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Ozonation Products of Organophosphorous Pesticides in Water

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Primary degradation products of organophosphorous pesticides by ozonation in water and the oxidation pathways of diazinon, fenthion (MPP) and edifenphos (EDDP) were studied by gas chromatography-mass spectrometry. Mass-spectra of ozonation products of the 17 organophosphorous pesticides evaluated suggested that they were oxons. Organophosphorous pesticides were converted to oxons in accordance with production of sulfate ion as their thiophosphorile bonds were oxidized by ozone into phosphorile bonds. Although oxons were stable against ozonation, they were further hydrolyzed into trialkyl phosphate and other hydrolysis products. However, in MPP, thiomethyl radicals were oxidized prior to thiophosphorile bonds and MPP-sulfoxide was produced. MPP-sulfone, MPP-sulfoxide-oxon and MPP-sulfone-oxon were also generated from MPP. Two major oxidation products were obtained from bis-dithio type ethion. EDDP of the phosphate type was resistant to ozonation, but its oxidation products were detected after hydrolysis.

Since the oxons of these organophosphorous pesticides are resistant to ozonation and are toxic, they should be monitored.

Keywords — ozonation product ; organophosphorous pesticide ; oxon ; GC/MS

Introduction

As a result of agricultural chemical surveys of environmental water carried out on the occasion of formulation of the tentative standards governing the use of pesticides on golf courses, trace amounts of organic pesticides were detected in surface water, causing apprehension about the contamination of drinking water. Organophosphorous pesticides are often used in Japan for a wide variety of products and in large amounts, some of which have been detected in river water.¹⁾ These organophosphorous pesticides, even if detected in the source of the water, are known to be undetectable after treatment of the water by chlorination and ozonation.²⁾

Onodera *et al.* reported that the cholinesterase inhibitor activity of organophosphorous pesticides is enhanced as they are converted to oxons by ozonation in water.³⁾

The new standards on drinking water quality set reference values for 4 organophosphorous pesticides, namely, diazinon, isoxathion, EPN and fenitrothion. These pesticides and their oxidation products are expected to be eliminated by treatment with activated carbon^{4,5)} coupled with ozonation in the process of water purification, but monitoring is still needed not only of the organophosphorous pesticides themselves but also their degradation products to ensure the safety of water.

In this context, we identified the major ozonation products of 17 organophosphorous pesticides widely used today, and propose here the degradation pathways of diazinon,

fenthion (MPP) and edifenphos (EDDP).

Methods and Materials

Reagents — Acetone, dichloromethane, anhydrous sodium sulfate, the 17 organophosphorous pesticides shown in Table I, MPP-sulfoxide and MPP-sulfone used were made by Wako Pure Chemical Industries, Ltd. for analysis of residual agricultural chemicals. Triethyl phosphate, thiophenol, diphenyl disulfide and sodium thiosulfate were also purchased from Wako Pure Chemical Industries, Ltd. *N*-paraffins with carbon numbers of 11–26 were products of Sperco Co. Oxons of diazinon, isoxathion, EPN and fenitrothion were purchased from Hayashi Pure Chemical Industries Co., Ltd. 2-Isopropyl-6-hydroxymethylpyrimidine (HOP) standard was provided at no charge by Nihon Kayaku Co., Ltd. Water was prepared by Millipore Labo. Ion Pure-12 and Milli-Q PLUS system, and had a resistivity of 18.2 M Ω .

Apparatus — The ozone generator was a Mitsubishi OS-1N, and the source gas was pure oxygen S from Nippon Sanso Co., Ltd. The gas chromatograph-mass spectrometer (GC/MS) was a HP 5980-5970 with a selective ion detector (SIM) or a II-MS-AUTOMAS150 of the HP5980 Series (DELSI NERMAG). Column was a DB-5 from J&W Scientific, Inc. (internal diameter 0.25 mm, film thickness 0.1 μ m, length 30 m). The splitless injection method was used. The column temperature was maintained initially at 60°C for 1 minute, programmed to rise to 280°C at 10°C/min, and held there at 280°C for 5 min. The injection port temperature of the GC was 220°C, and the transfer line temperature was 280°C. Helium was used as the carrier gas, and the head pressure was adjusted to 7.5 psi. Ionization was made by the electron impact mode at an ionization energy of 70 eV or by the chemical mode, using isobutane as the reactant gas, at an ionization energy of 150 eV.

