Taylor & Francis
Taylor & Francis Group

Journal of Environmental Science and Health, Part B
Pesticides, Food Contaminants, and Agricultural Wastes

ISSN: 0360-1234 (Print) 1532-4109 (Online) Journal homepage: https://www.tandfonline.com/loi/lesb20

A sensitive LC-MS/MS method for measurement of organophosphorus pesticides and their oxygen analogs in air sampling matrices

Jenna L. Armstrong , Russell L. Dills , Jianbo Yu , Michael G. Yost & Richard A. Fenske

To cite this article: Jenna L. Armstrong , Russell L. Dills , Jianbo Yu , Michael G. Yost & Richard A. Fenske (2014) A sensitive LC-MS/MS method for measurement of organophosphorus pesticides and their oxygen analogs in air sampling matrices, Journal of Environmental Science and Health, Part B, 49:2, 102-108, DOI: 10.1080/03601234.2014.846744

To link to this article: https://doi.org/10.1080/03601234.2014.846744

Journal of Environmental Science and Health, Part B (2014) **49**, 102–108

ISSN: 0360-1234 (Print); 1532-4109 (Online)
DOI: 10.1080/03601234.2014.846744

Taylor & Francis
Taylor & Francis Group

A sensitive LC-MS/MS method for measurement of organophosphorus pesticides and their oxygen analogs in air sampling matrices

JENNA L. ARMSTRONG[1], RUSSELL L. DILLS[1,2], JIANBO YU[1,2], MICHAEL G. YOST[1] and RICHARD A. FENSKE[1]

[1]*Department of Environmental and Occupational Health Sciences, University of Washington School of Public Health, Seattle, Washington, USA*
[2]*Environmental Health Laboratory and Trace Organics Analysis Center, University of Washington, Seattle, Washington, USA*

A rapid liquid chromatography tandem mass spectrometry (LC-MS/MS) method has been developed for determination of levels of the organophosphorus (OP) pesticides chlorpyrifos (CPF), azinphos methyl (AZM), and their oxygen analogs chlorpyrifos-oxon (CPF-O) and azinphos methyl-oxon (AZM-O) on common active air sampling matrices. XAD-2 resin and polyurethane foam (PUF) matrices were extracted with acetonitrile containing stable-isotope labeled internal standards (ISTD). Analysis was accomplished in Multiple Reaction Monitoring (MRM) mode, and analytes in unknown samples were identified by retention time (±0.1 min) and qualifier ratio (±30% absolute) as compared to the mean of calibrants. For all compounds, calibration linearity correlation coefficients were ≥0.996. Limits of detection (LOD) ranged from 0.15–1.1 ng/sample for CPF, CPF-O, AZM, and AZM-O on active sampling matrices. Spiked fortification recoveries were 78–113% from XAD-2 active air sampling tubes and 71–108% from PUF active air sampling tubes. Storage stability tests also yielded recoveries ranging from 74–94% after time periods ranging from 2–10 months. The results demonstrate that LC-MS/MS is a sensitive method for determining these compounds from two different matrices at the low concentrations that can result from spray drift and long range transport in non-target areas following agricultural applications. In an inter-laboratory comparison, the limit of quantification (LOQ) for LC-MS/MS was 100 times lower than a typical gas chromatography-mass spectrometry (GC-MS) method.

Keywords: Air monitoring, organophosphorus, pesticide, drift, oxon, polyurethane foam, XAD resin.

Introduction

There is growing interest among public health scientists and practitioners in the assessment of community exposures to airborne OP pesticides, primarily in agricultural regions where they are applied by aircraft or tractor-pulled spray systems.[1,2] Community studies differ from occupational studies because measurements of exposure to OP pesticides occur outside of application areas at varying distances from applied fields rather than at the source, and air concentrations are generally much lower.

Phosphorothioate pesticides such as chlorpyrifos (CPF) and azinphos methyl (AZM) undergo transformation to their oxygen analogs (also referred to as "oxons") *in vivo*[3] and in the atmospheric environment.[4] This is illustrated in Figure 1. Their formation in the atmosphere has been associated with higher temperatures, higher levels of ozone, dry weather, interaction with hydroxyl radicals, and photodegradation via ultraviolet light.[5–9] Studies conducted in California and Washington State have measured oxygen analogs of OP pesticides in air samples along with parent compounds.[10–16] On an average, these studies reported concentrations of oxon that were 10–100 fold lower than the parent compound. However, in the Washington State study, the oxon represented as much as 94% percent of total pesticide mixture in some cases.[15] The failure to measure for these oxygen analogs in air will lead to the underestimation of total OP pesticide exposures. Furthermore, it is important quantify oxygen analogs in air because they are considered to have toxicity equivalents 5–100 fold those of the parent OP pesticides.[17–20] For example, as little as a 1% concentration of CPF-O relative to the parent CPF concentration can significantly increase overall toxicity and should be considered in health risk assessments.[4]

Address correspondence to Jenna L. Armstrong, Department of Environmental and Occupational Health Sciences, University of Washington School of Public Health, Box 357234, Seattle, WA 98195, USA; E-mail: jennaa@uw.edu; wse3@cdc.gov
Received June 26, 2013.

