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Original Article

Aerobic Aquatic Metabolism of Fenitrothion and Its Oxon Analog in Water-Sediment Systems

Rika KODAKA,* Terumi SUGANO, Toshiyuki KATAGI and Yoshiyuki TAKIMOTO

Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd., 2-1, Takatsukasa 4-chome, Takarazuka, Hyogo 665-8555, Japan

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The aerobic aquatic metabolism of fenitrothion and its oxon was studied in French lake and Japanese pond water-sediment to estimate their behavior in the natural aquatic environment. The effect of aeration on the metabolic profiles of fenitrothion was examined by either passing air over the surface or gently bubbling the water phase. Almost the same metabolic profiles were obtained under both conditions but the partition profiles of radioactivity between the aqueous and sediment phases differed slightly. There was no significant difference in the metabolism of fenitrothion between French and Japanese sediments. Fenitrothion mainly underwent cleavage of the P-O-aryl linkage to form the corresponding phenol and reduction of the nitro group followed by the subsequent acetylation of the amino group. The oxidative desulfuration of fenitrothion to form the oxon was a minor route. The increase in phenol and $^{14}CO_2$ with less reductive transformation under the bubbling of water with air was likely to originate at least in part from less anaerobicity. The oxon derivative of fenitrothion rapidly underwent cleavage of the P-O-aryl linkage to form the phenol in the water-sediment system.

Key words: biodegradation, fenitrothion, water sediment system, reduction, nitro group, ester hydrolysis, oxidative desulfuration.

INTRODUCTION

After a pesticide is applied to a field it partly enters the aquatic environment mainly *via* spray drift and/or run-off along with transformation. Once in the aquatic environment, a pesticide is considered to be first partitioned among an aqueous phase, various kinds of biota, and a non-biological phase such as dissolved organic matter and sediment before undergoing biotic and abiotic transformation. Among natural aquatic systems, ponds, swamps and lakes are considered characteristic of both a small mass of water with little exchange of water and an enriched population of aquatic species with a large amount of dissolved organic matter. For ecotoxicological risk assessment in these environments, water-sediment systems have been utilized as a promising model of the behavior and fate of a pesticide.[1)]

The physico-chemical properties and individual transformation processes of organophosphorus pesticides widely applied in the field have been investigated previously.[2)] Most of these agents are soluble in water at a ppm level[3,4)] have moderate hydrophobicity,[5)] and are susceptible to abiotic hydrolysis as well as biological metabolism. Therefore, they are considered to be degraded into many kinds of metabolites during a rather slow partition process in the water-sediment system.

In order to assess the design of a water-sediment system and clarify the complex behavior anticipated for organophosphorus pesticides, studies of aerobic aquatic metabolism have been performed.[1,6)] Based on these studies, an experimental design for a water-sediment system has been recently proposed in the SETAC guideline.[7)] There are two methods of application, that is, the water- and sediment-spiked methods assuming a different path of entry into the system and the aeration method such as the air-bubbling of an aqueous phase or air-flow over the water surface.[8,9)]

We first examined the impact of the aeration method on the behavior of a pesticide water-spiked in the French and Japanese water-sediment systems by using fenitrothion (**I**) [*O,O*-dimethyl *O*-(3-methyl-4-nitrophenyl) phosphorothioate] as a model compound. Furthermore, the oxon derivative of **I** was also examined as a potential degradate of **I** in the aquatic environment.

* To whom correspondence should be addressed.

