Analysis of Active Oxon Forms of Nine Organophosphorus Pesticides in Water Samples Using Gas Chromatography with Mass Spectrometric Detection

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We established a method for the simultaneous quantitative analysis of nine organophosphorus pesticides (OPs) and their active oxon forms in water samples using gas chromatography with mass spectrometric detection with solid-phase extraction (SPE). In this method, the lower limit of detection for the nine oxons ranged from 0.5 to 20 ng/ml. Each calibration curve had good linearity, with correlation coefficients (R^2) greater than 0.991. In comparing three SPE cartridges, the recovery rate of these compounds extracted from water was highly reproducible using a cartridge of packed silica bonded with C_{18}. The limit of quantification ranged from 2.5 to 200 ng/ml at 500-fold concentrations. When the OPs were examined after chlorination treatment to simulate the water treatment process, they decomposed rapidly and were converted to their oxon forms as primary reaction products of chlorination. Under these established analytical conditions, the behavior of oxons formed in the environment and after water treatment can be determined accurately.

Key words —— organophosphorus pesticide, oxon, Water Quality Standard, gas chromatography/mass spectrometry, tap water, chlorination

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INTRODUCTION

In recent decades, there have been several incidents involving various environmental pollutants which have drawn attention to their effects on human health. Among environmental pollutants, pesticides are commonly detected in river water and groundwater as the source of tap water,[1–5] giving rise to concerns about pesticide contamination of drinking water. Organophosphorus pesticides (OPs) of the thiono form with a P = S structure are used extensively. They are activated to the oxon form with a P = O structure by an oxidation reaction and are known to have adverse effects on living organisms through the inhibition of acethylcholinesterase.[6–10] Numerous papers have described the simultaneous detection of parent OP compounds.[1,11,12] However, there are few reports detailing the simultaneous analysis of their oxon forms. Furthermore, there were no standard methods for the analysis of oxons in the Water Quality Standard Guidelines of Japan.

Solid-phase extraction (SPE) is useful for the isolation of a wide spectrum of organic micropollutants in various fields, such as environmental sampling,[13] pharmaceuticals,[14] and food analysis.[15] The SPE extraction procedure is applied to carry out simultaneous extraction and concentration of pesticides in water samples.[16–20] The sorbents commonly used are C_{18} chemically bonded to slica, carbon black and polymeric resins, and styrene-divinylbenzene polymer. Gas chromatography with mass spectrometric detection (GC/MS) has been applied to detect a wide range of pesticides due to its superior sensitivity and specificity in identification and quantification.[5,15,19] The analysis employs electron-impact ionization using a full scan and selected characteristic ions for each compound. Our study focused on the combination of selective extraction and sensitive determination using SPE-GC/MS. We established an SPE method and employed GC/MS for the subsequent analysis of samples to detect parent OP compounds and their oxon forms in water samples.

OPs of the thiono form are converted to their respective oxon forms *via* oxidation, and we therefore investigated the influence of chlorination on the parent pesticides, since the compounds are exposed to chlorine during the water treatment process. Our goal is to examine the behavior of pesticides contaminating raw water under water treatment conditions. The nine OPs examined were isoxathion, isofenphos, EPN, chlorpyrifos, diazinon, tolclofos-methyl, fenitrothion (MEP), butamifos, and

prothiofos.

MATERIALS AND METHODS

Chemicals —— Butamifos, diazinon, diazinon oxon, EPN, EPN oxon, isofenphos, isofenphos oxon, isoxathion, isoxathion oxon, MEP, MEP oxon, prothiofos, and prothiofos oxon (pesticide residue analysis grade) were all purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Butamifos oxon, chlorpyrifos oxon, and tolclofos-methyl oxon (pesticide residue analysis grade) were obtained from Hayashi Pure Chemical Industries, Ltd. (Osaka, Japan), and chlorpyrifos and tolclofos-methyl were obtained from GL Sciences Inc. (Tokyo, Japan). EPA 525 Fortification Solution B containing phenanthrene-d_{10} used as an internal standard was supplied from Supelco (Bellefonte, PA, U.S.A.). Individual standard solutions were prepared in a volume of 1000 mg/l in acetone. A 0.1 mg/l solution of phenanthrene-d_{10} was prepared in dichloromethane as an internal standard. All standard solutions were stored at –20°C, and working solutions were prepared fresh for each use by diluting the standard stock solution.