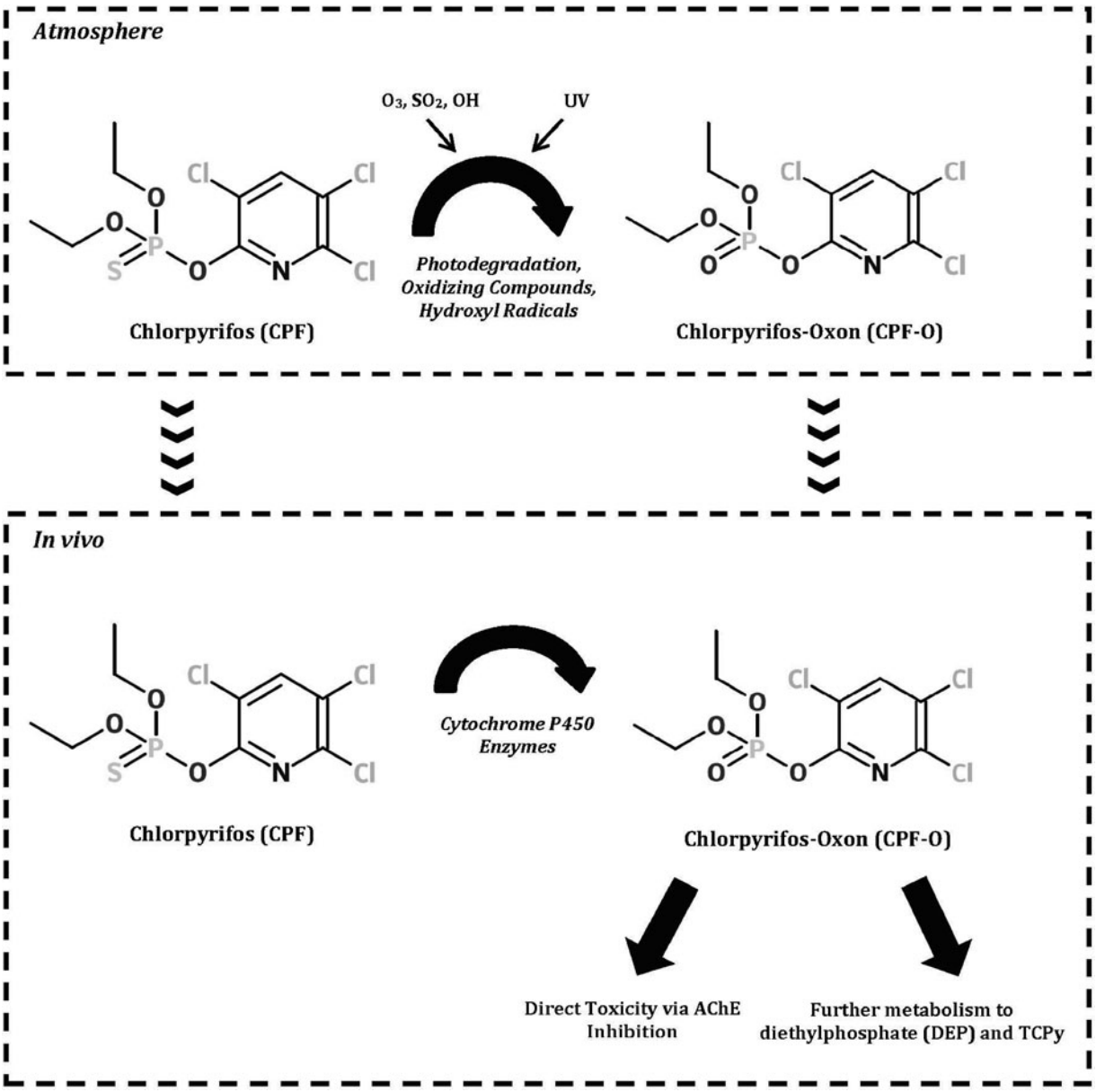

Fig. 1. Chlorpyrifos-oxon may be formed *in vivo* as a metabolic product through breakdown mechanisms involving cytochrome p450 enzymes, leading to eventual direct toxicity or metabolism to diethylphosphates (DEP) and TCPy. It may undergo photolysis or reaction with oxidizing agents such as hydroxyl radicals (OH, NO3) during atmospheric transport in the environment.[7]

The current recommended air sampling matrices for the parent OP pesticide compounds are XAD-2 (styrene-divinylbenzene) resin [used in OVS (OSHA Versatile Sampler) tubes] [21] and polyurethane foam (PUF).[22] Some studies cited above have provided data for active air sampling method validation and trapping efficiencies for OP pesticides. However, neither the NIOSH Method 5600 nor the EPA Method TO-10A requires sampling for the oxygen analogs. A study by the University of California, Davis, Trace Analytical Laboratory found that XAD resins have sufficient trapping efficiencies for OP oxygen analogs, although the tests were performed on XAD-4 resin rather than the XAD-2 resin recommend by NIOSH.[23]

