Arabian Journal of Chemistry (2015) 8, 624–631

King Saud University

Arabian Journal of Chemistry

www.ksu.edu.sa
www.sciencedirect.com

REVIEW

A review on sample preparation and chromatographic determination of acephate and methamidophos in different samples

Vijay Kumar [a], Niraj Upadhyay [b,*], Virender Kumar [a], Sitansh Sharma [a]

[a] Department of Chemistry, Lovely Professional University, Punjab, India
[b] Department of Chemistry, Dr. Hari Singh Gour University, Sagar, Madhya Pradesh, India

Received 22 January 2014; accepted 27 December 2014
Available online 3 January 2015

KEYWORDS

Acephate;
Methamidophos;
Toxicity;
Biological decomposition;
Methods of detection

Abstract Acephate and its metabolite methamidophos are common organophosphorus insecticide used for crop protection. High uses of acephate and methamidophos have induced health issues and environmental pollution. Their undesired presence in the environment is creating ecotoxicology and may harm human health. It is therefore essential to detect the presence of acephate and methamidophos even in trace level. In this review, we have tried to accommodate successful methods of detection of acephate and methamidophos in the different biological media. Their recovery and residue analysis in different media such as vegetables, human and animal tissues have also included. The most common method for their determination is based on chromatographic separation and identification. Among different chromatographic methods, LC and GC coupled with different detectors have used. But, they both need extensive pretreatment and cleanup procedure, before undergoing chromatographic separation and identification. LC coupled with mass spectrometry (LCMS) is sometime able to detect acephate and methamidophos in ppm level.

Contents

* Corresponding author.
E-mail address: nivij.res@gmail.com (N. Upadhyay).
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.arabjc.2014.12.007

1. Introduction

Acephate (*O,S*-Dimethyl acetylphosphoramidothioate) (Table. 1) is an organophosphate insecticide, introduced by Chevron Chemical in 1971 (Magee, 1973) and first time registered for use by the United States Environmental Protection Agency in 1973. It has been observed that the production of acephate increased quickly from last 5 years, surprisingly in India, 10% increase in production of technical grade was observed within one year (Standing Committee on Chemicals & Fertilizers, 2012-13).

It is an insecticide registered for use on food crops, agricultural seed and non-bearing plants, institutions and commercial buildings including public health facilities, sod, golf course turf, ant mounds, and horticultural nursery plants.

In soil, plants and insects approximate half-life period of acephate is 3–6 days, although in some soils the half-life may be increased to more than 13–60 days due to variation of properties (physical, chemical and biological) of soils (Antonious and Snyder, 1994; Bouchard and Lavy, 1982; Chuanjiang et al., 2010; Yen et al., 2000). Acidic nature of soil is responsible for long life spam of acephate in soils ((Antonious and Snyder, 1994; Bouchard and Lavy, 1982; Chuanjiang et al., 2010). It has been observed that, after the decomposition acephate generally converts into highly toxic methamidophos (O,S-dimethyl phosphoramidothioate) (Table 1) and methamidophos is also an efficient organophosphorus insecticide (Yen et al., 2000; Chuckwudebe, 1984). Methamidophos is the major metabolite of acephate. It is toxic, not only for insects but also in various components of the environment such as nontarget animals, plants, waters, and soils (Wang et al., 2013) (see Tables 2–4).

Physicochemical properties of acephate and methamidophos are signifying that both are hydrophilic and having low soil sorption with an order acephate > methamidophos (Roberts & Hutson, 1999; Tomlin, 2006). Consequently, their runoff through water medium can produce potential water contamination (Roberts & Hutson, 1999; Tomlin, 2006). Acephate leads to contamination of groundwater much more readily than methamidophos under normal environmental conditions. Studies highlight that wet soils are more conducive for the contamination of the aquatic environment with acephate and its metabolites (Chai et al., 2009; Wang et al., 2013).

2. Mode of action and toxicity

Acephate and methamidophos inhibit acetylcholinesterase enzyme (AChE) in nervous system tissues, although, acephate itself is a weak acetylcholinesterase inhibitor as compared to its decomposed product methamidophos (Wilson et al., 1990; Spassova et al., 2000). The toxicity of acephate and methamidophos varies with application of enantiomeric compound of them (Wang et al., 2013). Both acephate and its metabolite methamidophos have the stereogenic phosphorus atom. The enantioselective bioactivity of enantiomers of acephate and methamidophos has been observed during past years. The R-(+)-enantiomers of acephate and methamidophos were found to be more potent to houseflies than the optical antipodes and racemates, whereas the S-(−)-enantiomers were more toxic for German cockroaches soils (Miyazaki et al., 1988; Wang et al., 2006, 2013). Another experiment showed that (−)-methamidophos was about 8.0−12.4 times more potent to the bovine erythrocytes and Electrophorus electricus than its (+)-form, but the (+)-enantiomer was 7.0 times more toxic to *D. magna* in 48 h tests (Lin et al., 2006). It was also concluded that D-(+)-methamidophos should be responsible for the organophosphate-induced delayed poly-neuropathy when racemic methamidophos is given to hens (Lotti et al., 1995).

