

DETERMINATION OF SELECTED ORGANOPHOSPHORUS PESTICIDES USING GC-MS IN AQUEOUS SAMPLES.

^{1,2}Ukpebor, J. E., *²Ukpebor, E. E., and ¹Halsall, C. J.

¹Lancaster Environment Centre, Lancaster University, Lancaster, LA1, 4YQ, UK

²Chemistry Department, Faculty of Physical Science, University of Benin, Benin City Edo State, PMB 1154 Nigeria

*Author for correspondence; Email: jukpebor@yahoo.com

(Received: 7th November, 2011; Accepted: 13th March, 2012)

ABSTRACT

A reproducible analytical method utilising GC-MS is presented for the analysis of thirteen organophosphorus insecticides (OPs) often found in environmental aqueous samples. Extraction of filtered river and canal water (100 mL), fortified with the OPs, was conducted using solid-phase extraction and eluted with a variety of solvent combinations. The best recoveries (70-110%) were obtained using 1:1 acetonitrile/dichloromethane. The major OP ions and subsequent product ions used for both analyte confirmation and quantitative analysis are presented. Although chromatographic peak shape was poor on a low polarity column (5% phenyl methyl) for the more water soluble compounds of malathion and dimethoate, reproducibility of spiked water samples was <15% RSD for all compounds, with instrumental detection limits ranging over 0.046 - 0.176 ngL⁻¹. This makes the described method to be applied for OPs analysis in water samples.

Keywords: Insecticides, Solid Phase Extraction, Product Ion, Gas Chromatography, Mass Spectrometry

INTRODUCTION

Organophosphorus (OP) pesticides are widely used in agriculture and are present in environmental media, often well removed from the initial site of application. There is now evidence that some of these chemicals undergo long range transport and have been observed present in aqueous media in remote locations like the Arctic (Chernyak et al., 1996; Hermanson et al., 2005).

Organophosphorus insecticides are widely used in agriculture with approximately 37 compounds divided between phosphorothioates and phosphorodithioates with varying vapour pressures (1.3E-5 - 0.04Pa, 298K) and aqueous solubilities (0.0042 - 790 gL⁻¹; 293 - 298K) (Mackay et al., 1996; Barcelo and Hennion, 1997). Many of these compounds (e.g. chlorpyrifos, chlorfenvinphos, diazinon, dimethoate, malathion, fenitrothion, parathion ethyl, azinphos methyl, azinphos ethyl, fenthion) are now included in the Priority Pollutant Lists for legislation like the EU's Water Framework Directive which require environmental quality standards for water (EC, 2000, 2001). Common methods developed for the determination of organophosphorus pesticides in environmental samples include GC - NPD (Karamfilov *et al.*, 1996; Szymczyk and Malczewska, 1998) and GC-EI-MS (Cairns *et al.*, 1993; Lacorte *et al.*, 2000; Stan, 2000; Peck and Hornbuckle, 2005).

Here we present a reproducible method for the analysis of thirteen OPs in aqueous samples. The

pesticides are listed in Table 1 along with their physicochemical properties. In view of these varied properties (vapour pressures and solubilities range over two orders of magnitude), this study was designed to develop effective extraction and recovery experiments for water samples using solid-phase extraction (SPE) cartridges and examine matrix effects on chromatography.

METHODS

Chemicals and Materials

Pesticide standards: azinphos methyl, chlorpyrifos, diazinon, dimethoate, disulfoton, fenitrothion, fenthion, fonofos, imidan, malathion, methyl parathion, temephos and terbufos (=98% purity) were purchased from Sigma Aldrich, (UK). Analytical grade dichloromethane (DCM), methanol (MEOH), acetone (ACE) and acetonitrile (ACN) were obtained from Fisher Scientific (UK). EnviChrom 'P' Solid Phase Extraction (SPE) cartridges were purchased from Supelco (Sigma Aldrich, UK). All experiments were carried out with water from a MilliQ water purification system (resistivity = 18 M Ω cm) (Millipore, USA). Stock solutions of the pesticides (100 mgL⁻¹) were prepared by dissolving the standards in DCM. These standards were stored at <4°C and working solutions prepared by diluting aliquots of each pesticide stock solution to obtain a concentration range of 0.1 - 1 mgL⁻¹ respectively. Fresh solutions were prepared as

needed.