A recent study examined side-by-side sampling for CPF on XAD-2 and PUF.[24] In the laboratory, both matrices were spiked with the parent compound and air was pulled at 2 L/min (LPM) for 24 h. After sampling, spike recoveries were within the acceptable range of 60–120% (EPA, 1999); 78.5 and 100.6% on XAD-2 resins and PUF, respectively. The amount of CPF-O on the XAD-2 resin ranged from 10–32% of the total CPF recovered, whereas CPF-O was at or below the limit of detection (LOD) on the PUF samplers.

Subsequent field trials confirmed the artificial formation of CPF-O on XAD-2 resin, but also documented measurable amounts of CPF-O in community air with the PUF samplers.

Current analytical methods are adequate for most worker exposure scenarios in which the goal is to measure airborne concentrations of OP pesticides greater than 1 $\mu g/m^3$, but these methods were not designed to evaluate the oxons of these compounds, which may be present at concentrations 10–100 times lower than the parent compound. Most of the air sampling studies cited above have used gas chromatography-mass spectrometry (GC-MS) for analysis of air sampling matrices. In regard to community exposures, ambient concentrations of OP pesticides (even during active application seasons) tend to be less than 1 $\mu g/m^3$.[10–16] Sampling concurrently for the oxons thus requires air concentration limits of detection (LOD) in the 0.1–10 ng/m^3 range to be relevant for community health risk assessments. A 2006 literature review compared GC-MS and LC-MS/MS methods for the analysis of pesticides and found greater sensitivity for the LC-MS/MS method.[25] A study of pesticides in water samples used LC-MS/MS methods for CPF and AZ and reported detection limits lower than those typically reported in GC-MS studies.[26]

This research reports on the laboratory method development results stemming from two recent air monitoring studies conducted in Washington State. The first study was a large scale investigation ($n > 200$) for the Washington State Department of health to measure air concentrations of OP pesticides in residential areas of Central Washington. The study used XAD-2 sampling matrices.[15] The second study was the smaller side-by-side comparison study ($n \approx 30$), discussed earlier, which investigated PUF as an alternative sampling matrix.[24] As a result, the number of replicates in the sensitivity and storage stability studies reported in this paper differ among XAD-2 and PUF tubes because data from the two different studies are combined.

At the time of these studies, specific methods were used for each specific pesticide and oxon pair being studied, and the methodology is presented as such. The analysis of all compounds mentioned is now commonly done as a suite. Although the studies were different in design, both included an improved laboratory method for quantification of OP pesticides and their oxygen analogs from different types of air sampling media. This method should prove useful for future studies of these chemicals in community air. The analytical procedures described in this paper were used for the extraction and analysis of four OP pesticide compounds: chlorpyrifos (CPF), chlorpyrifos-oxon (CPF-O), azinphos-methyl (AZM), and azinphos methyl-oxon (AZM-O). These compounds were collected on both types of matrices during active air sampling for the two studies.

The paper describes an extraction method that was adapted from NIOSH 5600 method, with substitution of a solvent compatible with aqueous chromatography.[25,27,28] Instrumental analysis procedures were developed from published studies that analyzed pesticides in food, serum and urine samples using LC-MS/MS methods.[25,28] Compounds were desorbed from XAD-2 and PUF by ultrasonication of the sampling matrices with an acetonitrile solution containing stable-isotope labeled internal standards (ISTD). Extracted samples were then analyzed using LC-MS/MS with Multiple Reaction Monitoring (MRM) detection without any further sample preparation.

Materials and methods

Chemicals and materials

The stock solutions later used for spiking of 10 and 20 μg/mL were prepared in acetone using CPF (99.5%, 1,000 μg/mL in acetonitrile, ChemService, West Chester, PA, USA, PS-674), CPF-O (98.8%, ChemService, 1,000 μg/mL in acetonitrile, MET-674B), AZM (99.5%, 100 μg/mL in methanol, ChemService, F2055S), and AZM-O (98.5%, 100 μg/mL in toluene, ChemService MET-666A). The internal standards solutions were prepared using CPFdiethyl-D_{10} (99%, neat, 100 μg/mL in acetonitrile, Cambridge Isotope Labs, Andover, MA, USA, DLM-4360), $^{13}C_2$,^{15}N-CPF-O (99%, solid, donated by Dow Agro Sciences, Indianapolis, IN, USA), AZM-D6 (98.5%, 1,000 μg/mL in toluene, EQ Laboratories, Atlanta, GA, USA<XA10365100AC), and AZM-Odimethyl-D6 (99.3%, solid, Bayer Crop Science, Research Triangle Park, NC, USA, K-176). Solvents and mobile phase components were pesticide or LC-MS grade. Deionized water (Barnstead Nanopure II, USA, 18 MΩ) was used.