Acephate and methamidophos are extremely toxic to aquatic invertebrates, birds and mammals (Tomlin, 2006; Vyas et al., 1996; Mahajna et al., 1997). The most likely route of exposure to acephate and methamidophos for the public is

Table 1 Chemical structure and physicochemical properties of acephate and methamidophos (Roberts & Hutson, 1999; Tomlin, 2006).

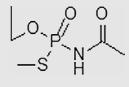
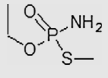
Name	Chemical structure	Molecular weight (g/mol)	Solubility g/L	Log K_{OW}	Henry's law constant (atm m^3/mol)
Acephate		183.16	790 in water at 25 °C	0.13 at 25 °C	5.1×10^{-13} at 25 °C
Methamidophos		141.13	200 in water at 25 °C	−0.65 at 25 °C	8.64×10^{-10} at 25 °C

Table 2 Toxicity of acephate in biological media.

Mammals	Aquatic organisms	Plants
Rat – LD_{50} 866 mg/kg, oral, and LC_{50} > 15 mg/m^3 (1 h), inhalation	**Goldfish** – EC_{50} 9550 mg/l for 96 h.	**Corn** – > 30% effect at 4.5 kg/ha. **Wheat** – 15–40% effect at 4.3 mg/l
Dog – LD_{50} 210 mg/kg, oral	**Rainbow trout** – LC_{50} 1000 mg/l (96 h)	**Lettuce** – > 40% effect at 0.5 kg/ha
Mouse – LD_{50} 360 mg/kg, oral	Perch – LC_{50} 16 mg/l (96 h)	**Millet** – 15–40% effect at 1.6 mg/l
Rabbit – LD_{50} > 10,000 mg/kg, oral and 7500 mg/kg, dermal	**Bluegill** – LC_{50} > 1000 mg/l (96 h)	**Soybean** – 15–40% effect at 1.1 mg/l

Table 3 Analytical techniques for determination of acephate and methamidophos in different media.

Authors	Technique	Medium	Metabolite	Extraction Techniques
Araoud et al. (2010)	Liquid chromatography tandem mass spectrometry	Human blood	Acephate methamidophos, etc.	Liquid–liquid extraction
Khan et al. (2010)	HPLC and GC with NPD detector	Plasma alanine aminotransferase, aspartate aminotransferase, creatinine, urea, and gamma glutamyltransferase	Methamidophos, etc.	Liquid–liquid extraction
Tanaka et al. (2005)	Liquid chromatography mass spectrometry	Human serum pseudocholinesterase	Acephate and methamidophos	Liquid–liquid extraction
Ueyama et al. (2006)	Gas chromatography mass spectrometry	Human and animal urine	Multiresidue including acephate and methamidophos	Liquid–solid phase extraction
Hardt and Angerer (2000)	Gas chromatography mass spectrometry	Human and animal urine	Multiresidue including acephate and methamidophos	Liquid–liquid phase extraction
(Agüera et al., 2002	Tandem mass spectrometry	Vegetables and fruits	Multiresidue including acephate and methamidophos	Liquid–solid phase extraction
Zhang et al. (2002)	Gas chromatography with flame photometric detection	Water, soil and plants	Multiresidue including acephate and methamidophos	Liquid–solid phase extraction

Table 4 Recovery data (%) for acephate and methamidophos in the matrix: lettuce, orange, apple, cabbage, grape and wheat flour respectively.

Matrix	Acephate		Methamidophos	
	Recovery (%)	RSD	Recovery (%)	RSD
Lettuce	81	11	93	11
Orange	95	5	77	11
Apple	84	5	70	7
Cabbage	81	7	83	5
Grape	85	9	78	8
Wheat flour	75	6	70	11

via residues in food (WHO, 2003, 2005). The prolonged or continued use of acephate and methamidophos in plant protection may lead to significant dermal exposure with an impact on cholinesterase, genotoxicity and cardiotoxicity activities (Farag et al., 2000;Spassova et al., 2000; Padungtod et al., 1998).