Instrumental Conditions

Separation of the thirteen pesticides was carried out via GC-MS on a Trace GC (ThermoFinnigan UK) interfaced with a Finnigan Trace MS (ThermoFinnigan UK). OP analyte separation was performed on a 50 m low polarity GC column (8% phenyl methyl) with a 1 μ L injection volume. Instrumental operating conditions of the GC-MS are also provided in Table 2.

Sample Preparation and Extraction

Recovery studies were carried out on spiked water samples extracted using reverse phase C_{18} SPE cartridges (3 mL Envi Chrom-P C_{18} cartridges (Supelco UK)). A pesticide solution containing all 13 OP chemicals (each 0.1 mgL^{-1}) was prepared in ACN and evaporated to dryness under a gentle stream of nitrogen (99.999%), and reconstituted with 100 mL MilliQ water. Environmental samples comprising river and canal water were first filtered using a 0.4 μm filter (Sigma Aldrich, UK), and the filtered water used to reconstitute the reduced ACN solution (containing the 13 OP analytes) to examine the effects of dissolved constituents on the chromatography, chemical ionization and recoveries of the OP analytes.

The SPE cartridges were mounted on a Supelco vacuum manifold (Visiprep Supelco TM) and sequentially conditioned with 10 mL methanol and 10 mL MilliQ water, before the sample (100 mL) was loaded onto the cartridge followed by 1 mL of MilliQ water. Each cartridge was then allowed to go dry by applying a partial vacuum for ~ 5 - 10 minutes. The sample extracts were eluted using a variety of solvent combinations to assess the best recoveries for the range of OPs selected for this study, the solvents being acetonitrile, methanol, DCM, acetone and binary combinations of acetonitrile: methanol, and DCM: methanol etc. The elution volume for each solvent type was 20 mL at a flow rate of ~1 mLmin^{-1} . For each solvent the eluate was evaporated to dryness under a gentle stream of nitrogen on a heating block maintained at 30 $^{\circ}\text{C}$ and reconstituted to 1 mL with DCM. The concentration of recovered analyte was determined by an internal calibration method using seven calibration standards and triphenyl phosphate used as the internal standard.

RESULTS AND DISCUSSION

The organophosphorus pesticides studied here were chosen because of their widespread occurrence and potential impact on the environment; specifically in the aquatic environment. Many of these compounds have been present in agricultural regions for a considerable time, and have recently been detected in more remote environments such as the Arctic snowpack (Hermanson et al., 2005) and, in some cases, are susceptible to (photo)degradation in aquatic systems resulting in transformation of products which require analysis (Weber et al., 2009b, a).

Instrumental Conditions and Performance

In this study GC-MS was employed because many of the 13 OPs used in this study are semi-volatile and have low to moderate polarity. The total ion gas chromatogram for the 13 compounds is shown in Figure 1, peak intensity and shape for the more polar compounds (e.g. dimethoate) were poor on the low-polarity column selected for this work. In order to effectively separate the 13 compounds, peak shape for the more polar compounds was compromised. Peaks were confirmed by injecting individual standards and triphenyl phosphate which was used as an internal standard. GC-MS retention times and major ions are shown in Table 3.

SPE Performance

Various solvents were used to obtain the most appropriate solvent for effective recovery of the OPs from the SPE cartridge. Solvents were chosen that ranged in polarity from methanol to the low polarity solvents like *n*-hexane and results are presented in Figure 2. Ethyl acetate gave the lowest recoveries for most of the OPs, especially chlorpyrifos. Acetonitrile generally gave good recoveries, but gave a lower recovery ~33.1 56.3% for the low polarity compounds (e.g. chlorpyrifos). A binary solvent system comprising 1:1 solution of dichloromethane and acetonitrile gave the best recoveries for both the polar and non-polar OPs. Chlorpyrifos gave the highest recovery (103%) while malathion the lowest (78.0%), although values obtained were within the 70 - 110% range for recovery as stipulated by the EU guidelines for evaluating the accuracy and precision of a method (EU, 1999).