The air sampling matrices were XAD-2 (OSHA Versatile Sampler [OVS], 13 × 75 mm outer diameter × length, 270/140 mg sorbent, SKC, Fullerton, CA, USA) and PUF (22 × 100 mm, outer diameter × length, 76 mg sorbent, SKC). Both the XAD-2 and PUF matrices were pre-cleaned with acetone by the supplier prior to purchase.

Instrumental analysis

Stable isotope-dilution quantification was performed on an Agilent (Santa Clara, CA, USA) 6410 liquid chromatography tandem mass spectrometer using a C_{18} reverse-phase column (Gemini, 3μ, 150 × 2.0 mm with 4 × 2.0 mm guard column; Phenomenex, Torrance, CA, USA). Electrospray ionization (positive polarity; nebulizer gas, N_2, 350°C, 9 L/min, 40 psi; capillary voltage 4,000) was used in multiple reaction mode (MRM; N_2 collision gas). MRMs and optimized acquisition parameters are listed in Table 1.

Isocratic elution (Agilent G1312A binary pump; mobile phase A, 0.1% formic acid/deionized water; B, 0.1% formic acid/acetonitrile; 0.2 mL/min flow) was used for both groups of compounds but with different%

Table 1. Acquisition parameters.

Compound	*Precursor ion (m/z)*	*Product ion (m/z)*	*Fragmentation (V)*	*Collision energy (V)*	*Retention time (min)*
CPF-D_{10}	362	201	90	20	8.28
CPF Quantifier	352	200	90	20	8.49
CPF Qualifier	352	97	90	20	
CPF-$^{13}C_2$-^{15}N-Oxon	339	283	90	10	4.02
CPF-O Quantifier	336	280	90	10	4.02
CPF-O Qualifier	336	308	90	10	
AZM-D_6	324	131	90	10	9.70
AZM Quantifier	318	125	90	10	9.81
AZM Qualifier	318	132	90	10	
AZM-Oxon-methyl-D_6	308	132	70	5	4.00
AZM-O Quantifier	302	132	70	5	4.01
AZM-O Qualifier	302	245	70	5	

composition for the separate analyses (CPF-related, 25% A, 75% B, runtime 12 min; AZ-related, 45% A, 55% B, runtime 13 min). An Agilent G1313A auto-sampler was used for injections (5 μL volume). Since we report aggregate results from two recent air monitoring studies conducted in Washington State, two separate isocratic methods were used for CPF/CPF-O and AZM/AZM-O. However, this may be also accomplished with a single method.

Calibrants were prepared in internal standard solution (CPF-D_{10} 260 ng/mL, CPF-$^{13}C_2$-^{15}N-O 27 ng/mL, AZM-D_6 100 ng/mL, and AZM-O-D_6 25 ng/mL). Calibration ranges were for 1—1,000 ng/mL CPF and AZM, 0.5—1,000 ng/mL for CPF-O and 0.1—1,000 ng/mL for AZM-O. Depending on sample loading, the upper calibration was lowered to 200–250 ng/mL. Weighted (1/concentration) linear regression was used with 6–10 calibrants depending on the range needed for analysis. Samples with analytes above the upper calibrant were diluted in acetonitrile and rerun. The lowest calibration observed valued was within 25% of nominal.

Recovery assay procedures

Spiking solutions for CPF, CPF-O, AZM, and AZM-O were diluted from stock solutions for fortification of XAD-2 and PUF at mass loadings of 5, 50, and 1,000 ng, except that there were no spike mass loadings of AZM or AZM-O on PUF tubes at low masses (5 ng) (Appendix Table 1). PUF matrices were spiked *in situ* with a 50-μL Hamilton positive displacement syringe into the approximate center of the matrix with the syringe; and XAD-2 matrices were spiked *in situ* by inserting the needle beyond the quartz fiber pre-filter into the first bed of XAD-2.

After the spiking procedure was completed, all samples were immediately capped and stored in a −20°C freezer. After freezer removal, sampling matrices were allowed to equilibrate at room temperature (20–23°C) in a lab hood for 30 min prior to extraction. Air sampling media were placed in 50-mL Corning® centrifuge tubes with 1-mL ISTD solution (25 μL of 100 ng/mL CPF-diethyl-D_{10}, $^{13}C_2$, ^{15}N-CPF-O 100 ng/mL, 4,000 ng/mL AZM-D_6, 1,000 ng/mL AZM-OD_6). Acetontirile was added to produce final volumes of 5 mL for XAD-2 and 10 mL for PUF). Samples were sonicated for 1.5 h at room temperature. The samples were then placed in a Zymark TurboVap II (McKinley Scientific, Sparta, NJ, USA) for 8–9 min @ 40°C, or until the solution was evaporated down to 0.5 mL. The front and back section of the XAD-2 tubes were extracted separately to conform to procedures from NIOSH method 5600.[21] Breakthrough was not observed since air was not drawn through the matrix. The inside of the glass sampling tubes that held the PUF matrices were rinsed with 5 mL of acetonitrile, which was then added to the extract (final volume of 15 mL for PUF). Quality control included matrix blanks, matrix spike recovery, instrument duplicate analysis (10%), and reagent blanks (acetonitrile, ISTD, and no air matrix).