Dichloromethane and acetone [pesticide residue polychlorinated biphenyl (PCB) analysis grade], methanol (HPLC grade), sodium hypochlorite solution, and L(+)-ascorbic acid sodium salt were purchased from Wako Pure Chemical Industries, Ltd. Laboratory water was purified with a Milli-Q gradient A10 Elix system with an EDS polisher (Millipore, Bedford, MA, U.S.A.).

GC/MS Analysis —— GC was carried out using an HP6890 Series Gas Chromatograph system (Hewlett Packard, Wilmington, DE, U.S.A.) with an HP6890 Series autosampler and split/splitless injector. The analytical column was a DB-5 fused-silica capillary column, 30 m × 0.25 mm i.d., 0.25 μm film thickness (J&W Scientific, Folsom, CA, U.S.A.). The oven temperature program was 70°C initial temperature for 2 min; ramped at 15°C/min to 190°C, 1.5°C/min to 220°C, and then 10°C/min to 290°C; and holding for 3 min at 290°C. The carrier gas (helium) flow was set at 1.2 ml/min. Pulsed splitless injection of a 2 μl volume was carried out at 250°C. MS was carried out using a 5973 Mass Selective Detector (Hewlett Packard) in electron-ionization mode with an ionization voltage of 70 eV and ion source temperature of 280°C. The instrument was operated in selected-ion monitoring (SIM) mode. Two selected ions for each compound were monitored for identification and quantification and are summarized in Table 1.

Analytical Procedures —— The standard solutions were diluted with acetone to 50 ng/ml for diazinon oxon and tolclofos-methyl oxon, 100 ng/ml for isofenphos oxon and prothiofos oxon, 200 ng/ml for butamifos oxon, 500 ng/ml for EPN oxon and MEP oxon, 1000 ng/ml for chlorpyrifos oxon, and 2000 ng/ml for isoxathion oxon. A 250 μl aliquot of sodium ascorbic acid solution100 g/l was added to 500 ml of tap water to remove the chlorine and then was fortified with 50 μl of each solution. The fortified water samples were concentrated following the SPE method described below with each of three cartridges: Oasis HLB Plus Extraction Cartridge, Sep-Pak Plus PS-2, and Sep-Pak Plus C18 (Waters, Milford, MA, U.S.A.). The cartridges were equilibrated with dichloromethane 5 ml, methanol 5 ml, and water 5 ml, respectively. The extraction of water samples was carried out at a flow rate of approximately 10 ml/min. Air was then pulled though the cartridges for 10 min, followed by nitrogen gas for 20 min. The analytes were eluted from the cartridges with dichloromethane 5 ml. After evaporating the samples to less than 1 ml under a gentle nitrogen steam, the eluate was added to 50 μl of internal standard solution and adjusted to a final volume of 1.0 ml with dichloromethane for GC/MS analysis. The automatic concentrator used was a Sep-Pak Concentrator Plus (Waters).

Chlorination Processing —— Standard solutions of pesticides were added to purified water to make a final concentration of 10 ng/l. After dissolving the pesticides at room temperature, a 100 ml of the solution was collected as a sample at the start time. Subsequently, sodium hypochlorite solution was added to produce 1 mg/l of free chlorine. After mixing in an incubator at 20°C, a 100 ml sample of each solution was collected at reaction times of 0.5, 1, and 2 hr after the addition of the sodium hypochlorite solution. A 250 μl aliquot of sodium ascorbic acid 100 g/l was added to the sample solutions in order to eliminate chlorine. OPs and their by-products were extracted with SPE and then eluted with dichloromethane for GC/MS analysis.

RESULTS AND DISCUSSION

GC/MS Validation Study

We established the analytical conditions for detecting nine OPs and their oxon forms in GC/MS.