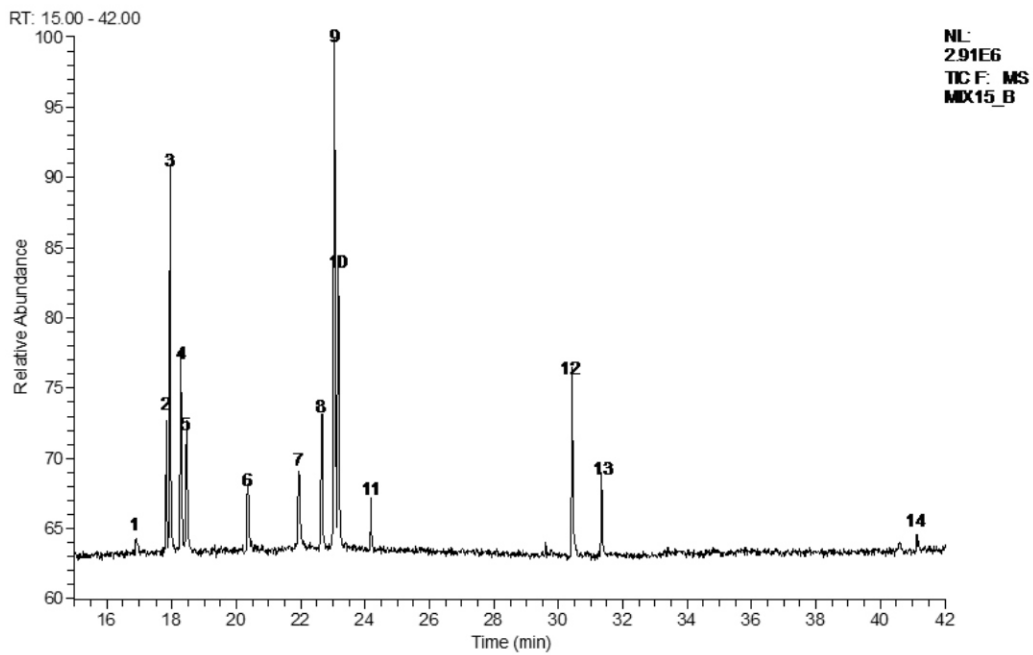


Figure 1: Chromatogram obtained from GC-MS for the 13 OP standards (1=dimethoate, 2=fonofos, 3=terbufos, 4=diazinon, 5=disulfoton, 6=methyl parathion, 7=fenitrothion, 8=malathion, 9=fenthion, 10=chlorpyrifos, 11=triphenyl phosphate (internal standard), 12=imidan, 13=azinphos methyl, 14 = temephos).

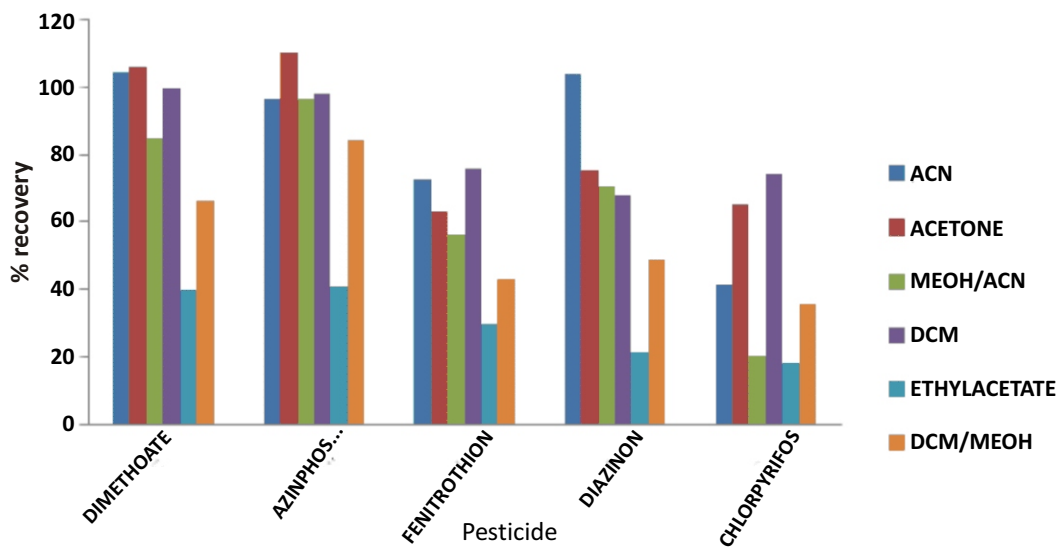


Figure 2: Recoveries obtained from different solvents for a selected OP pesticides, of varying polarity and aqueous solubility.