The ion chromatograph was a Yokogawa IC-500. Analytical conditions were: column, SAM3-125 (4.9 mm $\times$ 125 cm); eluent, 5 mM Na₂CO₃; flow rate, 2.0 ml/min; regenerate solution, 15 mM H₂SO₄; flow rate, 2.0 ml/min. An

electroconductive detector was used, and ozone concentration was measured by the iodometric method.⁶⁾

Screening of Major Degradation Products

— The standard solutions of the organophosphorous pesticides shown in Table I dissolved in acetone (1000 μ g/ml) were placed at 100 μ l each in 200-ml incubation bottles; the bottles were filled with 9.5 μ g/ml ozone water, sealed with stoppers, and allowed to stand in the dark for 30 min. After the reaction, 0.1 g sodium thiosulfate was added prior to extraction of degradation products, 50 ml of the reaction solution was extracted twice with the same volume of dichloromethane, and the extract was concentrated to 0.5 ml in an evaporator. Analyses were made of this sample solution by GC/MS. The programmed temperature retention index (PTRI) was determined by GC/MS after mixing the sample solution with 0.5 ml of standard solutions of *n*-alkans with carbon numbers of 11–26 (in 10 μ g/ml dichloromethane).⁷⁾ In case of diazinon, residual ozone concentration in sample was 6.1 μ g/ml.

Analysis of Reaction Products — The ozonation process and analysis of the degradation products of diazinon, MPP and EDDP were carried out as follows. Ozonation was done in an air-washing bottle, 6.5 cm in internal diameter and 40 cm in height, to which a sampling port was attached. One milliliter of the standard solution of a pesticide in acetone (1000 μ g/ml) was added to 1 liter of water, and the mixture was bubbled from the bottom with ozone at 43 mg/min through a G4 glass frit. A fixed volume of the reaction solution was sampled serially for up to 20–60 minutes after the ozonation, residual ozone was purged with nitrogen gas, and ions were analyzed by ion chromatography. For the analysis of degradation products, 0.1 g of sodium thiosulfate was added to 5 ml of the reaction solution, the reaction solution was extracted once with the same volume of dichloromethane, the extract was concentrated to 0.5 ml after dehydration, and degradation products were analyzed by GC/MS-SIM. Until 6 min after the beginning of the reaction, ozone concentration attained equilibrium at near-

TABLE I. Organophosphorous Pesticides and Their Main Ozonation Products

Original pesticide	M.W.	PTRI	Monitor ion (m/z)	Mass fragments of main products (m/z)			
				PTRI	M ⁺	base	other
1. Phosphorothioates							
salithion	216	1610	216,183	1577	200	185	122,121,78
cyanophos (CYAP)	243	1724	243,109	1637	227	96	109,79,115,119
chlorpyrifos-methyl	321	1845	286,288	1817	305	270	272,109
tolclophos-methyl	300	1855	265,267	1812	284	249	251,109,234
fenitrothion (MEP)	277	1872	277,125	*1832	261	109	244,79,127,245
fenthion (MPP)	278	1931	278,125	2113	278	263	262,109
				2122	294	294	215,109,279,104
				*2192	294	279	125,278,153,109,169
				*2202	310	310	125,136,109,105
chlorpyrifos	349	1949	197,125	1930	333	270	298,272,242,197,109
diazinon	304	1765	179,304	*1739	288	273	137,217,93,109
isoxathion	313	2145	105,177	*2115	297	161	105,77,125,81
2. Phosphorodithioates							
methidathion (DMTP)	302	2042	145, 85	1949	286	85	145,142,109,125,229
phenthoate (PAP)	320	2029	274,246	1935	304	258	125,121,231,109
ethion	384	2232	231,153	2071	352	171	184,109,81
				2150	368	171	322,215,97,109,125
phosmet (PMP)	317	2378	160,301	2260	301	160	109
3. Phosphonothioates							
EPN	323	2372	157,169	*2311	307	141	77,306,169,278,279
cyanofenphos (CYP)	303	2279	157,169	2173	287	141	77,169,286
4. Amidothioates							
isofenphos	(346)	2034	213,121	1959	(330)	229	201,58,271,314,200
butamifos	(333)	2107	286,200	2046	(317)	244	216,287,136

1. PTRI was measured using a 100% methyl silicone column.

2. Numbers in () are those for quasi-molecular ions (QM⁺) estimated by CI-MS.

3. Major degradation products marked with * were identified by comparison with authentic standards.

ly 10 $\mu\text{g/ml}$ in every experiment.

Results and Discussion

Major Degradation Products of Organophosphorous Pesticides

The data shown in Table I were obtained by GC-MS analysis of degeneration products of the 17 organophosphorous pesticides. Two peaks of degeneration products were observed in ethion, and 4 peaks were observed in MPP. The other pesticides produced one major degradation product, and the decrease in molecular weights (16 amu smaller than the original pesticides) of the products suggest the oxidation of thiophosphoryl bond (P=S) into phosphoryl bond (P=O) by electron impact mass spectroscopy (EI-MS),

except for amidothioates. Molecular ions (M⁺) of amidothioates were too weak to be analyzed by EI-MS so that the analysis was made by chemical ionization mass spectroscopy (CI-MS).⁸⁾ The ozonation products of isofenphos and butamifos had quasi-molecular ions (M⁺+1) 16 amu smaller than those of the original pesticides. The major degradation products of isoxathion, fenitrothion, EPN and diazinon were identified as their oxons because of the agreement with their standards in retention times and mass spectra.