MATERIALS AND METHODS

1. Chemicals

Fenitrothion (**I**) [*O,O*-dimethyl *O*-(3-methyl-4-nitrophenyl) phosphorothioate][10] and its oxon derivative (**II**) [*O,O*-dimethyl *O*-(3-methyl-4-nitrophenyl) phosphate] uniformly labeled with ^{14}C at the phenyl ring were synthesized in our laboratory. **II** was synthesized by treating **I** with a 1.1 molar equivalent of *m*-chloroperbenzoic acid in dichloromethane at room temperature as reported previously.[11] **I** and **II** were purified by thin-layer chromatography (Silica gel $60F_{254}$ thin-layer plates; 20×20 cm, 0.25-mm layer thickness, E. Merck) in toluene/ethyl acetate (4/1, v/v; Rf=0.57 and 0.20, respectively). The specific activity and radiochemical purity were 6.66 MBq/mg and 97.9% for **I**, and 7.81 MBq/mg and 98.1% for **II**, respectively. The non-labeled **I** and **II** and the following potential degradates were also synthesized in our laboratory according to the reported method[12]; *O*-(4-amino-3-methylphenyl) *O,O*-dimethyl phosphorothioate (**III**) and *O*-(4-acetylamino-3-methylphenyl) *O,O*-dimethyl phosphorothioate (**IV**). The chemical purity of each compound was determined to be >95% by high-performance liquid chromatography. The corresponding phenol, 3-methyl-4-nitrophenol (**V**), was purchased from Tokyo Kasei Kogyo Co. Ltd. (Tokyo, Japan) and used without further purification.

2. Radioassay

The radioactivity in the water layer, extracts of water and sediment, and trapping media was individually measured by mixing an aliquot of each liquid sample with 10 ml of Packard Emulsifier-Scintillator Plus® and quantified by liquid scintillating counting (LSC) with a Packard model 2000CA liquid scintillation analyzer. The unextractable sediment residues were powdered after drying in a vacuum desiccator and the aliquots were subjected to combustion analysis using a Packard model 307 sample oxidizer. The $^{14}CO_2$ produced was absorbed into 9 ml of Packard Carb-CO_2® adsorber, which was then mixed with 15 ml of Packard Permafluor® oxidizer scintillator, and the radioactivity was quantified by LSC. The radiocarbon in ethylene glycol was determined as follows: a 1-ml aliquot was mixed well with 1.5 ml of methyl alcohol followed by 10 ml of Emulsifier Scintillator Plus® (Packard) in a low potassium glass vial in duplicate and subjected to radioanalysis. The 1-ml aliquot of 0.5 M NaOH solution in the alkaline trap was first radioassayed in duplicate. Carbonate ions in the alkaline solution including any due to $^{14}CO_2$ were precipitated by addition of 10 ml of $BaCl_2$ aqueous solution (2 mol/l) to 30 ml of the alkaline solution. The 1-ml aliquot of the supernatant fraction separated by centrifugation was radioassayed in duplicate to quantify the soluble ^{14}C species. The amount of $^{14}CO_2$ was determined by subtraction of the radioactivity in the supernatant fraction from that in the alkaline solution.

3. High-Performance Liquid Chromatography

The extracts from water and sediment were analyzed by reversed phase high-performance liquid chromatography (HPLC) for preparative and analytical purposes. A Hitach L-6200 liquid chromatograph equipped with a Sumipax ODS A-212 column (5 μm, 6-mm i.d.×15 cm, Sumika Analytical Service, Ltd., Osaka) was operated at a flow rate of 1 ml min^{-1}. The composition of the mobile phase was changed stepwise as follows: 0 min, % A (acetonitrile)-%B (0.01% trifluoroacetic acid), 10: 90; 0–10 min, linear, 35: 65 at 10 min; 10–30 min, linear, 50: 50 at 30 min; 30–55 min, linear, 90: 10 at 55 min. The UV absorbance at 254 nm was monitored with a Hitachi model L-4000 UV detector. The radioactivity of the column effluent was monitored with a Packard Flow-one/Beta A-100 radio detector equipped with a 500-μl liquid cell using Ultima-Flo AP® (Packard) as a scintillator. Each ^{14}C peak was identified in HPLC co-chromatography by comparing its retention time with those of non-radiolabeled authentic standards detected by the UV detector. Typical retention times of **I**, **II**, **III**, **IV** and **V** were 46.6, 27.7, 23, 27.3 and 25.2 min, respectively.

4. Metabolism Experiments

French lake (Aire de Pique Nique Lake Haut Languedoc, France) and Japanese pond (Tondabayashi City, Osaka Prefecture) sediments and the corresponding surface water were used in this study. The sediment and water were passed through 2-mm and 250-μm sieves prior to use, respectively, to remove stones and plant debris. Their physical and chemical properties are listed in Table 1. A sediment sample equivalent to 49 g (French lake)

Table 1 Characteristics of sediment and the associated water.