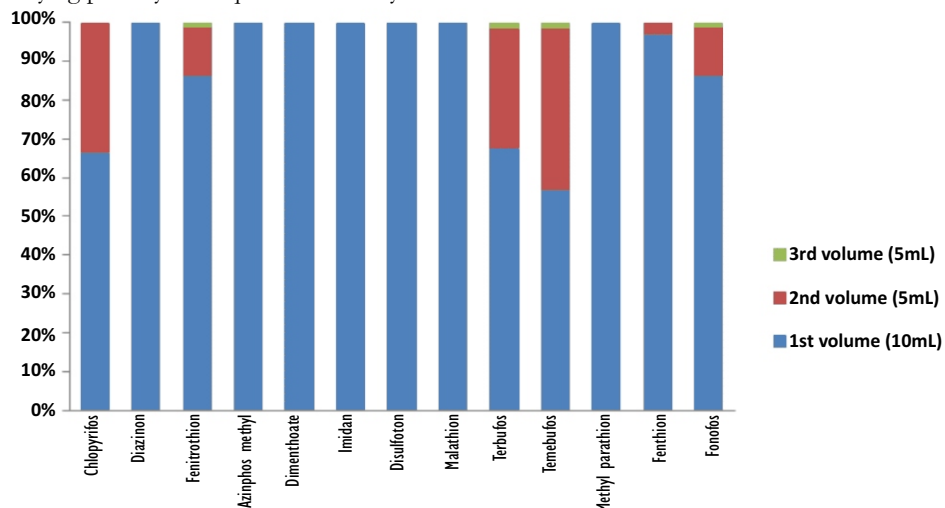


Figure 3: Eluting solvent volume for SPE cartridges

Table 1: Chemical Names and Selected Physical-Chemical Properties of 13 Op Insecticides

Name	Chemical name	CAS number	Molecular weight	Log K _{ow}	Solubility in water (mgL ⁻¹) ¹	Vapour pressure (Pa) ² 293- 298K
Dimethoate	O, O - dimethyl - S - methylcarbamoyl methyl phosphorodithioate	60-51-5	229	0.70	23	1.1 E-3
Fonofos	O - ethyl S - phenyl (RS) - ethyl phosphorodithioate	944-22-9	246	3.94	13	2.8 E-2
Methyl parathion	O, O - dimethyl - O - 4 - nitrophenyl Phosphorodithioate	298-00-0	263	3.00	55	0.2 E-3
Disulfoton	O, O - diethyl S - [2 - (ethylthio) ethyl] phosphorodithioate	298-04-4	274	3.95	12	7.2 E-3
Fenitrothion	O, O - dimethyl - O - 4 - nitro - m - tolyl phosphorodithioate	122-14-5	277	3.43	14	1.8 E-2
Fenthion	O, O - dimethyl O - [3 - methyl - 4 - (methylthio) phenyl phosphorothioate	55-38-9	278	4.84	4.2	7.4 E-4
Terbufos	S - tert - butylthiomethyl O, O - diethyl phosphorodithioate	13071-79-9	288	4.50	5.0	2.46 E-2
Diazinon	O, O - diethyl - O - 2 - isopropyl - 6 - methyl (pyrimidine - 4 - yl) phosphorothioate	333-41-5	305	3.30	40	1.2 E-2
Azinphos methyl	S - (3,4 - dihydro - 4 - oxobenzo [d] - [1,2,3] - triazin - 3yl methyl O, O - dimethyl phosphorodithioate	86-50-0	317	2.96	29	1.8 E-4
Imidan/Phosmet	O, O - dimethyl - S - phthalimidomethyl phosphorodithioate	732-11-6	317	2.95	25	6.5 E-5
Malathion	2 - (dimethoxyphosphinothioylthio) butanedioic acid diethyl ester	121-75-5	330	2.75	145	5.3 E-3
Chlorpyrifos	O, O - diethyl - O - 3,5,6, trichloro - 2 - pyridyl phosphorothioate	2921-88-2	349	4.70	1.4	2.7 E-3
Temephos	O, O' - (thiodi - 4, 1 - phenylene) bis (O, O - dimethyl) phosphorothioate	3383-96-8	466	4.90	0.27	9.5 E-6*