Immense toxicity of acephate and methamidophos has been observed in environment including birds (Zinkl et al., 1981; Vyas et al., 1996), animals (Singh and Drewes, 1987), fishes (Szeto et al., 1979), soils and its microorganisms (Wu et al., 2010; Lo, 2010; Battu et al., 2009). Studies have revealed that acephate and methamidophos can persist on soils (Zhang et al., 2005; Battu et al., 2009), fruits and vegetables (Antonious and Snyder, 1994; Bouchard and Lavy, 1982; Chuanjiang et al., 2010; Zhang et al., 2008), in dietary products (WHO, 2003, 2005; Nougadère et al., 2012), cereal and other cash crops (Hiemstra & Kok, 2007; Antonious and Snyder, 1994; Bouchard and Lavy, 1982; Chuanjiang et al., 2010). Both, acephate and methamidophos having high leaching capacity, contaminate agriculture soils by leaching behavior (Chai et al., 2009, 2010). Studies have revealed that the concentrations of acephate and methamidophos in the rat tissues vary from 0.2 to 1.1 ppm. The highest concentrations were found in kidney (4.1–12 ppm), testes (2.4–3.9 ppm) and brain (2.1–2.5 ppm). There was no tendency for methamidophos to accumulate in blood, liver, muscle, fat or heart (Farag et al., 2000; Spassova et al., 2000; Padungtod et al., 1998; WHO, 2003, 2005). But, acephate and methamidophos are found highly toxic to bees and other beneficial insects. The LD_{50} for acephate was 1.2 μg/honeybee. The LD_{50} of methamidophos was 1.37 μg/honeybee (EPA, 2006). Exposure of acephate and methamidophos was checked on farmers by Maroni et al. (1990), acephate was detected in urine, red blood cell (RBC) and milk, but methamidophos was in minute amount. Daily maximum acephate urine concentrations range was found from 3 to 9 mg/L. Urinary concentration of acephate was non-detectable after 48 h of the exposure (Maroni et al., 1990).

3. Detection of acephate and methamidophos

Based on physicochemical properties of acephate and methamidophos, most of the researchers decided to use chromatographic technique for the detection of acephate and methamidophos. Because of low molecular mass, gas chromatography (GC) coupled with different detectors has been used

for analysis. Gas chromatography requires less pretreatment as well as cleanup methods and shows low detection limit, but at the same time needs derivatization of acephate/methamidophos. Because the high polarity reverse phase liquid chromatography (RPLC) is a method of choice for the detection of acephate and monocrotophos, it has merit of high degree of selectivity and sensitivity. But, the method requires extensive pretreatment and cleanup method(s). In addition, sometimes methods are pH dependent and require use of buffer to inhibit any change in pH. In this review, we propose analytical procedures and materials used in the determination of the acephate and methamidophos present in different sorbing media such as vegetables, human and animal tissues, blood, serum, soils, etc. Before going in detail about any method of detection, let us first discuss about some of the pretreatment and cleanup methods.

4. Pretreatment procedure

Real-world sample for analysis is always complex. In order to get best result with low interference of unwanted substances, pretreatment of the sample is the prime requirement. Because of their high solubility in water, they (acephate and methamidophos) can reach to plant as well as non-target species through absorption as well as adsorption processes. In order to remove acephate and its degraded product from the complex matrix various researchers have established different pretreatment methods.

In soil, acephate and its metabolites adsorb over silica or alumina. For this purpose, the soil samples are collected in the field, are air-dried, grounded and sieved through a mesh with a grain size of 2 mm. This follows mostly liquid–solid extraction technique (prominently Soxhlet based technique) (Araoud et al., 2010; Montesano et al., 2007; Prasad et al., 2013; Schindler et al., 2009). Because of the polarity factor water or methanol is used as an extractant for acephate, although in the more complex soil media other solvents are also used such as, ethyl acetate, hexane, buffered water and cyclohexane–acetone in ratio (1:1).

After pretreatment different cleanup methods have employed to avoid interference with other organic motifs. Depending on types of interference, different groups incorporated different sorbents for isolation of organic compounds from the extracted solutions, including alumina, Florisil, ion-exchange resins, silica gel/silica-based sorbents (e.g., octadecyl-, octyl-, phenyl-, and diol-bonded silica) and graphitized black carbon (Araoud et al., 2010; Montesano et al., 2007; Schindler et al., 2009). Whole procedure of extraction, cleanup and sample loading is summarized in Fig. 1.

4.1. Determination of acephate and methamidophos in serum and blood

Inoue et al. (1998) have determined ten organophosphorus pesticides including acephate and methamidophos concentrations in serum of acute poisoning patients by LCMS. In the reported method, compounds are extracted in acetonitrile, evaporated to 0.5–0.9 mL, and allowed for liquid chromatographic mass spectroscopic (LCMS) analysis using methanol as mobile phase and ammonium formate as a buffer. Studies have shown recovery in serum over the range between 60.0% and 108.1%, the limits of detection ranged from 0.125 to 1 μg/mL, and the limits of quantification ranged from 0.25 to 1.25 μg/mL.

An analytical and multiple reactions monitoring method for the determination of multiresidue of pesticides was developed including the acephate and methamidophos; method involves a liquid–liquid extraction procedure followed by liquid chromatography tandem mass spectrometry (LC–TMS) for the identification and quantification of compounds. Ionization of molecules was performed by the electrospray