Location	French Lake	Japanese Pond
Sediment		
Soil texture (%)		
Sand	93.0	63.7
Silt	4.9	17.1
Clay	2.1	19.2
Soil classification	sand	Sandy clay loam
Organic carbon (%)	1.6	1.3
Cation exchange capacity (meq/100g dry sediment)	9.2	8.1
pH (H_2O)	5.8	5.3
Biomass (μg C/g)	30.0	n.a.[a]
Associated water		
DOC (mg/l)	11.6	n.a.
pH	6.57	6.40

[a] not analyzed.

or 30 g (Japanese pond) on a dry-weight basis was added to a two-necked cylindrical glass vessel (5-cm diameter) to a depth of 2.5 cm. The associated water was added to the vessel to a depth of 6 cm above the sediment in accordance with the BBA guideline.[9] The water-sediment system was then pre-incubated in darkness at 20±1°C for 50 days.

The rate of application of **I** or **II** to each system was adjusted to 39 μg/vessel taking into account the surface area of water in the vessel (19.6 cm^2) at an application rate of 1000 g ha^{-1}, assuming that **I** or **II** uniformly distributes in the water phase at a depth of 30 cm. After pre-incubation, the 95-μl acetonitrile solution of **I** or **II** was fortified dropwise to the water surface using a microsyringe. Each vessel containing sediment and water was placed in an incubator and kept at 20±1°C in darkness. CO_2-free air was passed through the vessel in sequence to one gas washing bottle containing 300 ml of ethylene glycol and one containing 350 ml of 0.5 M NaOH solution to trap the volatile ^{14}C.

The effect of aeration on the distribution and metabolism of ^{14}C was first examined for **I** by using the French lake water and sediment. Pre-moistened carbon dioxide-free air was passed moderately over the surface of the water in System-1 and the water phase was alternatively bubbled with a gentle flow of air not causing a disturbance of the sediment (System-2). Second, the effect of water-sediment characteristics was examined for **I** using the same aeration method as in System-1 but with the Japanese water-sediment system (System-3). Furthermore, the behavior of **II** was also examined under the same conditions as in System-1 (System-4).

At appropriate intervals, the surface water was separated from the sediment by decantation and passed through a glass filter (ϕ60 m/m, Kiriyama) under suction. The water combined with filtrate from the sediment was then extracted by partitioning three times with 80 ml of ethyl acetate. The organic layer was evaporated to dryness *in vacuo* for further analysis. The sediment was filtered under suction through the same glass filter used for filtration of the water. The sediment was transferred to a centrifugal bottle and extracted with 60 ml of ethyl acetate and 30 ml of 0.2 M HCl by mechanical shaking for 10 min. After shaking, centrifugation was conducted for 10 min at 5000 rpm and 4°C. The extraction was repeated twice in the same manner as above. The organic layer was evaporated to dryness *in vacuo* for further analysis. The sediment residue was dried in a vacuum dessiccator and approximately 100 mg was combusted in duplicate using the sample oxidizer prior to LSC to quantify the bound ^{14}C. The volatile radioactivity in the ethylene glycol and alkaline traps was measured by radioassay. The possible adsorption of ^{14}C onto the inner surface of the glass vessel was examined by rinsing the surface with 100 ml of methanol and analyzing a 10-ml aliquot of rinsate by LSC.

The concentrated extract from either the water or sediment phase was subjected to HPLC co-chromatography with the non-radiolabeled reference standards to quantify **I, II** and metabolites together with chemical identification of each peak. The degradation rate constant, half-life (DT_{50}) and period of 90%-degradation (DT_{90}) of **I** and **II** were calculated with Microsoft Excel 2000 by the least-square approximation method, assuming that the degradation followed first-order kinetics.

