T. Miyata for their valuable suggestions in preparing this paper and to Mr. H. Honda for his assistance of the experiments. This work was supported in part by a Grant in Aid for Scientific Research from the Japanese Ministry of Education.

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Fate of Organophosphorus Insecticides in Soils. Part II. The Changes of the Retention and the Metabolism of ^{32}P-Labeled Disulfoton and Dimethoate in the Soils. Ikuro KAWAMORI, Tetsuo SAITO and Kisabu IYATOMI (Laboratory of Applied Entomology and Nematology, Faculty of Agriculture, Nagoya University, Chikusa, Nagoya, Japan) Received January 9, 1971. *Botyu-Kagaku* **36**, 12, 1971.

3. 有機燐殺虫剤の土壌施用に関する研究. 第2報 ^{32}P 標識 Disulfoton 及び Dimethoate の土壌における保持及び代謝の経時的変動. 川森郁郎, 斎藤哲夫, 弥富喜三 (名古屋大学農学部害虫学教室, 名古屋市千種区不老町) 46. 1. 9. 受理.

^{32}P 標識 Disulfoton 及び Dimethoate の湛水土壌における保持と代謝の経時的変動を調べた.

施用直後に土壌に保持された Disulfoton の量は Dimethoate より多く, 特に火山灰土壌では他の土壌より多量に保持された. また, Disulfoton を施用した土壌による放射活性の保持は時間の経過に伴ない漸次増加した. 一方, 施用直後における Dimethoate の土壌による保持量は非常に少なかったが施用後2日間で急激に増加した.

土壌有機物抽出法で得られる抽出液中の放射活性は Dimethoate 保持の土壌よりも Disulfoton 保持の土壌で高く, それらの値は時間の経過に伴なって増加した.

Dimethoate を施用した土壌からの水溶出物中から得られるクロロホルム可溶物質 (加水分解を受けていない物質及び誘導体) の相対量は Disulfoton を施用した土壌からのそれより多かった. しかし, それらの相対量の土壌間での差違は Disulfoton を施用した土壌で見られ沖積土壌>頁岩風化土壌>火山灰土壌の順であったが, Dimethoate を施用した土壌間では明らかな差が見られなかった. Disulfoton 施用後10日経過した土壌からの水溶出物中から得られるクロロホルム画分に Disulfoton の sulfoxide 誘導体が検出された. また, Disulfoton の相対量が減少する一方, Disulfoton thiol 化合物の sulfone 体が増加した. しかし, Disulfoton 及び Dimethoate の thiol 化合物は本実験条件下では検出されなかった.

Introduction

Systemic insecticides can be applied in various ways: foliar spray, bark, seed and soil treatments, etc. In soil application, most of the insecticides may have no direct contact to plant roots, and their behaviours are affected by water flow in soils and nature of soils. It is therefore important to investigate the interaction between soil and insecticide in order to get informations on the

persistence of systemic insecticides applied to soils and uptake of them from soils.

The purpose of this work is to study the retention and the metabolism of Disulfoton and Dimethoate in various soils under the submerged condition.

Materials and Methods

Chemicals

The syntheses of ^{32}P-labeled insecticides were undertaken as described in the previous report[1], which follows methods reported by Dauterman *et al* (1959)[2] for Dimethoate (*O, O,* -dimethyl *S*-(*N*-methylcarbamoyl methyl) phosphorodithioate), and by Metcalf *et al* (1957)[3] and O'Brien (1960)[4] for Disulfoton (*O, O,* -diethyl *S*-ethyl-2-mercapto-ethyl phosphorodithioate). Purities, specific radioactivities and formulations of these insecticides have been described in the previous report.[1]

Soils

Loamy sand (organic matter content (O.M.) 1.1%, cation exchange capacity (C.E.C.) 5.4meq./100g soil, pH 4.7), clay loam (O.M. 3.6%, C.E.C. 13.3meq., pH 4.9) and silty clay loam (O.M. 15.5 %, C.E.C. 33.2meq., pH 5.3) were used for the experiments. Further detail on nature of the soils has been described in the previous report.[1]