Sensitivity

For each compound, the method detection limit (MDL) was defined as the standard deviation × $t_{N-1,99}$ of the recovered analyte mass in replicate (N) spiked blank matrix samples; the LOD was equated to the MDL. The limit of quantification (LOQ) was defined as 10 times this standard deviation or lowest valid calibrant, whichever was higher. The LOD and LOQ, expressed in air concentrations, were calculated by dividing the mass LOD and LOQ by typical air sampling volumes. In the California and Washington State studies, low-volume community air samples typically operated at 2 L/min (LPM) for 24 h.[13–15] This was equivalent to 2,880 L or 2.88 m^3.

Storage stability

The storage stability results on XAD-2 and PUF active sampling matrices are aggregate results from the two air monitoring studies conducted in Washington State. The timing of the storage stability analysis was dependent on analysis of field sampling results, which are not presented in this study. For the first study, the XAD-2 air sampling matrices were spiked in the same fashion as for the matrix spike recovery experiments at mass loadings CPF and CPF-O of 25 ng, AZM and AZM-O of 50 ng, and stored at −20°C for 171–236 days. For the second study, the PUF air

sampling matrices were spiked at mass loadings CPF and AZM of 20 and 50 ng, and stored at −20°C for 72 days.

Inter-laboratory comparison

An inter-laboratory comparison study was performed with the California Department of Pesticide Regulation (CDPR) laboratory and the University of Washington (UW) laboratory. CDPR continually analyzes air samples for pesticides, including CPF, CPF-O, AZM, and AZM-O. CDPR uses a GC-MS method similar to NIOSH Method 5600 for XAD-2 resins [29,30] and the California Department of Food and Agriculture (CDFA) method for XAD-4 resins.[31] The UW laboratory shared analytical standards (see Chemicals and Materials) and a set of six XAD-2 (OVS) tubes spiked with either 400 or 800 ng of CPF and CPF-O, while retaining duplicates. The CDPR laboratory received samples from UW frozen on dry ice. Samples were stored at between −5° and 0°C until analysis. Samples analyses by the two laboratories were conducted within one month of preparation. The UW laboratory followed extraction and analytical procedures described above. The CDPR laboratory used the following procedures: the front section of the OVS tube was poured into a test tube with 10 mL acetonitrile; the test tube with the sample was stirred using a vortex mixer for 1 min. The sample and the acetonitrile were allowed to stand and then placed into autosampler vials for analysis. The reported sample concentrations were calculated using the final 10 mL volume. No comparison was performed for PUF matrices since CDPR typically uses XAD resin as a sample matrix.

Results

LC-MS-MS determination

Analysis was accomplished in MRM mode, and analytes in unknown samples were identified by retention time (ą 0.1 min) and qualifier ratio (ą 30% absolute) as compared to mean of calibrants. Table 1 reports the precursor and product ion mass/charge (m/z), fragmentation (volts), and collision energy (volts), and retention time (min).

Linearity of response and detection limit

Calibration performance was based on the linearity of the calibration curve. Linearity was determined through weighted regression analysis, as indicated previously. Calibration curve R^2 values were >0.999 for XAD-2 tubes and 0.997 to >0.999 for PUF tubes. On XAD-2 tubes, the LOD were 0.6 ng/sample for CPF, 0.5 for CPF-O, 0.4 for AZM, and 0.6 for AZM-O. On PUF tubes, the LODs were 0.5 ng/sample for CPF, 0.8 for CPF-O, 0.2 for AZM, and 1 for AZM-O. LOQ values were 2 ng/sample for all four analytes on both matrices based on the lowest valid calibrant. The LOQ can be translated to an air concentration of 0.7 ng/m^3 using the 2.88 m^3 sample volume mentioned earlier (2 L/min × 24 h).

Table 2. Percent recoveries and coefficients of variation (CV%) at different spiking levels for CPF, CPF-O, AZ, and AZ-O from XAD (OVS) tubes, PUF tubes, and PUF-PAS disk. Each spiking level was performed in triplicate unless otherwise noted.