Table 1. Analytical SIM Conditions for GC/MS Analysis of Nine Parent OPs and their Oxons

Compound	Retention time[a)] (min)	Quantitation ion (*m*/*z*)	Identification ion (*m*/*z*)
Butamifos	20.3	286	200
Chlorpyrifos	16.3	199	197
Daizinon	13.0	179	137
EPN	30.4	157	169
Isofenphos	18.2	213	121
Isoxathion	22.3	177	105
MEP	15.4	277	260
Prothiofos	20.7	309	267
Tolclofos-methyl	14.6	265	125
Butamifos oxon	18.8	244	216
Chlorpyrifos oxon	16.0	270	197
Daizinon oxon	12.6	273	137
EPN oxon	26.7	141	169
Isofenphos oxon	16.6	229	201
Isoxathion oxon	20.7	161	105
MEP oxon	14.1	244	109
Prothiofos oxon	18.1	162	139
Tolclofos-methyl oxon	14.0	249	109
Phenanthrene-d_{10}	12.9	188	—

a) On a DB-5 column.

The two monitored ions were selected based on their relative abundance in the MS spectrum, characteristic fragment ions, and lack of interference with nearby peaks. If the selected ions of the parent compound and its oxon were the same, we chose the next most relatively abundant ion. All compounds studied were separated with high sensitivity and selectivity in GC/MS on the DB-5 column. Retention times and selected ions are summarized in Table 1.

The quantitative analysis of oxon forms of the nine OPs was performed using GC/MS in the SIM mode under the conditions described above. For quantification, an internal standard solution was added to each sample. Standard curves showed good linearity, with correlation coefficients (R^2) greater than 0.991 for all oxons in the concentration ranges studied. The limit of detection (LOD) was calculated as 3-fold the standard deviation of the slope of the calibration curve. LOD values were 0.5 ng/ml for diazinon oxon and tolclofos-methyl oxon, 1.0 ng/ml for isofenphos oxon and prothiofos oxon, 2.0 ng/ml for butamifos oxon, 5.0 ng/ml for EPN oxon and MEP oxon, 10 ng/ml for chlorpyrifos oxon, and 20 ng/ml for isoxathion oxon. Correlation coefficients, concentration ranges, and LOD values of the nine oxons are summarized in Table 2. The LOD values of the parent compounds were 0.5 ng/ml for tolclofos-methyl; 2.0 ng/ml for butamifos, chlorpyrifos, diazinon, EPN, MEP, isofenphos, and prothiofos; and 50 ng/ml for isoxathion. Under the present conditions, the nine oxons were analyzed with nearly equal sensitivity as the parent compounds.

Optimization of SPE

A recovery test was performed with tap water to determine the presence of matrix in water samples. Standard solutions in acetone were added at 5-fold the LOD levels to dechlorinated tap water. Recovery rates and relative standard deviations (RSDs) were assessed at 500-fold concentrations. Dechlorinated tap water 500 ml was used as the blank control. For quantification, an internal standard solution was added to all samples. Table 3 shows the level of the standard, mean recovery rates, and RSDs obtained for all compounds. Recovery rates were satisfactory with RSDs of less than 20%. The recovery test of isoxathion oxon was performed at 10-fold the LOD level because the recovery rate at 5-fold the LOD level was low. Butamifos oxon and EPN oxon had in low recovery rates for all SPE cartridges. The lowest results were obtained for EPN oxon. Recov-

Table 2. Limit of Detection, Limit of Quantity, and Linear Range of Nine OP Oxons

Compound	Correlation coefficient (R^2)	Linear range (ng/ml)	Limit of detection (ng/ml)	Limit of quantity (ng/ml)
Butamifos oxon	0.996	1–50	2.0	10.0
Chlorpyrifos oxon	0.994	10–1000	10.0	50.0
Daizinon oxon	0.997	0.2–20	0.5	2.5
EPN oxon	0.998	5–100	5.0	25.0
Isofenphos oxon	0.995	1–100	1.0	5.0
Isoxathion oxon	0.991	20–2000	50.0	200.0
MEP oxon	0.998	5–500	5.0	25.0
Prothiofos oxon	0.996	1–100	1.0	5.0
Tolclofos-methyl oxon	0.999	0.5–50	0.5	2.5