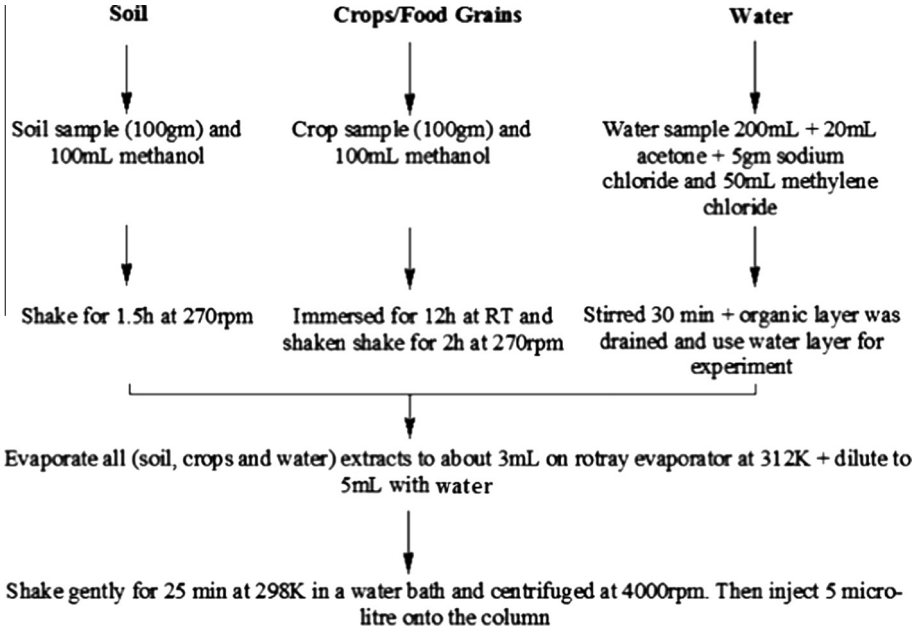


Figure 1 Flow steps of extraction, cleanup and sample loading of organophosphate pesticides including acephate and its metabolites with LC/GC–MS column. (*Here, RT is room temperature; LC is liquid chromatography; GC is gas chromatography; and MS is mass*).

mode. The average recoveries obtained, at three different fortification levels, ranged between 65% and 106% for most of the pesticides studied, except for methamidophos (lower than 25%). The linearity of the method was over the range of 5 to 50 μg/L with a correlation coefficient from 0.995 to 0.999, depending on the analyte. The estimated limit of detection and limit of quantification were 2 μg/L and 5 μg/L, respectively (Araoud et al., 2010).

4.2. Determination of acephate and methamidophos in human and animal urine

Acephate concentrations in the human urine were determined by different researchers. Mostly, method includes dilution by water and acetone, partitioned by acetone-methylene chloride (1 + 1, v/v) after adjusting urine to neutral pH. After using gas chromatography-pulsed flame photometric detection, limit of detection was established at 2 μg/L and limit of quantitation was 10 μg/L. The average recovery from urine fortified with 10–500 μg/L was 102 ± 12% (n = 32) (LePage et al., 2005; Olsson et al., 2003). When the analysis of urinary samples was done by LCMS method, the extraction efficiency ranged between 52% and 63%, relative recoveries were about 100%, and the limits of detection were over the range of 0.001–0.282 ng/mL (Montesano et al., 2007).

It is equally typical to analyze polar pesticides in urine, so their identification was done based on their stable metabolites. In reported gas chromatographic-mass spectrometric method (GCMS) (developed to check the metabolites of organophosphate pesticides including acephate and methamidophos), analytes were extracted from acidified urine into a mixture of diethylether and acetonitrile, where dibutylphosphate used as internal standard and derivatization is performed using pentafluorobenzylbromide at 40 °C overnight. After further liquid–liquid extraction, analysis is carried out by gas chromatography–mass spectrometry. The limits of detection were found to be 5 μg/L urine for dimethylphosphate and 1 μg/L for the other five metabolites, *viz*. diethylthiophosphate, O,O-dimethyldithiophosphate, and O,O-diethyldithiophosphate in human urine (Hardt and Angerer, 2000).

Another sensitive method namely gas chromatography–mass spectrometry (GC–MS) was developed for the simultaneous determination of urinary dialkylphosphates or metabolites of organophosphorus insecticides including dimethylphosphate (DMP), diethylphosphate (DEP), dimethylthiophosphate (DMTP), and diethylthiophosphate (DETP), using a pentafluorobenzylbromide derivatization. The limit of determination was approximately found to be 0.3 μg/L for DMP and 0.1 μg/L for each of DEP, DMTP, and DETP in human urine (Ueyama et al., 2006). In the above mentioned solid-phase extraction, derivatization was done with pentafluorobenzyl bromide and further solid-phase cleanup done, and the extracts were analyzed by gas chromatography–tandem mass spectrometry. The limits of detection were 0.25 μg/L for both analytes (Schindler et al., 2009).

