J. Pestic. Sci. **28**, 175–182 (2003)

Original Article

Comparative Metabolism of Organophosphorus Pesticides 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

(Received August 30, 2002; Accepted December 16, 2002)

The aerobic aquatic soil metabolism of three ^{14}C-labeled organophosphorus pesticides was examined in French lake water-sediment systems to estimate factors controlling their behavior in the natural aquatic environment. The more hydrophobic tolclofos-methyl and butamifos rapidly distributed between the aqueous and sediment phases even in the early stage of incubation, while partition of cyanophos to the sediment was found to be more gradual. The three pesticides were degraded in the water-sediment systems with half-lives of 8.8 to 24.5 days. Tolclofos-methyl and cyanophos commonly underwent cleavage of the P-O-methyl and P-O-aryl linkages the latter with a stepwise hydration of the cyano group, while butamifos was degraded to many unknown compounds each with less than 1.4% of the applied radioactivity. The evaporative loss of tolclofos-methyl and butamifos possibly due to azeotropic co-distillation was observed. The behavior of pesticides in the water-sediment system was considered to be mainly controlled by the balance among partition, degradation and evaporative processes, at least in part due to their chemical structures and physico-chemical properties.

Key words: biodegradation of organophosphorus pesticides, water-sediment system, ester hydrolysis, oxidative desulfuration.

INTRODUCTION

After a pesticide is applied in the field, it is considered to finally distribute in the aquatic environment *via* spray-drift and run-off events dependent on the type of formulation and application method. The aquatic environment mainly consists of water bodies, suspended matter, underlying sediment and various kinds of biota and hence, these components complicatedly affect the behavior of a pesticide.[1] Among the factors that control the behavior of a pesticide, partition and transformation processes are among the most important along with the pesticides own physicochemical properties. In order to investigate the environmental profiles of a pesticide in such an aquatic environment, a lab-scale water-sediment study was proposed[1] as a model system. Incidentally, organophosphorus pesticides have been widely utilized in the field and their physico-chemical properties and individual transformation processes have been investigated.[2] Most of them are soluble in water[3,4] with moderate hydrophobicity[5] and susceptible to abiotic hydrolysis as well as biological metabolism.

Taking account of these characteristics, we have recently examined the dominant experimental conditions controlling the behavior of a pesticide using fenitrotion-spiked water in French and Japanese water-sediment systems.[6] Through this study, the partition and transformation processes were found to be rather insensitive to the water-sediment type but the aerobicity of the system greatly affected the metabolic profiles. The objective of the present study is to examine the effects of chemical structure and accompanying physico-chemical properties in a non-contaminated French lake water-sediment system where the behavior of fenitrothion has been thoroughly examined[6] by using the following three organophosphorus pesticides as model compounds; tolclofos-methyl (**I**) [*O,O*-dimethyl *O*-(2,6-dichloro-4-methylphenyl) phosphorothioate], cyanophos [*O,O*-dimethyl *O*-(4-cyanophenyl) phosphorothioate] (**II**) and butamifos [*O*-ethyl *O*-(5-methyl-2-nitrophenyl) *sec*-buthylphosphoramidothioate] (**III**).

MATERIALS AND METHODS

1. Chemicals

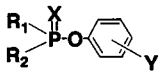
The chemical structures of **I**–**III** are listed in Table 1. **I**–**III** uniformly labeled with ^{14}C at the phenyl ring were synthesized in our laboratory from the corresponding ^{14}C-labeled phenol according to reported methods.[7,8] **I**–**III** were

* To whom correspondence should be addressed.

Table 1. Molecular formulae of pesticides and their possible degradation products