Procedure

Each of the air-dried soils, 12.0, 9.2 or 5.6g of loamy sand, clay loam or silty clay loam respectively, was placed in a 50m*l* beaker. One hundred μg of the ^{32}P-labeled insecticide emulsified in 10m*l* of distilled water was applied to the soil. The beaker was then kept at 25°±3°C and about 70% relative humidity. At 0 (initial stage), 2, 6 and 10 days after soil application of the insecticide, the soil was poured into a column (2.0cm diameter) with a glassfilter under suction and washed with 200m*l* of distilled water. The eluate and the washing collected through the glassfilter were combined (water-eluate). The insecticide-retaining soil was then transferred to an alminium-foil dish and well mixed. One ninth portion of the soil was placed in a planchet and dried up under an infrared lamp after adding 2 to 3 drops of 8% KOH methanol solution. The radioactivity measured was calculated as the amount of the radioactive materials retained in 1g of air-dried soil.

Extraction of organic matter from one ninth portion of the insecticide-retaining soil was conducted by the procedure reported by Kumada *et al* (1967).[5] The extracts of organic matter were centrifuged at 4500 r.p.m. for 20 minutes. An aliquot of the supernatant was pipetted into a planchet and dried up by the procedure mentioned above. The radioactivity was calculated as the amount of the radioactive materials extracted from 1g of air-dried soil.

The water-eluate from the soil column was concentrated to about 10m*l* at 40°±2°C under reduced pressure and extracted three times with chloroform. The chloroform extracts were combined and kept overnight at 5°C after adding the appropriate amount of anhydrous sodium sulfate. Aliquots of the chloroform and water extracts were respectively pipetted into planchets and dried up by the same procedure described above.

Radioactivity was measured by Aloka thin window gas flow automatic counter. Self-absorption of radioactivity was also corrected by weight for the soil sample.

The chloroform extract from the water-eluate was concentrated to a small volume under reduced pressure and chromatographed. Paper chromatography for Disulfoton was carried out by using 10% propylene glycol-impregnated filter paper (Toyo, No. 51A) and a solvent of 1 part toluene and 4 parts hexane saturated with propylene glycol. Each metabolite detected on the paperchromatogram was identically evaluated according to the method reported by Bull (1965).[6] For Dimethoate, untreated filter paper (Toyo, No. 51A) and acetonitrile : water : ammonium hydroxide (40:9:1) as solvent were used (Bull *et al* 1963,[7] Hackskaylo and Bull 1963,[8] Zayed *et al* 1968[9]). The radioactivity on the paperchromatogram was measured by Aloka 4π low background gas flow paperchromatogram automatic scanner. The relative amount of each metabolite was calculated from the total radioactivity detected on the paperchromatogram.

Results and Discussion

Disulfoton was retained more than Dimethoate in three soils, particularly in silty clay loam, at the initial stage, and afterwards the retained

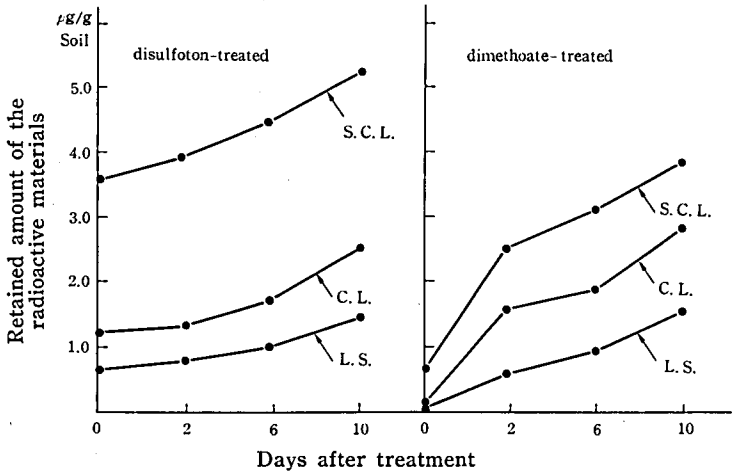

Fig. 1. The changes of the amount of the radioactive materials retained in the soils.
Note: L. S.: loamy sand, C. L.: clay loam, S. C. L.: silty clay loam. The retention just after soil application of the insecticides (0 day) was represented as initial stage.