		XAD (OVS) tubes		*PUF tubes*	
Spike level (ng)		*Recovery Mean (%)*	*CV (%)*	*Recovery Mean (%)*	*CV (%)*
Chlorpyrifos (CPF)					
Low	5	94.6	5.2	71.2	8.6
Medium	50	92.3	2.1	84.9[a]	NA
High	1,000	86.1	3.4	89.5	3.7
Chlorpyrifos-oxon (CPF-O)					
Low	5	97.6	3.3	82.6	1.0
Medium	50	93.9	3.6	92.3	2.1
High	1,000	85.8	7.7	107	7.5
Azinphos methyl (AZM)					
Low	5	109	4.8	NA	NA
Medium	50	103	1.9	77.2	5.7
High	1,000	89.7	1.6	102[a]	NA
Azinphos methyl-oxon (AZM-O)					
Low	5	111	2.0	NA	NA
Medium	50	104	3.0	86.3	7.3
High	1,000	94.7	1.5	108[a]	NA

[a]no quantifiable CV.
CV: Coefficient of variation; NA: duplicate sample; no quantifiable CV.

Recovery and repeatability of the extraction method

Table 2 presents the fortification recoveries from XAD-2 (OVS) tubes and PUF tubes. Four replicates were analyzed at each fortification spike level for XAD-2, and three replicates were analyzed at each fortification spike level for PUF tubes. Individual recoveries ranged from 78–113% from XAD-2 air sampling tubes, and were slightly higher for AZM and AZM-O than for CPF and CPF-O. Individual recoveries ranged from 69–108% from PUF air sampling tubes and recoveries were higher at larger spike volumes. The acceptable range for fortification spike recoveries in EPA Compendium Method TO-10A [22] for pesticides and PCBs in ambient air using low volume sampling is between 60–120%. Recoveries from all air sampling matrices were within this range.

The PUF sampling tubes are slightly bulkier than the XAD-2 (OVS) tubes recommended by NIOSH. During chemical extraction, the PUF matrices require a larger desorption volume and may require concentrated evaporation to achieve similar LOD/LOQ values. However, the difference in size of matrix did not substantially interfere with the ability to quantify low levels (2 ng). It is assumed that with this method, larger air sampling matrices may be potentially utilized with minimal effects on sensitivity.

Table 3. Storage stability for 2–10 months**:** CPF, CPF-O, AZ, AZ-O on three air sampling matrices.

				Recovery	
	*Replicates N**	*Spike level (ng/sample)*	*Storage time (days)*	*Mean (%)*	*CV (%)*
		XAD-2 (OVS) Tubes			
CPF	18	25	171–236	83.5	3.12
CPF-O	9	25	171–181	74.4	0.35
AZM	21	50	209–294	92.1	7.35
AZM-O	21	50	209–294	80.8	6.18
		PUF Tubes			
CPF	4	20–50	72	85.6	10.2
AZM	4	20–50	72	94.3	8.3

N: Number of replicates; CV: coefficient of variation.

Storage stability

Storage stability tests also yielded acceptable recoveries ranging from 73–98% after time periods of 2–10 months (Table 3). There was no measurable difference in percent recovery between samples that had been stored for ~10 months (294 days) or samples that had been stored for ~2 months (72 days).

Inter-laboratory comparison

The CDPR laboratory found <1% difference between its own standards and the UW standards when measured by GC-MS (0.97% for CPF; 0.45% for CPF-O). The LOQ values were 200 ng/sample for the CDPR laboratory's standard GC-MS method and 2 ng/sample for the UW laboratory's LC-MS/MS method for both analytes. Average CPF recovery from OVS tubes was 94.5% (2.1% SD) for the UW laboratory and 80.7% (3.6% SD) for the CDPR laboratory (*t*-test, $P < 0.0001$). The average CPF-O recoveries were 78.3% (1.9% SD) and 71.8% (4.8% SD) for the UW and CDPR laboratories, respectively. Differences in recoveries may be partially attributable to differences in extraction procedures (i.e., 1.5 h sonication at UW; 1 min vortex mixing at CDPR). Also, the UW laboratory has found that when using GC/MS, OP pesticides and their oxygen analogs can be susceptible to GC inlet degradation and that variability increases with the number of runs due to build up of active material on the GC liner. This problem has not been observed with the LC-MS/MS method. These findings indicate that the UW LC-MS/MS method performed well in regard to sensitivity, accuracy and precision when compared to the traditional GC-MS method and that it can be considered an appropriate alternative analytical method.

The measurement of trace levels of pesticides by LC-MS/MS is not necessarily a new technique. However, this analysis method has not been used previously to quantify pesticides from air samples. The availability of the method presented here makes it likely that more researchers will consider measurement of the OP oxygen analogs to a routine part of air sample analysis and begin to explore new ways of sampling for these compounds. Our research has demonstrated the need for this analysis in future studies, especially when considering OP pesticide drift and residential exposure.

Analysis with LC-MS/MS with MRM generally involves the use of labeled internal standards. The internal standards for CPF and AZM, chlorpyrifos diethyl-D_{10} and azinphos-methyl-D6, are available from Cambridge Isotope and EQ Laboratories. Unfortunately, there is a lack of commercially available internal standards for the oxon compounds ($^{13}C_2$, ^{15}N-chlorpyrifos-oxon, AZM-O dimethyl-D_6), and the chemical synthesis of these oxon internal standards is costly. We recommend that standards for the OP oxon compounds be made generally available for researchers in this field.