Compound[a]	R_1	R_2	X	Y	Rt (min)[b]
I	CH_3O	CH_3O	S	2,6-Cl_2-4-CH_3	66.3
Ia	CH_3O	CH_3O	O	2,6-Cl_2-4-CH_3	40.4
Ib	CH_3O	HO	S	2,6-Cl_2-4-CH_3	22.9
Ic	CH_3O	HO	O	2,6-Cl_2-4-CH_3	19.5
Id	-	-	-	2,6-Cl_2-4-CH_3	36.1
II	CH_3O	CH_3O	S	4-CN	33.0
IIa	CH_3O	CH_3O	O	4-CN	25.6
IIb	CH_3O	HO	S	4-CN	21.0
IIc	CH_3O	HO	O	4-CN	13.5
IId	CH_3O	CH_3O	S	4-$CONH_2$	27.0
IIe	CH_3O	CH_3O	S	4-COOH	30.0
IIf	-	-	-	4-CN	23.0
IIg	-	-	-	4-$CONH_2$	8.0
IIh	-	-	-	4-COOH	19.0
III	C_2H_5O	*iso*BuNH	S	2-NO_2-5-CH_3	38.5
IIIa	C_2H_5O	*iso*BuNH	O	2-NO_2-5-CH_3	32.2
IIIb	C_2H_5O	*iso*BuNH	S	2-NH_2-5-CH_3	34.8
IIIc	C_2H_5O	*iso*BuNH	S	2-$NHCOCH_3$-5-CH_3	35.6
IIId	C_2H_5O	*iso*BuNH	S	2-NHCHO-5-CH_3	34.8
IIIe	-	-	-	2-NO_2-5-CH_3	32.5

[a] The basic chemical structure is shown below.



Compounds **Id**, **IIf** - **IIh** and **IIIe** are Y-substituted phenols.

[b] Typical HPLC retention time.

purified prior to application by silica gel thin-layer chromatography ($60F_{254}$; 20×20 cm, 0.25-mm layer thickness, E. Merck) in solvent systems of toluene/acetic acid (7/1, v/v; Rf=0.57) for **I**, toluene/ethyl formate/formate (5/7/1, v/v/v; Rf=0.59) for **II**, and *n*-hexane/acetone (4/1, v/v; Rf=0.25) for **III**. The specific activities and radiochemical purities were 8.1 MBq/mg and 99.1% (**I**), 4.6 MBq/mg and 98.1% (**II**), and 3.3 MBq/mg and 99.1% (**III**). The non-labeled pesticides and their potential metabolites as listed in Table 1 were also synthesized in our laboratory according to reported methods.[9-12] The chemical purity of each standard was determined to be >95% by high-performance liquid chromatography. The corresponding phenols, *p*-cyanophenol (**IIf**) and 5-methyl-2-nitrophenol (**IIIe**), and *p*-hydroxybenzoic acid (**IIh**) were purchased from Tokyo Kasei Co., Ltd. (Tokyo, Japan) and Wako Pure Chem. Ind., Ltd. (Osaka, Japan), respectively, 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 liquid scintillation counting (LSC) with a Packard model 2000 CA liquid scintillation analyzer. The unextractable sediment residue was powdered after drying in a vacuum desiccator and a portion subjected to combustion analysis using a Packard model 307 sample oxidizer. Details of these measurements have previously been reported.[6]

The radiocarbon in polyurethane foam plugs was soaked in 5-ml of methanol and a 1-ml aliquot was quantified by LSC in duplicate.

3. *High-Performance Liquid Chromatography*

The extracts from water and sediment were individually analyzed by reversed phase high-performance liquid chromatography (HPLC) for 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}. Different mobile phase compositions were developed; Method #1 for **I** and Method #2 for **II** and **III** (Table 2). 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. The typical retention times of **I**–**III** and their potential metabolites are listed in Table 1.

4. *Metabolism Studies*

Natural sediment and the corresponding associated surface water collected from a French lake (Aire de Pique Nique Lake, Haut Languedoc, France)[6] were used in this

Table 2. HPLC mobile phase

Method #1

time (min)	% A[a)]	% B[a)]	% C[a)]	curve
0	0	30	70	isocratic
5	0	30	70	linear
15	15	30	55	linear
30	15	35	50	linear
45	15	40	45	linear
55	20	40	40	linear
65	30	50	20	isocratic
80	30	50	20	linear
85	0	30	70	isocratic
90	0	30	70	

[a)] Component of mobile phase. A, acetonitrile: B, methanol: C, 0.01% TFA.

Method #2

time (min)	% A[a)]	% B[a)]	curve
0	5	95	linear
10	10	90	linear
40	100	0	isocratic
45	100	0	linear
55	5	95	isocratic
60	5	95	

[a)] Component of mobile phase. A, acetonitrile: B, 0.1% TFA.

study. The sediment and water were passed through 2-mm and 250-μm sieves prior to use, respectively, to remove stones and plant debris. A sediment sample equivalent to 49 g on a dry-weight basis was taken into a two-necked cylindrical glass vessel (5-cm diameter) to a depth of 2.5 cm. Associated water was added to each vessel to a depth of 6 cm above the sediment in accordance with the method proposed by the BBA guideline.[13] The water-sediment system was then pre-incubated in darkness at 20±1°C for 30 days.