RESULTS

1. Effect of Aeration Method

The distribution of radioactivity in the French water-sediment system depending on the method of aeration is summarized in Table 2. Throughout the experiment, the total amount of ^{14}C recovered from the systems ranged from 92.8% to 107.1% with the ^{14}C adsorbed onto the glass vessel amounting to less than 1.2% of the applied amount. These results showed that almost all of the radiocarbon distributed only between the water and sediment phases. Upon application of **I** to the aqueous phase, the radioactivity gradually decreased with incubation irrespective of the aeration method, but a slightly faster partition of ^{14}C to the sediment was observed under air-bubbling (System-2). After 31-day incubation, 72.4% and 65.7% of the applied ^{14}C was partitioned to the sediment in System-1 and 2, respectively, and most was found to be bound to the sediment. More carbon dioxide, amounting to 6.9% after 31 days, was detected in System-2 but the formation of other volatiles was insignificant.

In System-1, **I** in the aqueous phase rapidly decreased with a DT_{50} value of 4.4 days and was undetectable at 31 days, while in the sediment, **I** gradually increased to a maximum value of 21.0% at day-7 and then decreased to 2.4%. The major metabolites detected in the water phase were **III, IV** and **V**, each amounting to less than 4.2% (day-14), 5.9% (day-14) and 8.1% (day-7), respectively. These metabolites were also detected in the sediment but in greater amounts especially for **III** (11.8% at day-14). Throughout the study, **II** amounted to less than 1% in total and hence, the oxidative desulfuration of **I** was of minor importance in the tested water-sediment system.

Under air-bubbling (System-2), a smaller DT_{50} value (2.8 days) was obtained for **I** in the aqueous phase. Almost the same metabolic profiles of **I** were observed but with the relative amount of each metabolite being different from that in System-1. Less **III** (0.7%) and **IV** (<2.4%) were detected in the aqueous phase but more **V** (<13.9%). The maximum partition of **I** to the sediment phase was observed after 3 days (37.8%) with a gradual decrease to 3.3% at day-31.

The initial redox potentials of the aqueous and sedi-

Table 2 Effect on **I** of aeration method in the French water-sediment system.

	Percentage of applied ^{14}C (Days after application)									
	0		3		7		14		31	
	A[a]	B[b]	A	B	A	B	A	B	A	B
Volatile	n.a.[c]	n.a.	n.d.	0.2	0.2	0.8	0.4	2.0	2.1	6.9
Ethylene glycol	n.a.	n.a.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
NaOH soln.($^{14}CO_2$)	n.a.	n.a.	n.d.	0.2	0.2	0.8	0.4	2.0	2.1	6.9
Aqueous	97.8	97.8	78.7	46.4	48.2	34.0	32.1	27.7	31.4	24.0
I	97.8	97.8	74.1	35.7	33.4	17.3	4.4	0.5	n.d.	n.d.
II	n.d.[d]	n.d.	0.5	0.3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
III	n.d.	n.d.	n.d.	n.d.	n.d.	0.7	4.2	0.4	0.7	0.3
IV	n.d.	n.d.	n.d.	n.d.	1.1	1.2	5.9	1.8	1.2	2.4
V	n.d.	n.d.	2.1	8.0	8.1	10.9	4.1	13.9	0.9	2.4
others	n.d.	n.d.	2.0	2.4	5.6	3.9	13.7	11.1	28.6	18.9
Sediment	1.3	1.3	19.8	54.2	45.2	63.0	59.5	62.3	72.4	65.7
Extractable ^{14}C	1.2	1.2	18.6	49.1	33.6	47.3	30.5	26.1	17.2	17.0
I	1.2	1.2	15.1	37.8	21.0	29.6	6.3	6.2	2.4	3.3
II	n.d.	n.d.	0.2	0.3	0.3	0.3	n.d.	0.4	0.3	0.5
III	n.d.	n.d.	n.d.	n.d.	1.0	3.0	11.8	4.3	5.5	3.5
IV	n.d.	n.d.	0.1	0.1	0.1	0.6	2.6	0.8	1.5	1.7
V	n.d.	n.d.	1.4	8.5	9.7	11.8	4.7	10.8	2.0	4.8
others	n.d.	n.d.	1.8	2.4	1.5	2.0	5.1	3.6	5.5	3.2
Bound ^{14}C	0.1	0.1	1.2	5.1	11.6	15.7	29.0	36.2	55.2	48.7
Rinse	0.4	0.4	0.8	0.7	0.8	0.4	1.1	0.8	1.2	0.3
Total ^{14}C	99.5	99.5	99.3	101.5	94.4	98.2	93.1	92.8	107.1	96.9