Supporting information

Information on spike solutions and calibration curves is available free of charge via the Internet at http://pubs.acs.org.

Acknowledgments

This work was supported by the Washington State Department of Health Pesticide Program, the Pacific Northwest Agricultural Safety and Health Center (PNASH) (NIOSH Agricultural Centers Program 2 U50 OH07544), and the Child Health Center Pesticide Exposure Pathways Project at the Center for Child Environmental Risks Research NEIHS-P01 ES009601, EPA-RD-83451401. A special thanks to Dow Agro Sciences LLC and Bayer Crop Sciences for supplying labeled internal standards, and to Randy Segawa at the California Department of Pesticide Regulation (California EPA) for the inter-laboratory comparison.

References

[1] Lee, S.; McLaughlin, R.; Harnly, M.; Gunier, R.; Kreutzer, R. Community exposures to airborne agricultural pesticides in California: ranking of inhalation risks. Environ. Health Perspect. **2002**, *110*, 1175–1184.

[2] Harnly, M.; McLaughlin, R.; Bradman, A.; Anderson, M.; Gunier, R. Correlating agricultural use of organophosphates with outdoor air concentrations: a particular concern for children. Environ. Health Perspect. **2005**, *113*, 1184–1189.

[3] Timchalk, C.; Busby, A.; Campbell, J.A.; Needham, L.L.; Barr, D.B. Comparative pharmacokinetics of the organophosphorus insecticide chlorpyrifos and its major metabolites diethylphosphate, diethylthiophosphate and 3, 5, 6-trichloro-2-pyridinol in the rat. Toxicol. **2007**, *237*, 145–157.

[4] Armstrong, J.L.; Fenske, R.A.; Yost, M.G.; Galvin, K.; Tchong-French, M.; Yu, J. Presence of organophosphorus pesticide oxygen analogs in air samples. Atm. Environment **2013**, *66*, 145–150.