The application rates of **I** or **III** to each water-sediment system were adjusted to 78.4 and 47 μg/vessel based on the field application rates (2 kg and 1.2 kg a.i /ha), respectively, assuming a uniform distribution in the water phase to a depth of 30 cm.[13] For easier comparison of the metabolic profiles among the three pesticides, the application rate for **II** was conveniently adjusted to 100 μg/vessel, about one-twentieth of the field rate. The application rates did not exceed the water solubility values (1.1 ppm, **I**; 6.2 ppm, **III** at 25°C, 46 mg/l, **II** at 30°C).[14] After pre-incubation, a 90-μl acetonitrile solution of each pesticide was dropwisely fortified to the water surface in each vessel 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 a polyurethane foam plug (except in the experiment with **I**), with one gas washing bottle containing 300 ml of ethylene glycol and the other containing 350 ml of 0.5 M NaOH solution to trap the volatile ^{14}C.

At appropriate intervals, the surface water, sediment and trapping media were analyzed as described previously.[6]

RESULTS

1. Aerobic Aquatic Soil Metabolism of I

The distribution of radioactivity in the French water-sediment system is summarized in Table 3. The total ^{14}C recovered from the system gradually decreased from 103.3% at day-0 to 78.7% at day-31. When the polyurethane foam plug was used as an additional trap in a separate study, it collected 17.1% of the applied ^{14}C after a similar incubation for 10 days and then, the total ^{14}C was greatly improved to 102.1%. The volatile ^{14}C trapped was identified as **I** by HPLC analysis of the extract. A portion of **I** was considered to escape from the water-sediment system probably *via* azeotropic co-distillation by the humidified air flow. The amounts of volatile ^{14}C collected in the other traps were less than 1.2% of the applied ^{14}C in total after 31 days and the adsorption of ^{14}C onto the vessel was found to be insignificant. Upon application of ^{14}C-**I**, the radioactivity in the aqueous phase rapidly decreased to about half of the applied ^{14}C followed by a slower partition to the sediment phase. After a 31-day incubation, radiocarbon partitioned to the sediment amounted to 49.2%, most of which was identified as the parent compound.

HPLC analysis of the extracts of sediment and associated water showed that **I** was gradually degraded with a DT_{50} value of 24.5 days in total (in sediment plus water) to several metabolites especially in the aqueous phase, as listed in Tables 3 and 6. In the aqueous phase, a more rapid degradation of **I** was observed (DT_{50}=7.7 days). The main metabolite was **Ib** produced *via* *O*-demethylation of **I**. In the later stage of incubation, **Ic** and **Id** appeared but accounted

Table 3. Degradation of **I** in the French lake water-sediment system

	Percentage of applied ^{14}C (Days after application)					
	0	1	3	7	14	31
Volatile	n.a. [a]	<0.1	<0.1	<0.1	<0.1	1.2
Ethylene glycol	n.a.	<0.1	<0.1	<0.1	<0.1	<0.1
NaOH soln. ($^{14}CO_2$)	n.a.	<0.1	<0.1	<0.1	<0.1	1.2 (1.2)
Aqueous	60.6	50.4	56.2	36.3	26.1	28.4
I	60.3	50.4	55.6	33.5	17.5	4.2
Ib	n.d.	n.d.	0.5	1.3	6.8	9.8
Ic	n.d. [b]	n.d.	n.d.	n.d.	0.8	3.2
Id	n.d.	n.d.	n.d.	1.1	0.5	2.1
others	0.4	n.d.	0.2	0.5	0.5	9.1
Sediment	42.7	50.1	39.6	51.8	58.8	49.2
Extractable ^{14}C	42.6	49.9	39.6	50.8	55.8	43.3
I	42.6	49.9	39.6	50.6	54.3	39.6
Id	n.d.	n.d.	n.d.	n.d.	n.d.	1.6
others	n.d.	n.d.	n.d.	0.2	1.6	2.2
Bound ^{14}C	0.1	0.2	<0.1	1.1	3.0	5.9
Rinse	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Total ^{14}C	103.3	100.4	95.8	88.1	84.9	78.7[c]

[a] Not analyzed. [b] Not detected. [c] Due to loss of volatile ^{14}C not trapped, as demonstrated by a separate study with a polyurethane foam plug.

