoring results. Occup. Med. (Lond.) 61 (August 5), 349–353.

Christiansen, A.; Gervais, J.; Buhl, K.; Stone, D. 2011. Acephate Technical Fact Sheet; National Pesticide Information Center, Oregon State University Extension Services. http://npic.orst.edu/factsheets/acephatech.pdf(accessed 13.03.14.)

Fakhr, I.M.I., Abdel-Hamid, F.M., Afifi, L.M., 1982. In vivo metabolism of 32P Tamaron in the rat. Isot. Rad. Res. 14, 49–55.

Garfitt, S.J., Jones, K., Mason, H.J., Cocker, J., 2002. Exposure to the organophosphate diazinon: data from a human volunteer study with oral and dermal doses. Toxicol. Lett. 134, 105–113.

IPCS (2014). 370. Methamidophos (Pesticide residues in food: 1976 evaluations) http://www.inchem.org/documents/jmpr/jmpmono/v076pr17htm (accessed 08.10.14.).

Jaga, K., Dharmani, C., 2004. Sources of exposure to and public health implications of organophosphate pesticides. Rev. Panam Salud. Publ. 14, 171–185.

Jayatilaka, N.K., Montesano, M.A., Whitehead Jr, R.D., Schloth, S.J., Needham, L.L., Barr, D.B., 2011. High-throughput sample preparation for the quantitation of acephate, methamidophos, omethoate, dimethoate, ethylenethiourea and propylenethiourea in human urine using 96-well-plate automated extraction and high-performance liquid chromatography-Tandem mass spectrometry. Arch. Environ. Contam. Toxicol. 61, 59–67.

JMPR (2002). Pesticide residues in food – (2002) -Joint FAO/WHO Meeting on Pesticide Residues-METHAMIDOPHOS, http://www.inchem.org/documents/jmpr/jmpmono/2002pr10.htm#1.3 (accessed 07.07.2014.).

Kamanyire, R., Karalliedde, L., 2004. Organophosphate toxicity and occupational exposure. Occup. Med. 54, 69–75.

Mason, H., 2000. The recovery of plasma cholinesterase and erythrocyte acetylcholinesterase after over exposure to dichlorvos. J. Occup. Med. 50, 343–347.

Montesano, M.A., Olsson, A., Kuklenyik, P., Needham, L.L., Bradman, A., Barr, D.B., 2007. Method for determination of acephate, methamidophos, omethoate, dimethoate, ethylenethiourea and propylenethiourea in human urine using high-performance liquid chromatography atmospheric pressure chemical ionisation tandem mass spectrometry. J. Expo. Sci. Environ. Epidemiol. 17, 321–330.

Olsson, A.O., Nguyen, J.V., Sadowski, M.A., Barr, D.B., 2003. A liquid chromatography/electrospray ionization-tandem mass spectrometry method for quantification of specific organophosphorous pesticide biomarkers in human urine. Anal. Bioanal. Chem. 376, 808–815.

Quinn, L.P., de Vos B, J., Fernandes-Whaley M., Roos C., Bouwman H., Kylin H., Pieters R., van den Berg, J., Chapter 4 - Pesticide use in South Africa: One of the largest Importers of Pesticides in Africa. Agricultural and Biological Sciences–Pesticides in the modern world–Pesticides use and management. ISBN 978–953-307–459-7, DOI: 10.5772/16995 http://www.intechopen.com/books/pesticides-%20in-the-modern-world-pesticides-use-and-management/pesticide-use-in-south-africa-one-of-the-largest-importers-of-pesticides-in-africa (accessed 11.06.14.).

Savieva, C., Aprea, C., Tumino, R., Masala, G., Salvini, S., Frasca, G., Giurdanella, M.C., Zanna, I., Decarli, A., Sciarra, G., Palli, D., 2004. Twenty-four hour urinary excretion of ten pesticide metabolites in healthy adults in two different areas of Italy (Florence and Ragusa). Sci. Total Environ. 332, 71–80.

Salama, A.K., Bakry, N.M., Aly, H.A., Abou-Donia, M.B., 1992. The metabolism of a single dose of methamidophos by pregnant Sprague-Dawley rats. J. Occup. Med. Toxicol. 1, 275–291.

Sams, C., Jones, K., 2011. Human volunteer studies investigating the potential for toxicokinetic interactions between the pesticides deltamethrin, pirimicarb and chlorpyrifos-methyl following oral exposure at the acceptable daily intake. Toxicol. Lett. 200, 41–45.

The Pesticide Manual (2012). A world compendium. Sixteenth Edition. ISBN 978-1-901396-86-7.

Tomaszewska, E., Hebert, V.R., 2003. Analysis of *O,S*-dimethyl hydrogen phosphorothioate in urinr, a specific biomarker for methamidophos. J. Agric. Food Chem. 51 (October 21), 6103–6109.

U.S. Environmental Protection Agency (U.S. EPA) (2009). Disulfoton and methamidophos; product cancellation order. September 23, (2009). http://www.epa.gov/fedrgstr/EPA-PEST/2009/September/Day-23/p22921htm (accessed 08/07/14.).