amount of the radioactive materials increased gradually. On the other hand, the amount of Dimethoate retained initially in the soils was very small, but the retained amount of the radioactive materials increased rapidly during the first two days (Fig. 1). At the initial stage, both the insecticides would be retained in the soils without degradation, but they seem to be decomposed gradually in the soils during the advance of the experimental periods. It is therefore suggested that the radioactive materials retained in the soils contain the insecticides and/or their degradation products. The different retention of the radioactive materials among three soils also appears to depend on the adsorptive capacity to the soil components. The results shown in Fig. 1 also indicate that at ten days after soil application of the insecticides, the total amount of the retained radioactive materials in silty clay loam treated with Disulfoton was larger than that treated with Dimethoate.

Most of the data on the fate of organophosphorus insecticides in soils have been interpreted in relation to clay and organic matter content (Casida *et al* 1952,[10] Getzin and Chapman 1959[11] and 1960,[12] Zaki and Reynolds 1961[13]). Kawamori *et al* (1971)[1] have reported that the amount of the radioactive materials obtained from Disulfoton-retaining soils by the extraction procedure for organic matter was related to the content of organic matter rather than clay in three soils. Table 1 showed that the radioactive materials extracted from the insecticide-retaining soils increased up to ten days after soil application of the insecticides, and also that the similar patterns as the retention of the insecticides and/or their degradation products as shown in Fig. 1 were observed in the fractions obtained by the

Table 1. The changes of the radioactive materials in the fractions obtained by the extraction procedure for organic matter.

Soils	Applied amount (μg/g soil)	μg/g soil							
		Disulfoton				Dimethoate			
		0[a)]	2	6	10	0	2	6	10
loamy sand	8.33	0.57	0.61	0.70	0.73	Trace	0.33	0.37	0.51
clay loam	10.87	0.75	0.83	0.92	1.21	Trace	0.33	0.38	0.67
silty clay loam	17.85	2.34	2.66	2.85	3.20	0.76	1.01	1.15	1.71

a): Days after soil application of the insecticides.

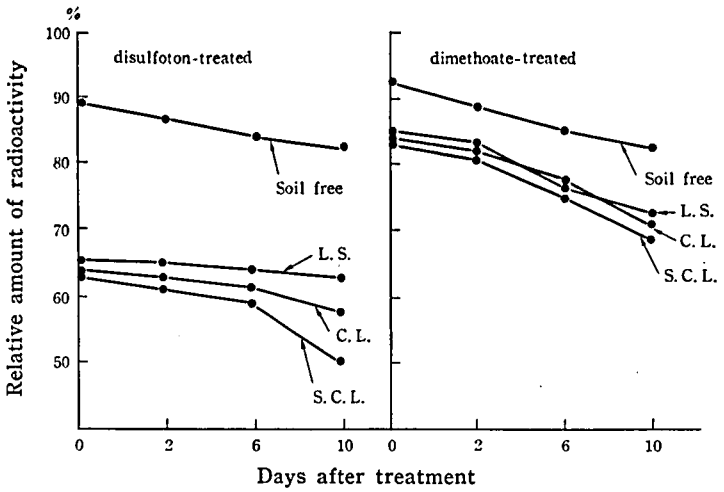

Fig. 2. The changes of the relative amount of the radioactivity in the chloroform extracts from the water-eluate of the insecticide-treated soils.
Note: The abbreviations of the soil name are the same as used in Fig. 1.
Soil free; The insecticides were incubated without adding the soils.

extraction procedure for organic matter. The different retention among three soils is supported indirectly by the evidence that several insecticides were absorbed more abundantly by plant roots from sand than clay loam, silty loam or muck (Getzin and Chapman 1959[11] and 1960[12]).