Table 3. Average Recovery Rates of Nine OP Oxons from Tap Water Using Three Solid-Phase Extraction Cartridges

Compound	Spike level (ng/ml)	Recovery (%) (RSD%)		
		HLB	PS-2	C18
Butamifos oxon	10.0	57.3 (8.1)	60.0 (0.3)	67.3 (4.2)
Chlorpyrifos oxon	50.0	69.0 (1.9)	62.0 (0.2)	92.5 (16.7)
Daizinon oxon	2.5	96.1 (8.3)	110.5 (7.3)	107.2 (6.5)
EPN oxon	25.0	58.7 (2.6)	38.7 (4.9)	58.9 (2.3)
Isofenphos oxon	5.0	80.0 (9.0)	77.9 (7.2)	78.7 (1.0)
Isoxathion oxon	200.0	118.1 (8.0)	82.5 (1.8)	72.1 (2.9)
MEP oxon	25.0	83.6 (9.2)	84.2 (5.0)	89.9 (8.0)
Prothiofos oxon	5.0	71.1 (0.6)	68.5 (0.9)	84.6 (10.2)
Tolclofos-methyl oxon	2.5	84.7 (11.3)	120.8 (6.2)	118.2 (11.4)

ery was less than 60% with an RSD of less than 5%. To improve this, additional investigation was carried out on the pretreatment method with C18-HLB, C18-C18, and HLB-C18 cartridges. Tandem cartridges were equilibrated separately. After extraction of the water samples, tandem cartridges were dried separately and elution was carried out as described above. However, not all compounds were detected from the second of the tandem cartridge pairs. It is thus necessary to take the recovery rate into account when calculating the concentration based on the calibration curves to ensure precision in the analysis of compounds for which the recovery rate is low.

The performance of each cartridge was compared based on extraction efficiency. The cartridge packed with C_{18} bonded to silica (Sep-Pak Plus C18) was a suitable for the extraction of oxons from water samples. Most compounds were extracted with high reproducibility and good recovery rates of more than 70%, and the RSDs for the studied compounds were 12% lower when using Sep-Pak Plus C18.

The limit of quantification (LOQ) was calculated to be 10-fold the standard deviation and as the lowest concentration that provided RSDs of less than 10% in the recovery test. LOQ values obtained at 500-fold concentrations were 2.5 ng/ml for diazinon oxon and tolclofos-methyl oxon, 5.0 ng/ml for isofenphos oxon and prothiofos oxon, 10 ng/ml for butamifos oxon, 25 ng/ml for EPN oxon and MEP oxon, 50 ng/ml for chlorpyrifos oxon, and 200 ng/ml for isoxathion oxon. Actual sample concentrations converted those LOQ values were 5 ng/l for diazinon oxon and tolclofos-methyl oxon, 10 ng/l for isofenphos oxon and prothiofos oxon, 20 ng/l for butamifos oxon, 50 ng/l for EPN oxon and MEP oxon, 100 ng/l for chlorpyrifos oxon, and 400 ng/l for isoxathion oxon. The LOQ values of their parent compounds were obtained at 5-fold the LOD level with the Sep-Pak Plus C18 cartridge, corresponding to 1% or less of the guideline values, except for the values of isoxathion and isofenphos.

Behavior of OPs upon Chlorination

Free chlorine 1 mg/l was added to water samples containing each OP 10 ng/l. Parent compounds rapidly decomposed, and their oxons were detected as