4.3. Determination of acephate and methamidophos in vegetables

The observed residues of acephate and methamidophos have found tremendous increase in their residues in year 2008 than year 2006, as per the study that was done on Applesauce, Bananas, Carrots, Cranberries, Eggplant, Grape, Green Collard, Greens Kale, Orange Juice, Peaches, Plums, Potatoes, Raisins, Spinach, Summer Squash, Sweet Peas, Frozen, Watermelon and Winter Squash. Comparative study was done by using chromatographic methods namely liquid or gas chromatography–mass spectrometry, when 8515 samples were analyzed in year 2006 and 8686 samples in year 2008. Out of all these samples, acephate was detected in 1.11% samples in year 2006, while 5.65% in year 2008. On the other hand, methamidophos was found in 1.02% samples of year 2006, which increased to 4.89% in year 2008. Acephate and methamidophos were analyzed on spinach, summer squash, nectarines, blueberries, collard greens, strawberries, and tomatoes (PDPAS, 2006) (Fig. 2). A straightforward extraction method based on liquid chromatography–mass spectrometry (LC–MS) was reported; in which ethyl acetate was used as extractant and 0.1% acetic acid/water mixture was used as mobile phase without undergoing any cleanup procedure. The method was validated at the 0.01 and 0.5 mg/kg level, for both cabbage and grapes. Recoveries were reported in between 80% and 101% with R.S.D. < 11% (n = 5). The limit of quantification was 0.01 mg/kg and limit of detection was in between 0.001 and 0.004 mg/kg (Mol et al., 2003).

Another liquid chromatography-tandem quadrupole mass spectrometry multi-residue method for the simultaneous target analysis of a wide range of pesticides and metabolites in fruit, vegetables and cereals (lettuce, orange, apple, cabbage, grape and wheat flour, etc.) was reported by using acetone/dichloromethane/light petroleum as extraction solvents. As per report recoveries ranged from 70% to 110%, with relative standard deviations (RSD) better than 15%, were obtained (Hiemstra and Kok, 2007). The observed values of recoveries and RSD for acephate and methamidophos in lettuce, orange, apple, cabbage, grape and wheat flour matrix are tabulated below at fortification level 0.01 mg/kg (Hiemstra and Kok, 2007).

A gas chromatography (by using a combination of positive chemical ionization and electron impact ionization modes) tandem mass spectrometry method was employed for the analysis of organophosphates (including acephate and its metabolites) in vegetables, the limits of detection obtained in a range of 0.07 to 4.21 μg/kg. Average recoveries were in between 52% and 114% while the RSD values greater than or equal to 29% in all the cases were reported (Agüera et al, 2002). A simple, fast and inexpensive, gas chromatography–mass spectrometry method was introduced for the pesticide determination by single-phase extraction of sample (10 g) with acetonitrile (10 mL), followed by liquid–liquid partitioning after addition of 4 g anhydrous $MgSO_4$ + 1 g NaCl. Recoveries were obtained in between 85% and 101% (mostly > 95%) and repeatabilities typically < 5% were achieved for a wide range of fortified pesticides (Anastassiades et al., 2003). Another GC based method

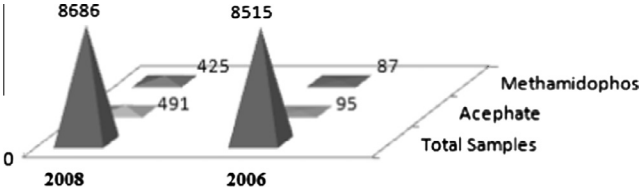


Figure 2 Study of acephate and methamidophos in food Grains (PDPAS) during 2008 and 2006.

was developed for the sensitive determination of acephate and other organophosphate pesticides by using 5% acetone in hexane as extracting solvent and 1-chloro-4-fluorobenzene as an internal standard in fruits and vegetables. The limit of detection by the method was found in between 0.005 and 0.01 mg/kg and the limit of quantification was 0.01 mg/kg (Lal et al., 2008).

4.4. Determination of acephate and methamidophos in soils and waters

A gas chromatography with flame photometric detection method was developed for the determination of concentrations of organophosphate pesticides including acephate and methamidophos in water, soil, sediment and plants. The concentrations of the total organophosphate insecticides ranged from 92.77 to 229 ng/L in river water, 1.61 to 9.93 ng/g dry weights in soil, 1.24 to 7.56 ng/g dry weights in sediment and 75.28 to 326 ng/g dry weights in plants were obtained by the researchers (Zhang et al., 2002). An analytical methodology for the analysis of methamidophos in water and soil samples incorporating a molecularly imprinted solid-phase extraction process using methamidophos-imprinted polymer was developed, where the confirmation of the imprinting done by FT-IR analysis due hydrogen bonding between the COOH in the polymer cavities and the NH_2 and P = O of the template is the origin of methamidophos recognition. The use of molecularly imprinted solid-phase extraction was found to improve the accuracy and precision of the GC method and lowered the limit of detection. The recovery of methamidophos extracted from 10.0 g soil sample at the 100 ng/g spike level was 95.4%. The limit of detection was 3.8 ng/g. The recovery of methamidophos extracted from 100 mL tap and river water at 1 ng/mL spike level was 96.1% and 95.8%, and the limits of detection were 10 and 13 ng/L respectively (Kumar et al., 2013; Shen et al., 2011).