^{1,2}[Extoxnet, 1996; Mackay et al., 1996; Barcelo and Hennion, 1997], ³(USEPA)

Table 2: Instrumental Conditions for GCMS

GC MS	
Injection	<i>Split flow, 275°C; surge pressure = 80kPa for 1.5min</i>
Column	<i>CP Sil 8, CB 50m, 0.25mm x 0.12 m, helium carrier gas</i>
Oven programme	<i>Start at 70°C, hold for 2mins, then ramp at 10°C/min to 190°C (held for 8 mins) then 10°C/min to 290°C (held for 15mins).</i>
Ionization	<i>Electron impact ionization (EI) mode</i>
Interface temperature	<i>300°C</i>
Source temperature	<i>250°C</i>
Emission current	<i>350 A</i>

才

Table 3: Retention Time and Masses of the Major Op Ions for the Gcms Loq is Determined as the Ratio of S/n = 10

Pesticide	GC - MS			
	Retention Time (min)	Quantitation ion (m/z)	Instrument detection limit (μgL^{-1})	LOQ (μgL^{-1})
Dimethoate	16.97	229,125,93	0.0462	0.1539
Methyl parathion	20.38	263, 247,137,125,109	0.0157	0.0524
Azinphos methyl	31.37	160,132,105	0.1764	0.5879
Imidan	30.45	317,192,161	0.0115	0.0385
Malathion	22.65	330,256,173,158	0.0042	0.0141
Fenitrothion	21.96	277,260,214,177,109	0.0198	0.0659
Fonofos	17.95	247,174,137,109	0.0016	0.0053
Fenthion	23.05	278,169,125,109	0.0055	0.0184
Diazinon	18.29	304,276,199,179,93	0.0618	0.2060
Disulfoton	18.46	274,245,142,97,88	0.1090	0.3632
Terbufos	17.84	286,231,186,153	0.0051	0.0172
Chlorpyrifos	23.18	351,316,314,298,199	0.0031	0.0102
Temephos	41.03	468, 203, 109	0.0740	0.2220

Table 4: Percentage Recovery of Pesticides Obtained from the River and Canal Water Samples

Pesticide	% Recovery	
	River	Canal
Dimethoate	91.66	77.54
Methyl Parathion	82.79	76.16
Azinphos methyl	92.54	109.39
Imidan	60.28	63.60
Malathion	96.14	84.61
Fenitrothion	92.01	73.90
Diazinon	72.30	100.12
Terbufos	78.80	82.94
Chlorpyrifos	80.96	95.36
Fenthion	75.72	83.84
Fonofos	108.0	97.978
Disulfoton	78.21	75.13
Temephos	99.4	64.32

Spiked SPE cartridges were eluted with 20 mL of 1:1 DCM/acetonitrile and the eluate divided into three aliquots at a volume ratio of 2:1:1, with each aliquot analyzed separately. The pesticides diazinon, azinphos methyl, dimethoate, imidan, disulfoton, malathion and methyl parathion eluted in the first aliquot (i.e. 10 mL) while chlorpyrifos and fenthion were present in the second aliquot (5 mL) and fenitrothion, terbufos, temephos and fonofos were present in the final 5 mL. Increasing the total volume of solvent (>20 mL) however did not yield a significant increase in analyte recovery (Fig. 3).

To examine the effect of an environmental matrix, river and canal water sample, fortified with the 13 OPs were analysed using GC-MS, after filtration and SPE extraction. The pH of the two water types was close with values of 7.13 (river) and 7.77 (canal), but with marked differences in the concentrations of total dissolved solids (TDS) and conductivity (e.g. TDS of 162 and 234 mg/L; conductivity of 331 and 478 μ S/cm, for river and canal respectively. 'Native' OPs were not detected in these samples. For both water types, most of the pesticides exhibited good recoveries (75–100%) (Table 4), except imidan, which gave relatively low recoveries (60–63%). This may be due to its relatively rapid degradation via base-driven hydrolysis in water at pH>7, as previously observed by Lartigues and Garrigues (1995).

Limits of Detection (LOD)

The instrumental limits of detection (LOD) were determined for all compounds with values given in Table 3. The GC-MS limits of detection were low and ranged from 0.046–0.176 ngL⁻¹ with fonofos and azinphos methyl exhibiting the highest and lowest sensitivity, respectively.