[a] System-1 (no bubbling). [b] System-2 (air bubbling). [c] not analyzed. [d] not detected.

ment phase were 220 mV and 150 mV, respectively. After the application, this decreased by *ca.* 70 mV in the aqueous phase of System-1 but remained almost constant in System-2. In contrast, almost a constant redox potential was observed for the sediment layer of System-1 though an increase of 70–120 mV was seen in System-2. These results showed that air-bubbling effectively maintained aerobicity in the system. The air-bubbling did not significantly change the formation profiles of each metabolite in the sediment but more **V** was formed as in the aqueous phase. **II** was also a trace metabolite in the sediment.

2. *Effect of Water-Sediment Characteristics*

The distribution of ^{14}C and **I** in System-3 (non-bubbling) is summarized in Table 3. Although the characteristics of sediment between the two water-sediment systems were very different, almost the same partitions and metabolic profiles were observed. The DT_{50} value of **I** in the aqueous phase was estimated to be 4.3 days and the maximum partition of **I** to sediment (23.7%) was detected after 7 days. The same three major metabolites (**III, IV** and **V**) with a trace amount of **II** were also formed in the Japanese pond system but in slightly different amounts. Less **III** as compared with **IV** was detected in the Japanese pond system.

3. *Degradation of II*

A similar profile of the distribution of ^{14}C was observed for **II** except for a more rapid dissipation in the aqueous phase with a DT_{50} value of 2.4 days, as summarized in Table 4. Slightly more carbon dioxide was also

Table 3 Degradation of **I** in Japanese pond water-sediment (System-3).

	Percentage of applied ^{14}C (Days after application)				
	0	1	7	21	31
Volatile	n.a.[a]	n.d.	0.3	2.0	5.6
Ethylene glycol	n.a.	n.d.	n.d.	n.d.	n.d.
NaOH soln. ($^{14}CO_2$)	n.a.	n.d.	0.3	2.0	5.6
Aqueous	97.8	92.5	55.0	51.2	19.9
I	97.8	87.6	41.0	10.8	0.4
II	n.d.[b]	0.5	0.4	n.d.	n.d.
III	n.d.	n.d.	0.7	7.4	0.9
IV	n.d.	n.d.	1.3	2.9	1.2
V	n.d.	3.3	8.8	6.5	0.9
others	n.d.	1.1	2.9	23.6	16.5
Sediment	1.2	8.4	40.2	43.1	62.6
Extractable ^{14}C	1.2	8.1	29.8	11.4	7.6
I	1.2	7.6	23.7	2.0	0.6
II	n.d.	n.d.	n.d.	n.d.	n.d.
III	n.d.	n.d.	n.d.	3.9	1.4
IV	n.d.	n.d.	1.0	0.8	0.2
V	n.d.	0.5	4.5	0.9	0.3
others	n.d.	n.d.	0.6	3.8	5.1
Bound ^{14}C	0.1	0.3	10.4	31.7	55.0
Rinse	0.4	0.9	1.2	1.9	0.2
Total ^{14}C	99.5	101.8	96.7	98.2	88.3

[a] not analyzed. [b] not detected.

found. The HPLC of extracts from the aqueous and sediment showed that **V** was the only metabolite in both phases. In the aqueous phase, the amount of **V** increased to 32.6% of the applied ^{14}C after 8 days but gradually decreased thereafter. In the sediment, most of the extractable ^{14}C was found to consist of **V** and the amount of **II** was less than 2.9% throughout the study.

DISCUSSION

After application to the aqueous phase, both compounds were degraded in each system with DT_{50} values

ranging from 2.5 to 7.9 days as listed in Table 5. The DT_{90} values of **I** were estimated to be 16.4–26.3 days, indicating that most of **I** in a biologically available state would disappear from the water-sediment system within a month and most of **II** in a much shorter period.