In salithion, m/z 201 (M⁺-15) derived by cleavage of the methyl moiety was observed,⁹⁾ but its major degradation product had a fragment ion at m/z 185, suggesting the oxidization of sulfur.

Of the major degradation products of MPP, those which had a fragment ion at m/z 125 derived from the P=S bond were identified with MPP-sulfoxide (PTRI, 2192) and MPP-sulfone (PTRI, 2202) by comparison with their standards. The other products were estimated to be MPP-sulfoxide-oxon (PTRI, 2113) and MPP-sulfone-oxon (PTRI, 2122) from the mass spectral data of photo-degradation products.¹⁰⁾

For phosphorodithioates, Laplanche *et al.* noticed that oxons are not the main products and a competitive reaction occurs between the cycloaddition of ozone on the double bond P=S and the electrophilic attack of oxidant on the second sulfur.¹¹⁾ In this study, however, the obtained main degradation products of methidathion (DMTP), phenthoate (PAP) and phosmet (PMP) were estimated to be oxons from the mass spectral data of photo-degradation products.¹²⁻¹⁴⁾

Of two major degradation products of ethion, in one ($M^+ m/z$ 368) one of the P=S bonds is considered to be oxidized to the P=

O bond, and in the other ($M^+ m/z$ 352) both of its 2 P=S bonds to be oxidized.

For phosphonothioates, the mass-spectra of major degradation products of EPN and cyanofenphos (CYP) were observed to be three common fragment ions derived from $C_2H_5O(C_6H_5)P=O$ (m/z 169) and $C_6H_5(OH) \cdot P=O$ (m/z 141),¹⁵⁾ suggesting oxidation of P=S bond and $M^+ - 1$.

Degradation Pathways of Organophosphorous Pesticides

Degradation of Diazinon by Ozonation

—After 20 min ozonation of $3.3 \mu M$ diazinon in water, diazinon oxon and triethylphosphate were identified by GC-MS based on agreement with the retention times and mass spectra of the standards shown in Fig. 1, and the hydrolysis product HOP was identified by its standard. No oxidized product of the isopropyl group found by ultraviolet-irradiation of diazinon¹⁶⁾ was detected.

We therefore measured the production of diazinon oxon, HOP, and sulfate ion, which are major degradation products of diazinon. The changes in their concentrations are shown in Fig. 2. All three products were generated from 1 min after the beginning of

Fig. 1. Mass Spectra of Ozonation Products Obtained from Diazinon

1. diazinon oxon, 2. triethyl phosphate, 3. 2-isopropyl-6-hydroxymethylpyrimidine (HOP).

Fig. 2. Serial Changes in Ozonation Products of Diazinon in Water

—■— Diazinon, —□— Diazinon oxon, —▲— HOP, —○— Sulfate ion.

the reaction. After 4 min, diazinon was decreased, yielding sulfate ion and $1.6 \mu\text{M}$ of diazinon oxon. Sulfate ion was nearly equivalent in amount to degraded diazinon. Diazinon oxon degraded slowly until 20 min after the beginning of the reaction, but it was considerably resistant to ozonation in water. HOP is considered to be produced from hydrolysis of diazinon oxon, but its production was not expected to increase because it was believed to be degraded as soon as it was generated. In another experiment, triethyl phosphate was confirmed to increase with progression of hydrolysis. Sulfate ion, acetate ion, and formate ion were identified in the same reaction solution by ion chromatography based on the agreement of their retention times with those

of the standards. Production of formic ion was confirmed by ion chromatography after ozonation of aqueous HOP solution, and the degradation pathway shown in Fig. 3 was conceived. However, diazinon oxon is thought to remain in conventional drinking water without being degraded to organic acids upon treatment with a low dose of ozone.

Degradation of MPP by Ozonation

The mass spectra of four ozonation products

Fig. 3. Proposed Degradation Pathways of Diazinon in Water by Ozonation

1. diazinon, 2. diazinon oxon, 3. diethyl phosphate, 4. HOP, 5. acetic acid, 6. formic acid, 7. triethyl phosphate.