[5] Racke, K.D. Environmental fate of chlorpyrifos. Rev. of Env. Contam. and Tox. **1993**, *131*, 1–150.
[6] Spear, R.C.; Lee, Y.; Leffingwell, T.; Jenkins, D. Conversion of parathion to paraoxon in foliar residues: effects of dust level and ozone concentration. J. Agric. Food Chemistry **1978**, *26*, 434–436.
[7] Winer, A.M.; Atkinson, R. Atmospheric reaction pathways and lifetimes for organophosphorus compounds. In *Long Range Transport of Pesticides*; Kurtz, D.A., Ed.; Lewis Publishers, Inc.: Chelsea, MI, 1990; 115–139.
[8] Aston L.S.; Seiber, J.N. Fate of summertime airborne organophosphate pesticide residues in the Sierra Nevada Mountains. J. Environ. Qual. **1997**, *26*, 1483–1492.
[9] Bavcon Kralj, M.; Franko, M.; Trebš, P. Photodegradation of organophosphorus insecticides – investigations of products and their toxicity using gas chromatography – mass spectrometry and AChE-thermal lens spectrometric bioassay. Chemosphere **2007**, *67*, 99–107.
[10] Seiber, J.N.; McChesney, M.; Woodrow, J. Airborne residues resulting from use of methyl parathion, molinate, and thiobencarb on rice in the Sacramento Valley, California. Environ. Tox. Chem. **1989**, *8*, 577–588.
[11] California Air Resources Board (CARB). *Report for the Application and Ambient Air Monitoring of Chlorpyrifos (and oxon analogue) in Tulare Country During Spring/Summer 1996*. Author: Sacramento, CA, 1998.
[12] CARB. *Appendices for the Report for the Application and Ambient Air Monitoring of Chlorpyrifos (and the Oxon Analogue) in Tulare County during Spring/Summer, 1996*. California Air Resources Board: Sacramento, CA, 1998a.
[13] California Department of Pesticide Regulation (CDPR). *Report of Ambient Air Monitoring for Pesticides in Lompoc, California.* Author: Sacramento, CA, 2003.
[14] CDPR. *Environmental justice pilot project: Pesticide air monitoring in Parlier, Second Progress Report*. California Environmental Protection Agency, Department of Pesticide Regulation: Sacramento, CA, 2006. Available at http://www.cdpr.ca.gov/docs/envjust/pilot_proj/index.htm (accessed Sep 2012).
[15] Fenske, R.A.; Yost, M.; Galvin, K.; Tchong, M.; Negrete, M.; Palmendez, P.; Fitzpatrick, C. *Organophosphorus Pesticide Air Monitoring Project*, Final Report; University of Washington: Seattle, WA, June 2009. Available at http://www.doh.wa.gov/DataandStatisticalReports/EnvironmentalHealth/Pesticides/AirMonitoringStudy.aspx (accessed Sep 2012).
[16] LeNoir, J.S.; McConnell, L.L.; Fellers, G.M.; Cahill, T.M.; Seiber, J.N. Summertime transport of current-use pesticides from California's Central Valley to the Sierra Nevada mountain range, USA. Env. Tox. Chem. **1999**, *18*, 2715–2722.
[17] Chambers, J.; Carr, R.L. Inhibition patterns of brain acetylcholinesterase and hepatic and plasma aliesterases following exposures to three phosphorotionate insecticides and their oxons in rats. Toxicological Sci. **1993**, *21*, 111–119.
[18] Cole, T.B.; Beyer, R.P.; Bammler, T.K.; Park, S.S.; Farin, F.M.; Costa, L.G.; Furlong, C.E. Repeated developmental exposure of mice to chlorpyrifos oxon is associated with paraoxonase 1 (PON1)-modulated effects on cerebellar gene expression. Toxicol. Sci. **2011**, *123*, 155–169.
[19] Cole, T.B.; Walter, B.; Shih, D.; Tward, A.; Lusis, A.; Timchalk, C.; Richter, R.; Costa, L.G.; Furlong, C.E. Toxicity of chlorpyrifos and chlorpyrifos oxon in a transgenic mouse model of the human paraoxonase (PON1) Q192 polymorphism. Pharmacogenet. Genom. **2005**, *15*, 589–598.
[20] Sultatos, L.; Costa, L.G.; Murphy, S. Factors involved in the differential acute toxicity of the insecticides chlorpyrifos and methyl chlorpyrifos in mice. Toxicol. App. Pharmacol. **1982**, *65*, 144–152.
[21] National Institute of Occupational Safety and Health. NIOSH Method 5600, Organophosphorous Pesticides. *NIOSH Manual of Analytical Methods,* 4th Ed.; National Institute for Occupational Safety and Health: Cincinnati, OH, 1994.
[22] US Environmental Protection Agency (US EPA). EPA Compendium Method TO-10A. *Determination of Pesticides and PCBs in Ambient air using Low Volume Polyurethane foam (PUF) Sampling Followed by Gas Chromatographic/Multi-Detector Detection (GC/MD)*. Center for Environmental Research Information: Cincinnati, OH, 1999.
[23] Shibamoto, T.; Mourer, C.R.; Hall, G.L.; Hengel, M.J. *Method Development, Ambient Site and Application Site Monitoring for Chlorpyrifos and Chlorpyrifos-oxon in Air Samples Using XAD-4 Resin as a Trapping medium*. University of California-Davis Trace Analytical Laboratory: Davis, CA, 1996.
[24] Armstrong, J.L.; Fenske, R.A.; Yost, M.G.; Tchong-French, M.; Yu, J. Comparison of polyurethane foam and XAD-2 sampling matrices to measure airborne organophosphorus pesticides and their oxygen analogs in an agricultural community. Chemosphere **2013**, *92*, 451–457.
[25] Alder, L.; Greulich, K.; Kempe, G.; Vieth, B. Residue analysis of 500 high priority pesticides: better by GC-MS or LC-MS/MS? Mass Spectrom. Rev. **2006**, *25*, 838–865.
[26] Hernandez, F.; Sancho, J.V.; Pozo, O.; Lara, A.; Pitarch, E. Rapid direct determination of pesticides and metabolites in environmental water samples at sub-microg/l level by on-line solid phase extraction liquid chromatography-electrospray tandem mass spectrometry. J. Chromatogr. A **2001**, *939*, 1–11.
[27] Agilent. *Multi-residue Analysis of 301 Pesticides in Food Samples by LC/Triple Quadruple Mass Spectrometry*. Agilent Application Note, Publication Number: 5989-8614EN. Author: Santa Clara, CA, 2008.
[28] Sancho, J.; Pozo, O.; Hernández, F. Direct determination of chlorpyrifos and its main metabolite in human serum and urine by coupled column liquid chromatography/electrospray tandem mass spectrometry. Rapid Commun. Mass Spectrom. **2000**, *14*, 1485–1490.
[29] U.S. Occupational Safety and Health Administration (OSHA). *Sampling and Analytical Methods. Analytical Method for chlorpyrifos, DDVP, diazinon, malathion, and parathion in air (Method 62)*. Author: Washington, DC, 2011. Available at www.osha.gov/dts/sltc/methods/organic/org062/org062.html (accessed Dec 2011).
[30] US EPA. *Method 8260B: Volatile Organic Compounds by Gas Chromatography/Mass Spectrometry (GC/MS)*. Author: Alexandria, VA, 2006.
[31] California Department of Food and Agriculture, Center for Analytical Chemistry. Determination of selected pesticides collected on XAD-4 resin by high performance liquid chromatography ion trap mass spectrometry and gas chromatography mass spectrometry. Author: Sacramento, CA, 2008. Available at http://www.cdpr.ca.gov/docs/emon/pubs/em_methd_main.htm (accessed Dec 2012).

Appendix Table 1. Spiking solutions.

Spike Solution (CPF, AZM)	*μg/mL*
Low	
A	0.1
B	0.2
High	
A	10
B	20