Table 4 Degradation of **II** in the French lake water-sediment system.

	Percentage of applied ^{14}C (Days after application)					
	0	1	3	8	15	30
Volatile	n.a. [a]	n.d.	0.3	0.6	1.9	7.0
Ethylene glycol	n.a.	n.d.	n.d.	n.d.	n.d.	n.d.
NaOH soln. ($^{14}CO_2$)	n.a.	n.d.	0.3	0.6	1.9	7.0
Aqueous	101.8	96.6	70.6	52.1	28.5	22.6
II	101.2	91.1	39.3	11.8	n.d.	n.d.
V	0.3	4.4	30.8	32.6	17.5	2.7
others	0.3	1.1	0.5	7.8	10.9	19.9
Sediment	2.9	5.8	31.8	50.2	67.7	70.9
Extractable ^{14}C	2.9	4.7	24.8	24.5	26.3	13.0
II	2.9	1.4	2.2	0.9	0.2	0.2
V	n.d. [b]	3.3	22.5	18.0	17.8	5.8
others	n.d.	n.d.	0.1	5.7	8.3	7.1
Bound ^{14}C	<0.1	1.1	7.0	25.7	41.5	57.9
Total ^{14}C	104.7	102.4	102.8	103.0	98.1	100.5

[a] not analyzed. [b] not detected.

Table 5 DT_{50} and DT_{90} values in the water sediment systems.

	I						II	
	System-1 [a]		System-2 [b]		System-3 [c]		System-4 [a]	
	water layer	total system	water layer	total system	water layer	total system	water layer	total system
DT_{50}	4.4	7.9	2.8	6.5	4.3	4.9	2.4	2.5
DT_{90}	14.8	26.3	9.4	21.5	14.2	16.3	8.0	8.2
r^2 [d]	0.964	0.925	0.969	0.999	0.927	0.945	0.933	0.938

[a] French lake system, no bubbling. [b] French lake system, bubbling. [c] Japanese pond system, no bubbling. [d] coefficient of correlation.

Degradation pathways of **I** and **II** in the systems tested are proposed in Fig. 1 based on the products identified. One of the major pathways for **I** was cleavage of the P-O-aryl linkage to form **V** possibly *via* both biotic and abiotic processes as known for organophosphorus pesticides.[13–15] The phenol formed was bound to the sediment and mineralized finally to carbon dioxide. Another pathway was reduction of the nitro group to form **III** which was successively acetylated to **IV**. This pathway has been reported under anaerobic conditions[16,17] and is most likely due to microbial metabolism[16,18]. The corresponding reduction of **V** was not observed in this study, possibly due to a faster degradation to smaller molecular fragments and more rigid association with the sediment than in the reductive degradation. The oxidative desulfuration of **I** to **II** was of minor importance, indicating less aerobicity of the system under the tested aeration conditions. More **V** and $^{14}CO_2$ with less **III** and **IV** was observed in System-2, than System-1. These differences were considered to originate at least in part from the extent of anaerobicity of the system. To quantitatively examine the effect of air-bubbling on each degradation pathway of **I**, the degradation profile of **I** in the total system was analyzed by a compartment model as shown in Fig. 2. "Others" means carbon dioxide and the remaining minor metabolites. To simplify the model, a single compartment was assumed for **III** and **IV**. The rate constant of each pathway was estimated by using a ModelMaker program (version 2.0c, SB Technology), as listed in Table 6. The larger k_1 and smaller k_2 values in System-2, each representing the hydrolytic and reductive pathways, clearly demonstrated that the latter pathway was retarded