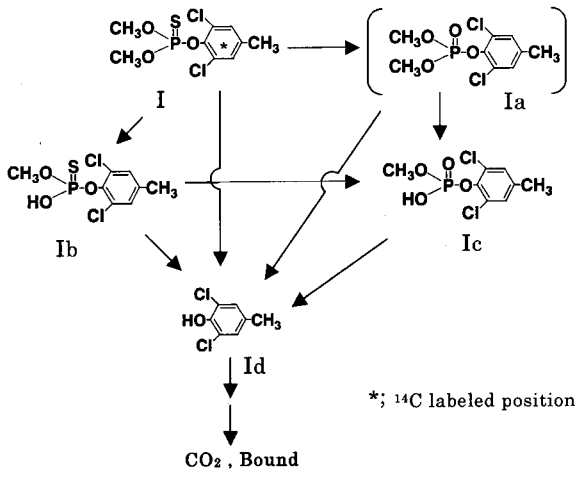


Fig. 1. Proposed degradation pathways of **I** in water-sediment systems.

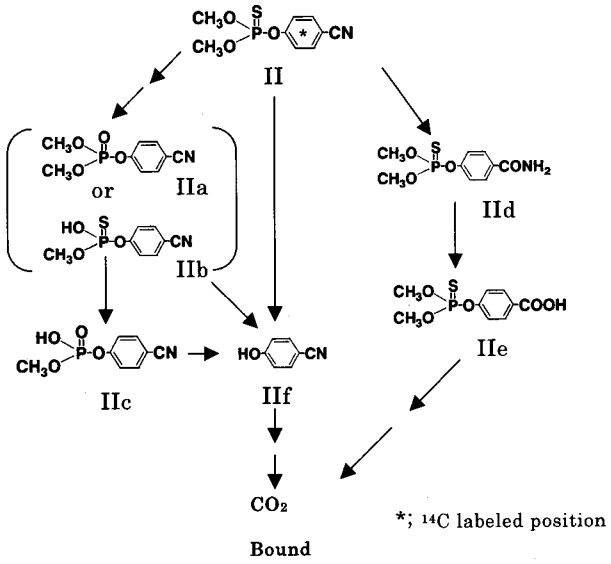


Fig. 2. Proposed degradation pathways of **II** in water-sediment systems.

for less than 3.7% of the total. Since the oxon analogue of **I** was not detected by HPLC analysis, the oxidative desulfuration and cleavage of the P-O aryl linkage were considered to be the minor degradation pathways. In contrast, **I** was scarcely degraded at all in the sediment with a trace formation of **Id** and bound residues. Degradation pathways of **I** in the French lake water-sediment system tested are proposed in Fig. 1 based on the products identified.

2. Aerobic Aquatic Soil Metabolism of II

The distribution of ^{14}C is summarized in Table 4. The total recovery ranged from 91.7% to 102.7% of the applied ^{14}C, better than for **I**. In contrast to the partition profiles of ^{14}C for **I**, **II** mostly remained in the aqueous phase in the early period of incubation and gradually decreased due to partition to the sediment phase with significant evolution of $^{14}CO_2$. The volatile ^{14}C other than $^{14}CO_2$ only amounted to less than 0.7% of the applied ^{14}C. The DT_{50} value of **II** was calculated to be 8.8 days in total, much shorter than that of **I**. As with incubation, **II** dominantly underwent cleavage of the P-O aryl linkage to form **IIf**, amounting to 16.3% of the applied ^{14}C in total after 14 days. The cyano group of **II** was hydrated stepwise *via* the carbamoyl derivative (**IId**) to the