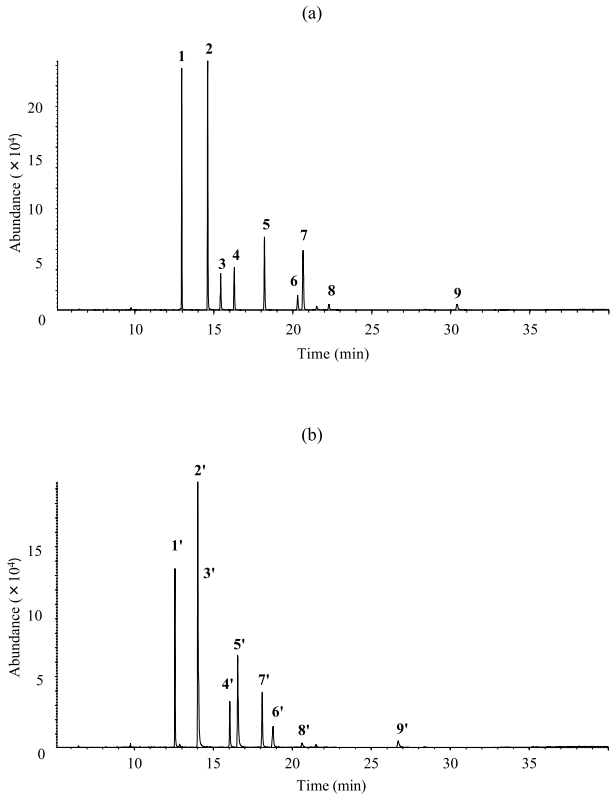

Fig. 1. Chromatograms of Nine OPs and their Products after Chlorination

(a) OPs: 1, diazinon; 2, tolclofos-methyl; 3, MEP; 4, chlorpyrifos; 5, isofenphos; 6, butamifos; 7, prothiofos; 8, isoxathion; 9, EPN. (b) Chlorinated products after 30 min: 1', diazinon oxon; 2', tolclofos-methyl oxon; 3', MEP oxon; 4', chlorpyrifos oxon; 5', isofenphos oxon; 6', butamifos oxon; 7', prothiofos oxon; 8', isoxathion oxon; 9', EPN oxon.

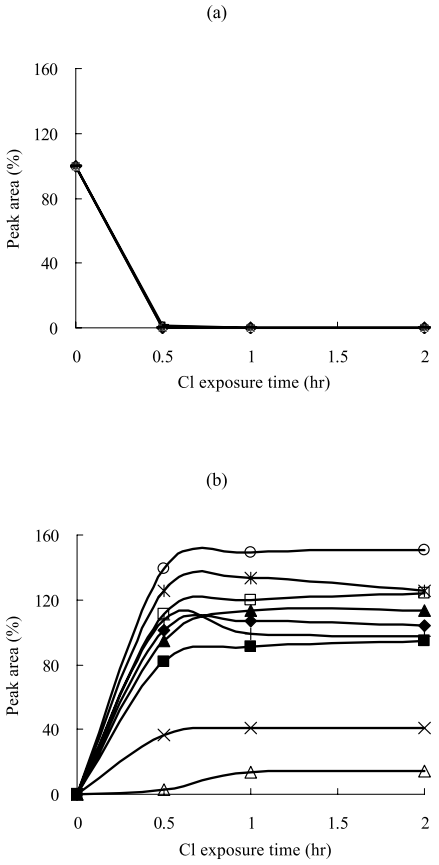

Fig. 2. Time-Dependent Behavior of OPs and their Oxon Forms after Chlorination

(a) Parent compounds, —○—, butamifos; —✳—, isofenphos; —□—, EPN; —▲—, MEP; —◆—, diazinon; —+—, prothiofos; —■—, tolclofos-methyl; —×—, chlorpyrifos; —△—, isoxathion. (b) Oxons, —○—, butamifos oxon; —✳—, isofenphos oxon; —□—, EPN oxon; —▲—, MEP oxon; —◆—, diazinon oxon; —+—, prothiofos oxon; —■—, tolclofos-methyl oxon; —×—, chlorpyrifos oxon; —△—, isoxathion oxon. The concentration at the start of the reaction was defined as 100%.

primary reaction products. Figure 1 shows the chromatograms of nine OPs and their oxons after chlorination at the start time and after 30 min. Figure 2 shows the time-dependent reactions of nine OPs and their oxons. The parent compounds decreased and the oxon forms increased upon chlorination in comparison with concentrations at the start time. The generation of oxons due to chlorination occurred rapidly. In addition, the effect of chlorination on diazinon was independently examined. The reaction was completed within 5 min at low concentrations of the parent compound (Figs. 3 and 4). It is known that the oxon forms of OPs are more toxic than their parent compounds.[6–8] These results highlight the necessity of detecting both forms to trace the presence of OPs and their oxons during the water treatment process.