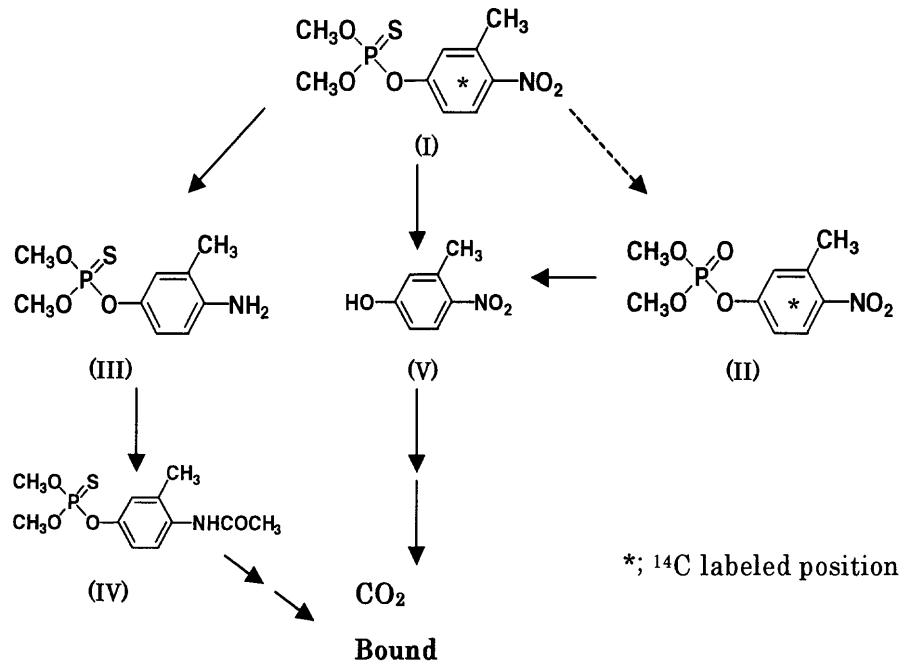

Fig. 1 Proposed degradation pathways for **I** and **II** in water-sediment systems.

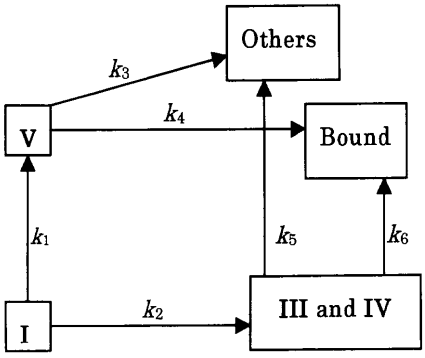

Fig. 2 Compartment model used for kinetic analysis of degradation of **I**.

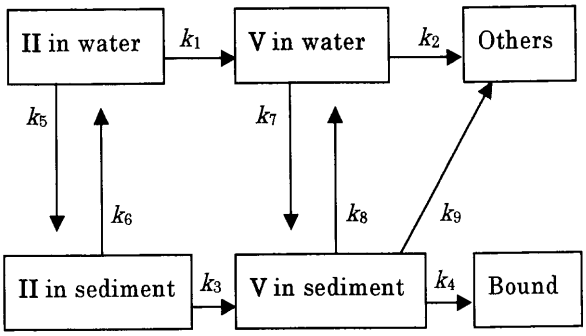

Fig. 3 Compartment model used for kinetic analysis of degradation of **II**.

Table 6 Kinetics analysis of degradation of **I**.

Rate constant (day^{-1})	System-1 [a]	System-2 [b]
k_1	0.069	0.107
k_2	0.028	0.011
k_3	0.126	0.044
k_4	0.136	0.122
k_5	0.003	0.048
k_6	0.050	10×10^{-13}
r^2 [c]	0.888	0.912

[a] French lake system, no bubbling.
[b] French lake system, bubbling.
[c] coefficient of correlation.

Table 7 Kinetic analysis of degradation of **II**.

Rate constant (day^{-1})	
k_1	4.17×10^{-5}
k_2	0.029
k_3	4.43
k_4	0.154
k_5	0.260
k_6	6.13×10^{-6}
k_7	3.503
k_8	5.104
k_9	0.046
r^2 [a]	0.957

[a] coefficient of correlation.

under the reduced anaerobicity caused by air bubbling. A comparison of the k_3 values showed that the further degradation of **V** would more easily occur under more anaerobic conditions (System-1). The k_5 and k_6 values seem to show that the bound process was favored over the biodegradation of **III** and **IV** under the more anaerobic conditions.