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

	Percentage of applied ^{14}C (Days after application)					
	0	1	3	8	14	29
Volatile	n.a. [a]	0.1	0.9	4.2	12.0	40.2
Polyurethane foam	n.a.	0.1	0.2	0.2	0.7	0.4
Ethylene glycol	n.a.	n.d.	<0.1	<0.1	<0.1	<0.1
NaOH soln. ($^{14}CO_2$)	n.a.	n.d.	0.7 (0.7)	4.0 (4.0)	11.3 (11.3)	39.8 (39.8)
Aqueous	96.8	97.6	79.0	51.2	31.7	8.9
II	95.4	94.0	72.5	34.3	14.5	1.9
IIc	n.d. [b]	0.3	0.6	1.1	1.0	1.1
IId	n.d.	0.8	1.7	2.4	1.5	0.4
IIe	n.d.	0.2	1.0	4.1	4.7	2.5
IIf	n.d.	0.7	1.6	7.6	9.1	1.9
others	1.4	1.8	1.8	1.7	1.0	1.3
Sediment	5.9	5.1	21.2	39.6	48.0	44.1
Extractable ^{14}C	5.9	4.6	19.0	31.3	30.8	17.3
II	5.7	3.3	15.0	20.7	17.5	9.1
IId	n.d.	0.6	1.8	3.1	2.9	1.9
IIe	n.d.	0.2	0.7	1.9	1.6	0.9
IIf	n.d.	0.3	1.2	4.8	7.2	4.0
others	0.2	0.2	0.4	0.9	1.7	1.3
Bound ^{14}C	<0.1	0.5	2.2	8.3	17.2	26.8
Total ^{14}C	102.6	102.7	101.1	94.9	91.7	93.1

[a] Not analyzed. [b] Not detected.

Table 5. Degradation of **III** in the French lake water-sediment system

	Percentage of applied ^{14}C (Days after application)					
	0	1	3	7	14	29
Volatile	n.a. [a]	0.3	0.9	3.9	4.2	12.6
Polyurethane foam	n.a.	0.3	0.9	3.9	4.2	11.1
Ethylene glycol	n.a.	<0.1	<0.1	<0.1	<0.1	<0.1
NaOH soln. ($^{14}CO_2$)	n.a.	<0.1	<0.1	<0.1	<0.1	1.6(0.9)
Aqueous	74.7	94.8	86.8	50.5	36.5	17.1
III	74.4	94.3	86.1	49.2	30.2	9.2
others	0.3	0.6	0.7	1.3	6.3	7.9
Sediment	26.0	5.9	11.3	41.2	54.6	67.7
Extractable ^{14}C	24.7	5.7	10.6	36.1	41.1	41.2
III	24.5	5.7	10.5	32.5	26.2	21.1
others	0.2	n.d. [b]	0.1	3.7	14.9	20.2
Bound ^{14}C	1.3	0.2	0.6	5.2	13.6	26.5
Total ^{14}C	100.7	101.0	99.0	95.7	95.3	97.4

[a] Not analyzed. [b] Not detected.

carboxyl one (**IIe**). These metabolites were detected in both phases and exhibited a maximum formation (5.5-6.3%) at day 8-14 but decreased afterwards. A trace amount of **IIc** was detected only in the aqueous phase but neither of its possible precursors, the *O*-demethylated or oxon derivative, was detected. Furthermore, the HPLC analysis demonstrated no formation of the corresponding phenols (**IIg** and **IIh**) from **IId** and **IIe**. Degradation pathways of **II** in the water-sediment system tested are proposed in Fig. 2 based on the products identified.

*3. Aerobic Aquatic Soil Metabolism of **III***

Good recovery of ^{14}C (95.3-101.0%) was obtained throughout the study, as summarized in Table 5. The immediate partition of **III** to the sediment was observed after application as in the case of **I**, but this seems to stem from capricious fluctuation because less partition was detected at day-1. **III** was degraded with a DT_{50} value of 16.2 days in total (Table 6) but none of the potential metabolites could be detected by HPLC analysis. As with incubation, the total amounts of unknown metabolites in the sediment phase increased finally to 20.2% of the applied ^{14}C at day-29 with a concomitant increase of the bound residues (26.8% at day-29). Each unknown metabolite was found not to exceed 1.4% of the applied ^{14}C throughout the study. As for **I**, **III** was volatilized during incubation and 11.1% of applied ^{14}C

Table 6. DT_{50} and DT_{90} values in water sediment system

	I		II		III	
	water layer	total system	water layer	total system	water layer	total system
DT_{50} (day)	7.7	24.5	5.0	8.8	8.7	16.2
DT_{90} (day)	25.6	81.3	16.5	29.3	29.1	53.8
r^2 a)	0.956	0.992	0.993	0.996	0.953	0.983

a) Coefficient of correlation.

was collected in the polyurethane foam plug as unchanged **III**.