In conclusions, we established a selective and sensitive method for the quantitative analysis of oxon forms of OPs in water samples using GC/MS with SPE. This method is suitable for the simultaneous detection and determination of OPs and their oxons and allows the tracing their reactions in water. Calibration curves for the oxons showed good linearity. The method yielded low LOD values and reproducible recovery rates for the accurate quantification, simultaneous extraction, and the determination of the concentration of 18 compounds in water. The LOD values of oxons were from 0.5 to 20 ng/ml. The LOQ values ranged from 5 to 400 ng/l in actual water samples. In addition, we found that OPs were rapidly converted to their oxon forms in the presence of chlorine.

Many OPs are highly toxic, and thus it is necessary to detect and control not only the parent compounds but also the oxon forms in the environment and after the water treatment process. Quality con-

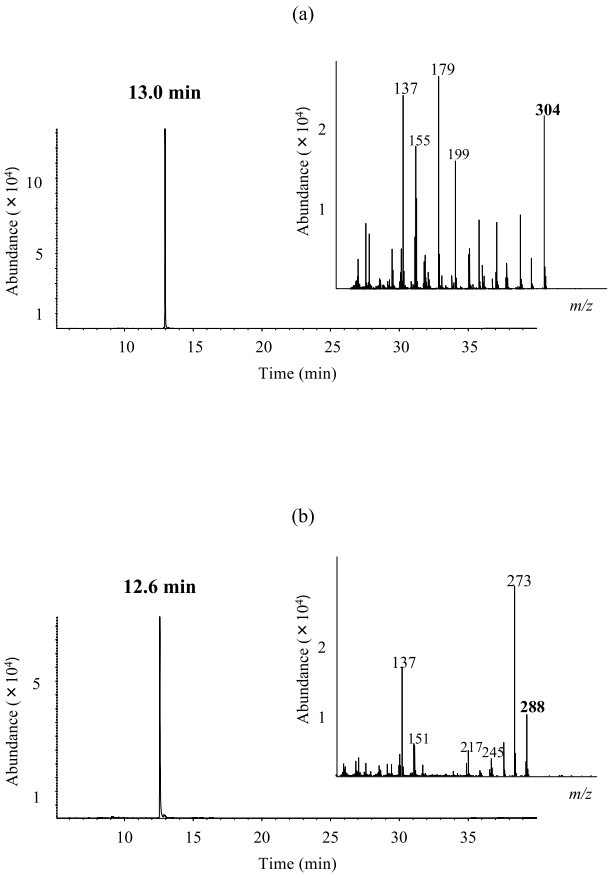

Fig. 3. Chromatogram and MS Spectra of Diazinon after Chlorination
Chlorine exposure time: (a) 0 min and (b) 5 min.

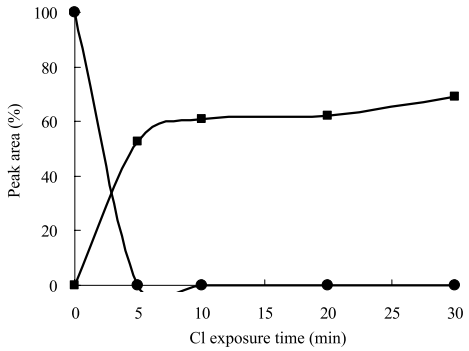

Fig. 4. Behavior of Diazinon and its Oxon after Chlorination
—●—, diazinon; —■—, diazinon xon.

trol of natural water has become an urgent issue. Regulations governing drinking water quality are meant to limit human risk and environmental pollution. However, regulations for OP oxons are not defined and no standard method for their detection has been given in Japan. Our approach can be applied as a screening method for field monitoring of OP parent compounds and oxons. The risk of these compounds to human health and ecosystems can be accurately evaluated using the present method. We believe that our results will be important in enforcing the Water Quality Standard of Japan.

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