It has been reported that the major product in hydrolysis at pH 7, close to the pH value of the water in this study, is the demethylated derivative of **I**.[19,20] Under aerobic soil conditions, **V** is the major metabolite, while under submerged conditions with a anaerobic atmosphere, **III, IV** and **V** predominate.[16] The demethylated derivative, **III** and **V** have been identified as metabolites in natural pond, running water and/or laboratory microcosms (aerobic atmosphere) with a trace amount of **II**.[21-24] Furthermore, the abiotic hydrolysis of **I** at neutral pH appeared to be a very slow reaction with a half-life of 180-186 days. In this study, **I** was not detected in the aqueous phase after 31 days due to rapid partition from water to sediment. This short existence in the aqueous phase resulted in an insignificant contribution of abiotic hydrolysis to the formation of the demethylated derivative, and hence, a resemblance of metabolic profiles to those for soil metabolism under the submerged conditions. In System-3, a similar pattern of degradation of **I** to that in System-1 was observed but with relatively different amounts of metabolites. Based on these results, the aeration method was considered to have a significant effect on the partition of **I** in the system and aerobicity rather than the type of sediment would have an effect on metabolic profiles.

The behavior of **II** was analyzed in more detail by assuming the compartments shown in Fig. 3, and each rate constant was estimated by the ModelMaker program as listed in Table 7. The k_5 (k_7) and k_6 (k_8) values represent the rate constants of adsorption from water to sediment of **II** (**V**) and desorption, respectively. The more hydrophobic **II** was easily partitioned to the sediment, while the hydrophilic **V** was easily desorbed to the aqueous layer. The k_5/k_6 and k_7/k_8 ratios are considered to correspond to the partition coefficient of **II** and **V** for the tested sediment and were calculated to be 4.24×10^4 and 0.686, respectively. The Koc values were estimated to be 236 (**II**) and 9 (**V**) by HPLC with a CN column (Waters), in methanol/0.01 M phosphate buffer pH7 (55/45, v/v)[25]. The larger Koc value for **II** than **V** was explained by this measurement. The Koc values in this study represent those in non-equilibrium and **V** would be considerably dissociated judging from its pKa value (7.33)[26], which might result in the difference of Koc values between the two methods. The larger k_3 value in Table 7 than the k_1 values in Table 6 shows the hydrolytic susceptibility of **II** is much greater than that of

I, revealing the small amount of **II** in the sediment phase even though **II** was rapidly partitioned to the sediment. The greater (8.9-fold) hydrolytic susceptibility of methyl paraoxon than methyl parathion[27] supports the profiles of **II** in the water-sediment system. **II** is known to be an essential compound exhibiting insecticidal activity[28] and has been reported to be produced not only by microbial metabolism but also via photodegradation in water, on soil and on plant surfaces.[16,19,20,29] Therefore, the possible contamination of the aquatic environment can be postulated by photodegradation in the surface water after spray-drift of **I** or run-off of **II** after the washing of soil and plant surfaces by rain. However, our study clearly indicated that **II** was unlikely to persist in the water-sediment phase even in the worst case scenario.

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要　　約

フェニトロチオンおよびそのオキソン体の水-底質系における代謝分解

小高理香，菅野輝美，片木敏行，瀧本善之

フェニトロチオン及びそのオキソン体のフランスの湖水及び日本の池水-底質系での好気的な代謝試験を行った．系への通気方法がフェニトロチオンの代謝経路に与える影響を見るために，水層中に穏やかに通気するかまたは水表面上に空気を流す方法で実験を行った．いずれの方法においても代謝経路はほぼ同じであったが水-底質間の放射能分布に若干の違いが認められた．水中通気による嫌気雰囲気の減少から，フェノール体と二酸化炭素の生成量が増加し還元的代謝が減少した．フランスと日本の水-底質系でのフェニトロチオンの代謝に顕著な差異は認められず主にP-O-アリール結合の開裂によるフェノール生成，ニトロ基のアミノ基への還元とそれに続くアセチル化により分解された．オキソン体の生成（<1%）は主要な分解経路ではなく，速やかにP-O-アリール結合が開裂しフェノール体に分解された．