The insecticides applied to the soils are neccessary to be absorbed initially by plant roots without their degradation for the toxicity to the phytophagous insect pests. The chloroform extracts in the water-eluate from the soil column were characterized at the given days after soil application of the insecticides. It is noted from Fig. 2 that the relative amount of the radioactivity in the chloroform extracts from the Dimethoate-treated soils was larger than that from the Disulfoton-treated soils, and also that the relative amount of the radioactivity in the chloroform extracts from the Disulfoton-treated soils increased in the order of loamy sand, clay loam and silty clay loam, while there was no remarkable difference among these soils treated with

Table 2. Relative amount of the chloroform extractable materials in the water-eluate obtained at the indicated periods after soil application of the insecticides.

Soils	Days after treatment	percentage of radioactivity							
		Disulfoton						Dimethoate	
		P. C.	PSO	PSO_2	POA	POAO	$POAO_2$	P. C.	POA
loamy sand	2	50.5	0	0	0	23.7	26.8	100	0
	10	22.2	Trace	0	0	19.1	58.7	100	0
clay loam	2	18.7	0	0	0	69.8	11.5	100	0
	10	2.9	7.5	Trace	0	10.5	79.1	100	0
silty clay loam	2	17.7	0	0	0	71.5	10.8	100	0
	10	1.9	6.9	5.8	0	22.6	62.8	100	0
soil free[a)]	10	21.6	24.8	12.0	Trace	23.8	17.8	100	0

a): The insecticides were incubated without adding the soils.
Abbreviations; P. C. (parent compound), PSO (the sulfoxide derivative from Disulfoton), POA (thiol types of Disulfoton and Dimethoate), POAO (the sulfoxide derivative from Disulfoton of thiol type), $POAO_2$ (the sulfone derivative from Disulfoton of thiol type).

Dimethoate. The relative amounts of both insecticides in the chloroform extracts decreased gradually, and their decreases seem to be explained by the negative correlation with the retention patterns as shown in Fig. 1.

Chromatographic separation of the chloroform extractable materials revealed that no thiol types (POAs) of Disulfoton and Dimethoate were detected in the water-eluate at 2 and 10 days after soil application of the insecticides. Such a evidence has also been reported by Metcalf *et al* (1957)[3] and Bull (1965)[6] using insects and plants treated with Disulfoton, and by Sato and Miyamoto (1967)[14] using the soil treated with Dimethoate. Table 2 also showed that the relative amount of the sulfone derivative from Disulfoton of thiol type ($POAO_2$) increased, whereas those of Disulfoton (P. C.) and the sulfoxide derivative from Disulfoton of thiol type (POAO) was decreased. The relative amounts of other metabolites (PSO and PSO_2) were very small.

The uptake and translocation of Disulfoton and its metabolites from the soils to plants are possibly influenced by the affinity of these toxicants with the plant roots and their metabolism in plants, although the fate of the insecticides in soils might be different from that in the presence of growing plants in soils.

Further evaluation for the suitable application of organophosphorus insecticides to soils should be undertaken considering soil moisture, which would affect the metabolism of the insecticides by soil micro-organisms under flooded or non-flooded conditions, and uptake of the insecticides by plants.

Summary

The retention and the metabolism of ^{32}P-labeled Disulfoton and Dimethoate in three soils, loamy sand, clay loam and silty clay loam, on the incubation for ten days were studied under the submerged condition.