5. Conclusion

The future perspective of the study is to check the decomposition of both acephate and its metabolite methamidophos in the presence of different microorganisms, especially in plant growth promoting bacteria. There is only one published information available on microbial degradation of acephate by *Pseudomonas sp. Ind01*, which uses acephate as a source of carbon to support cell growth and can promote the first step of acephate mineralization in soil microbial communities. Also there is information available on the microbial degradation of methamidophos with species *Hyphomicrobium species* MAP-1. However, in spite of the significant enantioselective bioactivity of acephate and methamidophos, no present investigations on their environmental behavior have taken their chirality into account. It should be recognized that the enantiomers of acephate and its metabolite methamidophos are independent entities which exhibit significant enantioselective bioactivity or toxicity to the target or nontarget organisms as described above and may be transformed by microbes or enzymes at different rates. Thus, information on the enantioselective degradation and environmental behavior of parent acephate as well as its metabolite methamidophos is essential to evaluate the risk of these two chiral insecticides to human and ecological health. This information cannot be obtained from conventional achiral analysis. However, to our knowledge, there was the only one published information available on the transformation and degradation of the enantiomers of acephate and its chiral metabolite methamidophos in soils (Wang et al., 2013). Study highlighted that degradation of racemates is enantioselective in unsterilized soils but not in the sterilized soils, thus confirming that enantioselectivity is microbial based. So there is a need to pay more attentions on chirality based study of acephate and its metabolite methamidophos on different soils under different conditions such as temperature and pH.

Finally, we have concluded that acephate and methamidophos are distributed throughout the environment because of its high water solubility and probable chances of water runoff. Various methods have been reported to analyze level of acephate and methamidophos in different media such as soil, water, vegetable, serum, urine, etc. Most popular among them is liquid chromatography mass spectrometry (LCMS) or gas chromatography mass spectrometry (GCMS). But, LCMS requires extensive pretreatment and cleanup procedure, while GCMS needs derivatization of the compounds. The enantioselective bioactivity of both acephate and methamidophos in future may produce new method(s) of detection including determination of extent of toxicity of these pesticides. It is desirable to develop new methods based on use of chromophores/fluorophores which should be selective for acephate and its degraded products. Because of high use of acephate and methamidophos these days, their easy and fast analysis is also required. A lot of scope for trend breaking research in this area is needed.

In biochemical aspects, decomposition pathway of acephate with selected rhizobacteria in the presence of trace and heavy metal ions, including the mechanisms is under experimentation. Our future work will address the development of spectrophotometric detection of acephate and its metabolite.

References

Agüera, A., Contreras, M., Crespo, J., Fernández-Alba, A.R., 2002. Multiresidue method for the analysis of multiclass pesticides in agricultural products by gas chromatography–tandem mass spectrometry. Analyst 127 (3), 347–354.

Anastassiades, M., Lehotay, S.J., Stajnbaher, D., Schenck, F.J., 2003. Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce. JAOAC Int. 86 (2), 412–431.

Antonious, G.F., Snyder, J.C., 1994. Residues and half-lives of acephate, methamidophos, and pirimiphos-methyl in leaves and fruit of greenhouse grown tomatoes. Bull. Environ. Contam. Toxicol. 52, 141–148.

Araoud, M., Douki, W., Najjar, M.F., Kenani, A., 2010. A simple analytical method for determination of pesticide residues in human serum by liquid chromatography tandem mass spectrometry. J. Environ. Sci. Health B 45 (3), 242–249.

Battu, R., Sahoo, S., Jyot, G., 2009. Persistence of acephate and cypermethrin oncotton leaves, cottonseed, lint and soil. Bull. Environ. Contam. Toxicol. 82, 124–128.

Bouchard, O.C., Lavy, T.L., 1982. Fate of acephate in the cotton plant. J. Econ. Entomol. 75, 921–923.

Chai, L.K., Mohd-Tahir, N., Bruun-Hansen, H.C., 2009. Dissipation of acephate, chlorpyrifos, cypermethrin and their metabolites in a