DISCUSSION

The water-sediment system used to examine the three organophosphorus pesticides consists of aqueous and sediment phases under aerobic conditions and hence, the partition and degradation processes are considered to mainly control the dissipation of pesticides in the system. Although the static conditions of the present system are different from the usual shaking method of measuring the adsorption coefficient to soil (Koc), it would be useful to estimate the partition profiles of these pesticides as well as the octanol-water partition coefficient (log P). The degradation processes can be classified as chemical and microbial and hydrolytic degradation is most important in the former.

The degree of partitioning can be evaluated qualitatively from the radioactivity in each phase during the very early stage of incubation but this type of analysis is sometimes difficult due to the fluctuation of data in the pre-equilibrium state. Similar amounts of **I** were present in the aqueous (50.4–60.3%) and sediment (42.6–49.9%) phases at day 0–1, while almost all of **II** (94.5–95.4%) was detected in the aqueous phase in the same period (Tables 3 and 4). This difference could be accounted for by the log P (4.56, **I**; 2.65, **II**) and Koc values (1650–6140, **I**; 160, **II**).[15] The Koc value of **III** is not available but its log P value (4.62)[14] is similar to that of **I** suggesting that almost half of **III** was immediately partitioned to the sediment phase. However, the degree of partitioning fluctuated in the early stage of incubation at 0–3 days and less **III** was detected in the sediment phase (Table 5). This may be due, at least in part, to the hydrophilic *iso*-butylamino moiety of **III** coincident with the higher water solubility of **III** than **I** (1.1 ppm, **I**; 6.2 ppm, **III** at 25°C).[14]

In order to examine the partition process in more detail, kinetic analysis based on assumed compartments was conducted using the Model-Maker program, as previously reported for fenitrothion.[6] The water-sediment system is basically composed of three compartments, sediment, water and atmosphere. Pesticides are partitioned among them with concomitant degradation in each compartment. Various compartment models including the unknown

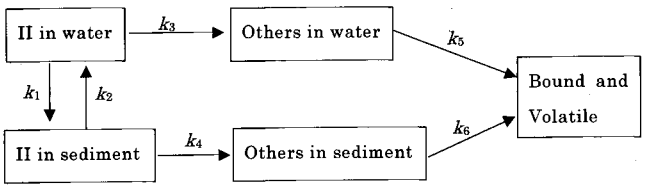


Fig. 3. Compartment model used for kinetic analysis of degradation of **II**.

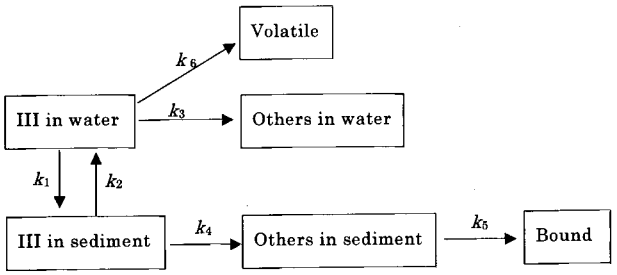


Fig. 4. Compartment model used for kinetic analysis of degradation of **III**.

metabolites, volatile ^{14}C and bound ^{14}C, were prepared and investigated by applying Model Maker to the ^{14}C distribution of each test material. Due to the complexity of the models and the low ^{14}C recovery, the kinetic analysis of **I** failed to reproduce the product distribution in the system. As for **II**, a simple five-compartment model as shown in Fig. 3 was utilized for kinetic analysis. The degradation products and unknowns in each of the water and sediment phases were conveniently dealt with as one compartment to avoid uncertainty in the metabolic process. It is considered that the microbial degradation results in the formation of $^{14}CO_2$ as a main component of volatiles in both phases and bound residues are formed in the sediment. Since the ^{14}C-bound residues are known to be partly converted into $^{14}CO_2$,[16,17] both components were combined as one compartment not relevant to further degradation for simplicity. In the case of **III**, slightly different compartments were assumed due to the significant amount of **III** escaping from the system as volatiles (Fig. 4). Various complex compartment models including the distribution of metabolites between water and sediment phases were examined for **II** and

Table 7. Kinetics analysis of degradation of **II** and **III**

Rate constant (day^{-1})	II	III
k_1	0.072	0.065
k_2	1×10^{-8}	1×10^{-11}
k_3	0.049	6.56×10^{-3}
k_4	0.111	0.0705
k_5	0.076	0.0777
k_6	0.126	0.0100
r^{2} [a]	0.986	0.952

[a] Coefficient of correlation.