As the result that the soil was eluted with water at a certain period after application of the insecticides to the soils, Disulfoton was retained more than Dimethoate in these soils, especially silty clay loam, at the initial stage, and afterwards the retained amount of the radioactive materials increased slowly. On the other hand, the amount of Dimethoate retained initially in the soils was very small, but the retained radioactive materials increased rapidly during the first two days. After ten days the total amount of the retained radioactive materials in silty clay loam treated with Disulfoton was larger than that treated with Dimethoate.

Radioactive materials obtained by the extraction of organic matter were larger in the Disulfoton-retaining soils than those in the Dimethoate-retaining soils. The retained amount of the radioactive materials increased progressively during ten days.

Therel ative amount of the chloroform extractable materials eluted with water from the Dimethoate-treated soils was larger than that of the Disulfoton-treated soils. In the case of Disulfoton treatment, the chloroform extractable materials were found in the following order: loamy sand>clay loam>silty clay loam. On the other hand such a clear difference was not observed among these soils treated with Dimethoate. In the chloroform extracts from the water-eluate, the sulfoxide derivative from Disulfoton was detected at ten days after soil application of the insecticide. The relative amount of the sulfone derivative from Disulfoton of thiol type increased, whereas the relative amount of Disulfoton itself decreased. However, thiol types of Disulfoton and Dimethoate were not detected under the present experimental condition.

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The Fundamental Research to the Application of Systemic Insecticides (II). The Absorption and Translocation of Several Insecticides in Rice plant by Soil application. Takeo Ishiguro* and Tetsuo Saito (Laboratory of Applied Entomology and Nematology, Faculty of Agriculture, Nagoya University, Nagoya) Received November 2, 1970. *Botyu-Kagaku* 36, 17, 1971.

4. 浸透殺虫剤の施用法に関する基礎的研究 (II) 土壌処理による各種浸透殺虫剤の水稲における浸透および移行について

石黒丈雄*, 斎藤哲夫 (名古屋大学農学部害虫学教室) 45. 11. 2. 受理

Vamidothion, Dimethoate, Disulfoton, Thiometon および Mecarbam の水稲における浸透移行性を ^{32}P-標識化合物を用いて検討した．すなわち，上記薬剤の乳剤稀釈液を水稲の移植した土壌表面に施用し，薬剤の浸透移行およびそれにともなう殺虫力を調べた．

その結果，Disulfoton は施用直後に水稲の地下部に薬量が多く，地下部への薬剤浸透が早く，また Dimethoateは 地上部および地下部の薬量はほぼ同じであり，植物体内の浸透および移行が極めて容易であると推察される．

これに対して Vamidothion の浸透移行性はゆるやかであり，Thiometon および Mecarbam のそれは弱い．同じ施用法で行なったヒメトビウンカに対する殺虫試験による死虫率の増加は地上部の薬量の増加とほぼ一致した．

実験終了時(施用後6日目)においてVamidothion と Mecarbam は土壌吸着物が多く Dimethoate は少なかった．

Introduction

Since Schrader and Kükenthal first found the systemic action of organophosphorus insecticides in 1935, many experiments have been made on absorption, translocation and metabolism in plants with various application methods. At present, systemic insecticides occupy a very important position in pest control for agricultural crops.

It was proved by David (1952),[3] Iyatomi and Saito (1967)[7] and Harris (1967)[6] that insecticidal effect of the applied chemicals were influenced by the type of soil. Further, Ching *et al.* (1961),[2] Bennett (1949)[1] and Wedding *et al.* (1952)[18] and Teitz (1954)[16] studied absorption, translocation and persistence of the several insecticides respectively on each crops. They reported that the absorption site in crop varies according to the application methods of chemicals.

This report is concerned with the absorption and translocation of the following five ^{32}P-labelled systemic organophosphorus insecticides in rice plants: Vamidothion, Dimethoate, Disulfoton, Thiometon and Mecarbam.

Meterials and methods

Test insecticides: Sulfur powder, methanol and

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