humid-tropical vegetable production system. Pest. Manage. Sci. 65 (2), 189–196.
Chai, L.K., Wong, M.H., Mohd-Tahir, N., Hansen, H.C.B., 2010. Degradation and mineralization kinetics of acephate in humid tropic soils of Malaysia. Chemosphere 79, 434–440.
Chuanjiang, T., Dahui, L., Xinzhong, Z., Shanshan, C., Lijuan, F., 2010. Residue analysis of acephate and its metabolite methamidophos in open field and greenhouse pakchoi (*Brassica campestris* L.) by gas chromatography-tandem mass spectrometry. Environ Monit. Assess. 165, 685–692.
Chuckwudebe, A.C., Hussain, M.A., Oloffs, P.C., 2000. Hydrolytic and metabolic products of acephate. J. Environ. Sci. Health B 19 (6), 501–522.
Farag, A.T., Eweidah, M.H., El-Okazy, A.M., 2000. Reproductive toxicology of acephate in male mice. Reprod. Toxicol. 14, 457–462.
Hardt, J., Angerer, J., 2000. Determination of dialkyl phosphates in human urine using gas chromatography–mass spectrometry. J. Anal. Toxicol. 24, 678–684.
Hiemstra, M., de-Kok, A., 2007. Comprehensive multi-residue method for the target analysis of pesticides in crops using liquid chromatography–tandem mass spectrometry. J. Chromatogr. A 1154 (1–2), 3–25.
Inoue, S., Saito, T., Mase, H., Suzuki, Y., Takazawa, K., Yamamoto, I., Inokuchi, S., 2007. Rapid simultaneous determination for organophosphorus pesticides in human serum by LC–MS. J. Pharm. Biomed. Anal. 44 (1), 258–264.
Khan, D.A., Hashmi, I., Mahjabeen, W., Naqvi, T.A., 2010. Monitoring health implications of pesticide exposure in factory workers in Pakistan. Environ. Monit. Assess. 168, 231–240.
Kumar, V., Upadhyay, N., Wasit, A.B., Singh, S., Kaur, P., 2013. Spectroscopic methods for the detection of organophosphate pesticides – a preview. Current World Environment. 8, 313–319.
Lal, A., Tan, G., Chai, M., 2008. Multiresidue analysis of pesticides in fruits and vegetables using solid-phase extraction and gas chromatographic methods. Anal. Sci. 24 (2), 231–236.
LePage, J.T., Hebert, V.R., Tomaszewska, E.M., Rothlein, J.E., McCauley, L., 2005. Determination of acephate in human urine. JAOAC Int. 88 (6), 1788–1792.
Lin, K., Zhou, S., Xu, C., Liu, W., 2006. Enantiomeric resolution and biotoxicity of methamidophos. J. Agric. Food Chem. 54 (21), 8134–8138.
Lo, C.C., 2010. Effect of pesticides on soil microbial community. J. Environ. Sci. Health B 45 (5), 348–359.
Lotti, M., Moretto, A., Bertolazzi, M., Peraica, M., Fioroni, F., 1995. Organophosphate polyneuropathy and neuropathy target esterase: studies with methamidophos and its resolved optical isomers. Arch. Toxicol. 69 (5), 330–336.
Magee, P.S., 1973. N-Acyl derivatives of phosphoramidothioates. U.S.Patent 3,716,600.
Mahajna, M., Quistad, G.B., Casida, J.E., 1997. Acephate insecticide toxicity: safety conferred by inhibition of the bioactivating carboxyamidase by the metabolite methamidophos. Chem. Res. Toxicol. 10, 64–69.
Maroni, M., Catenacci, G., Galli, D., Cavallo, D., Ravazzani, G., 1990. Biological monitoring of human exposure to acephate. Arch. Environ. Contam. Toxicol. 19, 82–788.
Miyazaki, A., Nakamura, T., Kawaradani, M., Marumo, S., 1988. Resolution and biological activity of both enantiomers of methamidophos and acephate. J. Agric. Food Chem. 36 (4), 835–837.
Mol, H.G., Van-Dam, R.C., Steijger, O.M., 2003. Determination of polar organophosphorus pesticides in vegetables and fruits using liquid chromatography with tandem mass spectrometry: selection of extraction solvent. J. Chromatogr. A 1015, 119–127.
Montesano, M.A., Olsson, A.O., Kuklenyik, P., Needham, L.L., Bradman, A.S., Barr, D.B., 2007. Method for determination of acephate, methamidophos, omethoate, dimethoate, ethylenethiourea and propylenethiourea in human urine using high-performance liquid chromatographyatmospheric pressure chemical ionization tandem mass spectrometry. J. Expo. Sci. Environ. Epidemiol. 17 (4), 321–330.
Nougadère, A., Sirot, V., Kadar, A., Fastier, A., Truchot, E., Vergnet, C., Hommet, F., Baylé, J., Gros, P., Leblanc, J.C., 2012. Total diet study on pesticide residues in France. Levels in food as consumed and chronic dietary risk to consumers. Environ. Int. 45, 135–150.
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 organophosphorus pesticide biomarkers in human urine. Anal. Bioanal. Chem. 376 (6), 808–815.
Padungtod, C., Lasley, B.L., Christiani, D.C., Ryan, L.M., Xu, X., 1998. Reproductive hormone profile among pesticide factory workers. J. Occup. Environ. Med. 40 (12), 1038–1047.
Pesticide Data Program Annual Summary (PDPAS), Calendar Year 2006; U.S. Department of Agriculture, Agricultural Marketing Service. Washington, DC, 2007.
Prasad, R., Upadhyay, N., Kumar, V., 2013. Simultaneous determination of seven carbamate pesticide residues in gram, wheat, lentil, soybean, fenugreek leaves and apple matrices. Microchem J. 111, 91–97.
Roberts, T.R., Hutson, D.H., 1999. Acephate. Metabolic Pathways of Agrochemicals - Part 2: Insecticides and Fungicides; The Royal Society of Chemistry: Cambridge, UK, pp. 201–204.
Schindler, B.K., Förster, K., Angerer, J., 2009. Quantification of two urinary metabolites of organophosphorus flame retardants by solid-phase extraction and gas chromatography–tandem mass spectrometry. Anal. Bioanal. Chem. 395, 1167–1171.
Shen, Z.L., Yuan, D., Su, Q.D., Zhang, H., Wang, J., Zhu, J.H., Liu, Y.M., 2011. Selective solid-phase extraction using molecularly imprinted polymer for analysis of methamidophos in water and soil samples. Biosci. Biotechnol. Biochem. 75 (3), 473–479.
Singh, A.K., Drewes, L.R., 1987. Neurotoxic effects of low-level chronic acephate exposure in rats. Environ. Res. 43, 342–349.
Spassova, D., White, T., Singh, A.K., 2000. Acute effects of acephate and methamidophos on acetylcholinesterase activity, endocrine system and amino acid concentrations in rats. Comp. Biochem. Physiol. Part C 126, 79–89.
Standing Committee on Chemicals & Fertilizers, 2012-13. (<http://www.downtoearth.org.in/dte/userfiles/images/pesticide%20-eport.pdf>).
Szeto, S.Y., Mac-Carthy, H.R., Oloffs, P.C., Shepherd, R.F., 1979. The fate of acephate and carbaryl in water. J. Environ. Sci. Health B 14, 635–654.
Tanaka, T., Tanaka, N., Kita, T., Kasai, K., Sato, H., 2005. Acephate in biological fluids of two autopsy cases after ingestion of the chemical. J. Forensic. Sci. 50 (4), 933–938.
Tomlin, C.D.S., 2006. The Pesticide Manual, A World Compendium, 14th ed. British Crop Protection Council, Alton, Hampshire, UK, pp. 5–6.
Ueyama, J., Saito, I., Kamijima, M., Nakajima, T., Gotoh, M., Suzuki, T., Shibata, E., Kondo, T., Takagi, K., Miyamoto, K., Takamatsu, J., Hasegawa, T., Takagi, K., 2006. Simultaneous determination of urinary dialkylphosphate metabolites of organophosphorus pesticides using gas chromatography–mass spectrometry. J. Chromatogr. B 832, 58–66.
Vyas, N.B., Kuenzel, W.J., Hill, E.F., Romo, G.A., Komaragiri, M.V.S., 1996. Regional cholinesterase activity in white-throated sparrow brain is differentially affected by acephate (Orthene®). Comp. Biochem. Physiol. 113C (3), 381–386.
Wang, P., Jiang, S., Liu, D., Zhang, H., Zhou, Z., 2006. Enantiomeric resolution of chiral pesticides by high-performance liquid chromatography. J. Agric. Food Chem. 54 (5), 1577–1583.
Wang, X., Li, Z., Zhang, H., Xu, J., Qi, P., Xu, H., Wang, Q., Wang, X., 2013. Environmental behavior of the chiral organophosphorus insecticide acephate and its chiral metabolite methamidophos: enantioselective transformation and degradation in soils. Environ. Sci. Technol. 47, 9233–9240.