III, but the simple model described above gave the best fit for the actual distribution. The results of kinetic analysis are listed in Table 7. The k_1 and k_2 values represent the rate constants of adsorption from water to sediment of **II** (**III**) and their desorption, respectively. The k_1/k_2 ratios corresponding to the partition coefficients of **II** and **III** for the tested sediment were calculated to be 7.2×10^6 and 6.5×10^9, respectively. Data fluctuation, volatilization, degradation in each phase and bound formation made the difference in partitioning in the system between **II** and **III** unclear, but the kinetic analysis clearly demonstrated a tendency in accordance with log P values (2.65, **II**; 4.62, **III**).[14] The k_3 and k_4 values represent the rate constants of degradation in the water and sediment phase, respectively. **II** and **III** were degraded faster in the sediment phase than the water phase.

Among the degradation processes in the water-sediment system, hydrolysis is one of the most important. Organophosphorus pesticides are known to undergo hydrolysis at both P-O-alkyl and P-O-aryl linkages with their contribution dependent on pH.[18] The hydrolysis rate is usually low in the acidic and neutral regions, but rapid hydrolysis occurs at a higher pH. Since the pH value of the associated water used in this study was 6.57, the hydrolytic degradation was considered low for **I**–**III**. The DT_{50} values have been reported to be 56 days for **I** in sterilized pond water[19] and the major degradate was the *O*-demethylated derivative (**Ib**). The corresponding phenol (**Id**) was a minor degradate. The product distribution in the water-sediment system was in good accordance with this profile. A similar DT_{50} value (ca. 48 days) could be estimated for **II** from a photolysis study in distilled water[20] because **II** was found to be resistant to direct photolysis. Ester hydrolysis together with *O*-demethylation occurred similarly as for **I**, indicating the significant involvement of hydrolysis. In addition, **II** was found to undergo successive hydration reactions of the cyano group at the 4-position of the phenyl ring, leading to the formation of the corresponding carbamoyl (**IId**) and carboyl (**IIe**) derivatives. This type of hydration is a common reaction for nitriles.[21] Hydration has been also reported to proceed biotically for benzonitriles.[22] From the various soil metabolism studies of organophosphrus pesticides, the hydrolysis of organophosphorus ester is known to proceed microbiologically and hence, both abiotic and biotic hydrolytic pathways were considered to play a major role in the tested water-sediment system. In contrast to that of **I** and **II**, the hydrolysis of **III** was expected to be extremely slow given its DT_{50} values in a previous study at pH 7 and 25°C (ca. 1500 days),[23] as demonstrated by the fact that the corresponding phenol derivative was not detected in the water-sediment system. Incidentally, reduction of the nitro group followed by acetylation or formylation of the amino group has been reported to be the main degradation pathway for fenitrothion.[24] **III** possesses a nitro group but no reduction product was detected. The steric hindrance over the nitro group at the *ortho*-position of the phenyl moiety might make a similar microbial reduction more difficult to proceed. Although its contribution to the dissipation of pesticides was small, oxidative desulfuration was observed for **I** and **II**, as previously reported for fenitrothion.[6]

One of the greatest differences from normal aerobic soil metabolism is the presence of a water layer over the sediment. The continuous flow of humidified air was found to enhance vaporization of **I** and **III** possibly due to azeotropic co-distillation. The vapor pressures[14] of these pesticides are reported to be 57 mPa (**I**), 105 mPa (**II**) and 84 mPa (**III**) and hence, a similar loss of ^{14}C from the system to the polyurethane foam plug was expected for **II**. However, the corresponding ^{14}C amounted to less than 1% of the applied ^{14}C. In the case of **II**, significant formation of $^{14}CO_2$ (39.8% at day-29) was detected, indicating a significant contribution by the microbial degradation process. Incidentally, the evaporation of the pesticides (**I** and **III**) seemed to increase gradually to a considerable amount after day-7. Therefore, the absence of **II** in the polyurethane foam plug might be accounted for by the greater biodegradation of **II** before it evaporated significantly.