Fig. 4. Mass Spectra of Ozonation Products of Fenthion (MPP) in Water and their Serial Changes

Fig. 4a) Mass Spectra of Ozonation Products of MPP in Water

1. MPP-sulfoxide, 2. MPP-sulfone, 3. MPP-sulfoxide-oxon, 4. MPP-sulfone-oxon.

Fig. 4b) Serial Changes in Ozonation Products of MPP in Water

The values of MPP-sulfoxide-oxon and MPP-sulfone-oxon were calculated from the fragment intensities of MPP-sulfoxide and MPP-sulfone

—■— MPP, —○— MPP-sulfoxide, —▲— MPP-sulfone, —△— MPP-sulfoxide-oxon, —□— MPP-sulfone-oxon.

from MPP were obtained, and their serial changes of concentration were analyzed as shown in Fig. 4. MPP-sulfoxide-oxon and MPP-sulfone-oxon, standards of which were not available, were calculated by conversion from the intensity of fragments of MPP-sulfoxide and MPP-sulfone.

MPP ($3.6 \mu\text{M}$) decreased rapidly until 10 s after the beginning of the reaction and disappeared within 5 min. Simultaneously, thiomethyl groups were oxidized quickly, and $2.7 \mu\text{M}$ of MPP-sulfoxide was generated after 10 s. MPP-sulfoxide reached a maximum concentration after 10 s and disappeared 20 min after the beginning of the reaction. MPP-sulfone reached a maximum concentration 1 min after the beginning of the reaction and disappeared after 20 min. MPP-sulfoxide-oxon reached a maximum concentration 5 min after the beginning of the reaction and decreased gradually thereafter. MPP-sulfone-

Fig. 5. Proposed Degradation Pathways of MPP in Water by Ozonation

oxon increased from 1 min after the beginning of the reaction and continued to increase until the end of the experiment. No MPP-oxon was detected. These findings suggest the degradation pathway of MPP as shown in Fig. 5 in which oxidation of thiomethyl groups preceded oxidation of P=S, little MPP-oxon was generated, and MPP-sulfoxide was oxidized into MPP-sulfone. However, as MPP-sulfone and MPP-sulfoxide-oxon, which resulted from oxidation of the P=S bonds, increased with the reduction in MPP-sulfoxide, oxidation to these degradation products is considered to occur simultaneously. Considering the amount of MPP-sulfoxide-oxon production, oxidation of MPP-sulfoxide to oxon-MPP-sulfoxide appears to have priority over oxidation to MPP-sulfone. MPP-sulfoxide-oxon decreased, and MPP-sulfone-oxon increased gradually. MPP-sulfone-oxon is considered to be fairly stable, because it increased even 60 min after the beginning of the reaction. MPP is known to

develop symptoms of poisoning much more slowly than other phosphorothioate type organophosphorous pesticides, and this may be explained by its conversion to sulfoxide and sulfone with P=S before P=S is oxidized to P=O. MPP-sulfone-oxon is thought to be 36 times more toxic than MPP.¹⁷⁾ The cholinesterase inhibiting activity is higher in these oxidized forms of MPP than MPP *per se*, so that analysis of these oxides is needed in the follow-up of MPP behavior in the process of water purification.

Degradation of EDDP by Ozonation

— EDDP used as a germicide is a pre-oxon type, and is reported to be resistant to ozonation,¹⁸⁾ but hydrolysis and oxidation products were generated after treatment with a high level of ozone. EDDP is resistant to ozonation, and part of it remained unchanged, but many degradation products were also confirmed. Among them, the hydrolysis product thiophenol and its oxidation product diphenyl disulfide were identified by comparison with the standards as shown in Fig. 6.

Other unidentified peaks were estimated by mass spectra to be diethylphenylmercaptophosphate, triphenylmercaptophosphate, and S-phenylphenylthiosulfonate with a common fragment ion at m/z 109 derived from C_6H_5S .

From these findings, oxons of organophosphorous pesticides oxidized to phosphates are believed to be resistant to ozonation. Onodera *et al.* reported that oxons in water cannot be degraded by ozonation alone, and that the inhibition of the cholinesterase activity is abolished by ozonation in combination with UV irradiation.³⁾ Highly toxic oxidation products of pesticides are expected to be eliminated when water is treated with activated charcoal coupled with ozonation. In general, however, the efficiency of activated charcoal adsorption is considered to be reduced in oxons, whose polarities are lower than the original pesticides. Therefore, con-

Fig. 6. Ozonation Products of EDDP Identified in Water

1. EDDP, 2. thiophenol, 3. diphenyl disulfide.

centrations of these oxons require monitoring in decontaminating water contaminated by organophosphorous pesticides.

Conclusion

The ozone degradation products and the degradation pathways of 17 organophosphorous pesticides in water were evaluated.

Mass-spectrometry indicated that major degradation products of these pesticides by ozonation were oxons derived by the oxidation of thiophosphorile bonds to phosphorile bonds. These oxons were resistant to ozonation, but they were hydrolyzed into trialkylphosphate and other degradation products.

More than one major degradation product was detected after ozonation of ethion with two thiophosphorile bonds and fenthion with a thioether bond.

Since these oxons were stable against ozonation and show stronger toxicity than their original chemicals, they must also be monitored to ensure the safety of drinking water.

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