WHO, 2003. Pesticide Residues in Food – Acephate; International Programme on Chemical Safety. World Health Organization, Geneva, Switzerland, pp. 3–35.

WHO, 2005. Pesticide Residues in Food – 2005: Acephate; International Programme on Chemical Safety. World Health Organization, Geneva, Switzerland, pp. 3–16.

Wilson, B.W., Henderson, J.D., Kellner, T.P., McEuen, S.F., Griffis, L.C., Lai, J.C., 1990. Acetylcholinesterase and neuropathy target esterase in chickens treated with acephate. Neurotoxicol 11, 483–492.

Wu, M., Li, X., Zhang, H., Cai, Y., Zhang, C., 2010. Effects of methamidophos on the community structure, antagonism towards Rhizoctonia solani, and phlD diversity of soil Pseudomonas. J. Environ. Sci. Health B 45 (3), 222–228.

Yen, J.H., Lin, K.H., Wang, Y.S., 2000. Potential of the insecticides acephate and methamidophos to contaminate groundwater. Ecotoxicol. Environ. Saf. 45, 79–86.

Zhang, Z.L., Hong, H.S., Zhou, J.L., Yu, G., 2002. Occurrence and behaviour of organophosphorus insecticides in the River Wuchuan, southeast China. J. Environ. Monit. 4 (4), 498–504.

Zhang, H., Zhou, Q., Zhang, Q., Zhang, C., 2005. Impacts of methamidophos, copper, and their combinations on bacterial community structure and function in black soil. Sci. China C Life Sci. 1, 14–25.

Zhang, X., Driver, J.H., Li, Y., Ross, J.H., Krieger, R.I., 2008. Dialkylphosphates (DAPs) in fruits and vegetables may confound biomonitoring in organophosphorus insecticide exposure and risk assessment. J. Agric. Food Chem. 56 (22), 10638–10645.

Zinkl, J.G., Roberts, R.B., Shea, P.J., Lasmanis, J., 1981. Toxicity of acephate and methamidophos to dark-eyed juncos. Arch. Environ. Contam. Toxicol. 10, 185–192.