In the water-sediment system, the tested pesticides, **I**–**III**, were not persistent and dissipated with DT_{50} values of 8.8–24.5 days close to or slightly greater than that of fenitrothion (8 days),[6] and finally bound to the sediment residues together with their metabolites and mineralized to carbon dioxide. It was found in the water-sediment system that a pesticide dissipated *via* various kinds of transformation such as hydrolysis depending on its chemical structure concomitant with partitioning between the aqueous and sediment phases.

REFERENCES

1) D. Bennett: "Progress in Pesticide Biochemistry and Toxicology, Volume 7 Environmental Fate of Pesticides," ed. by D. H. Hutson and T. R. Roberts, Chapter 7, John Wiley & Sons Ltd., Chichester, pp. 151–173, 1990.
2) M. Eto: "Organophosphorus Pesticides: Organic and Biological Chemistry," CRC, Cleveland, OH, pp. 1–287, 1974.
3) W. Y. Shiu, K. C. Ma, D. Mackay, J. N. Seiber and R. D. Wauchope: *Rev. Environ. Contam. Toxicol.* **116**, 1–13, 1990.

4) W. Y. Shiu, K. C. Ma, D. Mackay, J. N. Seiber and R. D. Wauchope: *Rev. Environ. Contam. Toxicol.* **116**, 15–187, 1990.
5) T. Katagi, M. Miyakado, C. Takayama and S. Tanaka: "Classic al and Three-Dimensional QSAR in Agrochemistry," ed. by C. Hansch and T. Fujita, Chapter 4, pp. 48–61, ACS, Washington D.C., 1995.
6) R. Kodaka, T. Sugano, T. Katagi and Y. Takimoto: *J. Pestic. Sci.* **27**, 235–241, 2002.
7) A. Yoshitake, F. Shono, T. Kamada and I. Nakatsuka: *J. Labelled Compd. Radiopharm.* **13**, 333–338, 1977.
8) A. Yoshitake, H. Kanamaru, F. Shono and I. Nakatsuka: *J. Labelled Compd. Radiopharm.* **16**, 477–482, 1978.
9) C. L. Carter and S. N. Slater: *J. Chem. Soc.*, 130–133, 1946.
10) M. J. Cook, E. J. Forbes and G. M. Khan: *Chem. Commun.* **5**, 121–122, 1966.
11) K. Mihara, H. Ohkawa and J. Miyamoto: *J. Pestic. Sci.* **6**, 65–74, 1981.
12) N. Mikami, Y. Takimoto, H. Kaneko and J. Miyamoto: Unpublished observations, 1985.
13) Biologische Bundesanstalt Guidelines, Part IV, Section 5-1, December 1990.
14) C. D. S. Tomlin: "The Pestic. Manual 12th Edition," British Crop Protection Council, Surrey, 2000.
15) T. Roberts: "Metabolic Pathways of Agrochemicals," The Royal Society of Chemistry, Cambridge, 1998.
16) N. Mikami, S. Sakata, H. Yamada and J. Miyamoto: *J. Pestic. Sci.* **9**, 697–702, 1984.
17) N. Mikami, S. Sakata, H. Yamada and J. Miyamoto: *J. Pestic. Sci.* **10**, 491–500, 1985.
18) R. Greenhalgh, K. L. Dhawan and P. Weinberger: *J.Agric. Food Chem.* **28**, 102–105, 1980.
19) N. Mikami, K. Imanishi, H. Yamada and J. Miyamoto: *J. Pestic. Sci.* **9**, 215–222, 1984.
20) N. Mikami, H. Ohkawa and J. Miyamoto: *J. Pestic. Sci.* **1**, 273–281, 1976.
21) W. J. G. M. Peijnenburg, K. G. M. de Beer, H. A. den Hollander, M. H. L. Stegeman and H. Verboom: *Environ. Toxicol. Chem.* **12**, 1149–1161, 1993.
22) O. Meth-Cohn and M. Wang: *J. Chem. Soc., Perkin Trans.* **1**, 1099–1104, 1997.
23) T. Katagi: *J. Agric. Food Chem.* **41**, 496–501, 1993.
24) Y. Takimoto, M. Hirota, H. Inui and J. Miyamoto: *J. Pestic. Sci.* **1**, 131–143, 1976.