CONCLUSION

A sensitive and effective method for the separation and analysis of organophosphorus insecticides in aqueous solutions has been developed using SPE and GC-MS as the main analytical tool. The extraction method gave good recoveries of the OP analytes from aqueous solutions and should also be applicable for a wider range of OP insecticides. River and canal water high in dissolved solids and presumably dissolved organic carbon did not provide interfering artefacts to analyte ionization using this method.

ACKNOWLEDGEMENT

We are grateful to Katerina Klagkou of Thermo Fisher UK for their technical support and assistance.

REFERENCE

Barcelo, D., Hennion, M. 1997. *Trace determination of pesticides and their degradation products in water*. Elsevier Science, Amsterdam.

- Cairns, T., Chiu, K.S., Navarro, D., Siegmunds, E. 1993. Multiresidue pesticide analysis by ion trap mass spectrometry. *Rapid Communication in Mass spectrometry* 7, 971-988.
- Chernyak, S.M., Rice, C.P., Mcconnell, L.L. 1996. Evidence of Currently-Used Pesticides in Air, Ice, Fog, Seawater and Surface Microlayer in the Bering and Chukchi Seas. *Marine Pollution Bulletin* 32, 410-419.
- EC 2000. European Commission Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for the community action in the field of water policy. *Official Journal of European Commission* L327.
- EC 2001. European Commission Decision 2455/2001/EC of 20 November 2001 establishing a list of priority substances in the field of water policy. *Official Journal of European Commission* L331, 12.
- EU 1999. Quality control procedures for pesticide analysis; Guidelines for residue monitoring in the European Union.
- Extoxnet. 1996. <http://extoxnet.orst.edu/pips/endosulf.htm>. 14th July 2008
- Hermanson, M.H., Isaksson, E.H., Teixeira, C., Muir, D.C.G., Compher, K.M., Li, Y.F., Igarashi, I., Kamiyama, K. 2005. Current-use and legacy pesticide history in the Austfonna ice cap, Svalbard, Norway. *Environmental Science & Technology* 39, 8163-8169.
- Karamfilov, V.K., Fileman, T.W., Evans, K.M., Mantoura, R.F.C. 1996. Determination of dimethoate and fenitrothion in estuarine samples by C - 18 solid phase extraction and high - resolution gas chromatography with nitrogen - phosphorus detection. *Analytica Chimica Acta* 335, 51-61.
- Lacorte, S., Guiffard, I., Fraisse, D., Barcelo, D. 2000. Broad spectrum analysis of 109 priority compounds listed in the 76/464/CEE council directive using solid phase extraction and GC/EI/MS. *Analytical Chemistry* 72, 1430-1440.
- Lartigues, S.B., Garrigues, P.P. 1995. Degradation kinetics of organophosphorus and organonitrogen pesticides in different water under various environmental conditions. *Environmental Science & Technology* 29, 1246-1254.
- Mackay, D., Shiu, W.Y., Ma, K. C. 1996. *Illustrated handbook of physical-chemical properties and environmental fate for organic chemicals*. CRC Press.
- Peck, A.M., Hornbuckle, K.C. 2005. Gas-phase concentrations of current-use pesticides in Iowa. *Environmental Science & Technology* 39, 2952-2959.
- Stan, H. J. 2000. Pesticide residue analysis in foodstuffs applying capillary gas chromatography with mass spectrometric detection: State-of-the-art use of modified DFG-multimethod S19 and automated data evaluation. *Journal of Chromatography A* 892, 347-377.
- Szymczyk, K., Malczewska, M. 1998. Gas chromatography analysis of organophosphorous pesticides in plant samples *Chromatographia* 48, 156-157.
- USEPA. http://www.epa.gov/oppsrrd1/REDS/temephos_red. 14th August 2008
- Weber, J., Halsall, C.J., Wargent, J.J., Paul, N.D. 2009a. The aqueous photodegradation of fenitrothion under various agricultural plastics: Implications for pesticide longevity in agricultural micro - environments. *Chemosphere* 76, 147-150.
- Weber, J., Halsall, C.J., Wargent, J.J., Paul, N.D. 2009b. A comparative study on the aqueous photodegradation of two organophosphorus pesticides under simulated and natural sunlight. *Journal of Environmental Monitoring* 11, 654-659.