原　著

Determination of Insecticide Residue in Animal and Plant Tissues. II. Metabolic Fate of Sumithion in Rice Plant Applied at the Preheading Stage and its Residue in Harvested Grains. by Junshi Miyamoto and Yoshishige Sato (Agricultural Chemical Research Department, Osaka Works, Sumitomo Chemical Co., Ltd. Kasugade-cho, Konohana-ku, Osaka, Japan.) Received Feb. 4, 1965. *Botyu-Kagaku*, 30, 45, 1965.

9. 動植物組織中における残留殺虫剤の定量 2. 水稲に散布したスミチオンの消長と米粒中における残留について　宮本純之・佐藤香重（住友化学工業株式会社 大阪製造所 農薬研究部 大阪市此花区春日出町）40. 2. 4 受理

1. 放射性リンで標識したスミチオン乳剤の1,000倍液を出穂前の水稲に散布し，スミチオンの組織中での消長をしらべた．この条件下で，水稲には生重量1gあたり約12 μgのスミチオンが付着（葉鞘へは，葉身への約5％が付着）し，散布後24時間で，その約半量が組織内へ浸入した．このスミチオンは，葉鞘，葉身部を通じて，ほぼ同程度に，比較的急速に（1週間後で最初の5％以下に減少し，脱メチルスミチオン，ジメチルチオリン酸，チオリン酸などに分解することが知られた．またスミチオンの酸化型であるスミオキソンの生成がみとめられた．
2. 放射性リンで標識したスミチオンを散布した水稲よりえられた穀粒中にはスミチオンはほとんどみとめられず，ジメチルチオリン酸，チオリン酸のごときスミチオンの代謝産物の含量も，ヌカ，精白米を通じて，1 ppm．もしくはそれ以下であつた．

さらに，ことなつた条件下でスミチオンを施用した3種類の水稲よりえられた穀粒中のスミチオン残留量は，ヌカ，精白米とも0.1 ppm．以下，また，*p*-ニトロクレゾールは，ヌカ中にわずかに存在したが，精白米中の含量は0.1 ppm．以下であつた．

以上の結果よりすれば，2化期のメイ虫防除に用いたスミチオンもしくはその代謝産物の米粒中への残留は，公衆衛生上問題になるほどの量でないことは明白である．

Introduction

For the control of the rice stem borer, *Chilo suppressalis*, Walker, one of the major pests of rice plant grown in Japan, ethyl-and methyl-parathion have been preferably used. Recently, however, Sumithion* has been rapidly replacing these insecticides, because this new organophosphorus compound has been proved to be almost as same effect against the said pest as parathion, and moreover can be safely used owing to its extremely low toxicity to mammals. In the experiments reported here, we applied phosphorus-32 labeled Sumithion to rice plant at the preheading stage corresponding to the period of the second hatching of the rice stem borer, and examined its metabolic fate in the plant. We also determined residual amount of Sumithion as well as of other metabolic products contained in rice grains from the view-point of public health.

* 0, 0-Dimethyl, 0-(3-methyl-4-nitrophenyl) phosphorothioate.

Materials and Methods

1) Radioactive Sumithion.

Phosphorus-32 labeled Sumithion was synthesized from phosphorus-32 trichloride, as described previously[1]. The purity of the preparation was more than 99 % and the initial specific activity was 9.0 mc/g.

2) Application of Sumithion to rice plant and methods of analysis.

Rice plants (variety Hatsushimo) were transplanted on Aug. 7, 1964 to a space under the similar conditions of a paddy field which had been prepared in a vinyl tent. On Aug. 25, phosphorus-32 Sumithion emulsion was sprayed uniformly on the plants under the pressure of 3.1 kg/cm^2 from a nozzle which was moving horizontally 50 cm above the top of the plants. The emulsion used was as follows ; emulsifiable concentrate composed of 50 parts of phosphorus-32

Sumithion, 30 parts of emulsifier Sorpol SM 100* and 20 parts of xylene, was diluted to 1,000 times (2,000 times, as compared with the active ingredient). Sprayed volume was enough to cover 75 *l*/10 ares. The weather was fine and the temperature inside the tent was 32°C. At appropriate intervals after application of Sumithion, 3 sheaves of rice plants (average weight ; leaf blade 140g, leaf sheath 230 g) were sampled at random by cutting down at the base, and leaf sheath and leaf blade were separated. Acetone was blown onto the surface to wash adhering Sumithion off. Subsequently leaf sheath and leaf blade were sliced finely with a scissor and thoroughly homogenized in a mortar with a pestle by addition of sea sand and dry ice. The homogenate obtained, after suspended in distilled water and acidified with 10% cold perchloric acid (the final concentration 2%), was extracted twice with chloroform. Phosphorus-32 in the combined chloroform layers was separated and identified by thin-layer chromatography, as described in detail elsewhere(in this case, Sumithion and Sumioxon that developed on the thin-layer plate were assayed radiochemically).[2] The aqueous layer was lyophilized and radioactive compounds were determined by co-chromatography with the authentic compounds on paper as shown in one of the previous papers.[1]

3) Determination of Sumithion and its metabolic products in rice grains.

Rice plants sprayed with radioactive Sumithion as mentioned above were reaped on Oct. 9, 1964 (46 days after application of Sumithion) and residue of Sumithion and its metabolic products in rice grains were determined. In addition, Sumithion and free *p*-nitrocresol** were analyzed out of the rice grains of the following three different varieties applied with Sumithion.

(A) Kin-nampu variety, sprayed with 700 times-diluted emulsion of 50% emulsifiable liquid at a rate of 50 *l*/10 ares on Aug. 28, 1964 and reaped on Oct. 29.

(B) Ginga variety, applied with 3 kg of 5% granule/10 ares on Aug. 3, 1964 and reaped on Sep. 10.

(C) Aichi-asahi variety, applied with 4 kg of 2% Sumithion dust per 10 ares on Aug. 23, 1964, and reaped on Oct. 28.

Those rice grains were divided into rice bran and polished rice, and Sumithion and *p*-nitrocresol in both parts were determined by means of chloroform extraction, followed by thin-layer chromatography and colorimetric measurement of sodium *p*-nitrocresolate under the alkaline condition, as described already. According to the preliminary test, *p*-nitrocresol is also extractable with chloroform and can be recovered satisfactorily by the same procedure. In the case of rice grains sprayed with phosphorus-32 Sumithion, the band of silica-gel of the thin-layer chromatoplate marked with the authentic Sumithion was stripped off and its radioactivity was counted. Control rice grains of variety Hatsushimo were not available, so content of *p*-nitrocresol could not be determined. Aqueous layer from the rice grains sprayed with radioactive Sumithion was chromatographed on Dowex-1 column, and degradation products of Sumithion containing phosphorus-32 were determined in the way as stated in the previous paper[1].

Results and Discussions

1. Metabolic fate of Sumithion in rice plant.

Persistence of the applied Sumithion on the plant surface and its penetration into the tissues were determined. The results are shown in Table 1, Figs. 1 and 2. Under the present experimental conditions, 11.79 ppm of Sumithion was attached to the surface immediately after spray, and during the subsequent twenty-four hours approximately one half of the amount was observed to have penetrated into the tissues. Only rone-tenth of the initial Sumithion attached re-

Table 1. Distribution of ^{32}P in leaf. (μg equivalent/g tissue)

Days after treatment	Total ^{32}P	Penetrated ^{32}P	^{32}P on the surface
0	11.79	0	11.79
1	6.97	5.68	1.29
2	5.79	5.01	0.78
4	5.51	4.97	0.54
7	5.00	4.68	0.32
10	4.80	4.47	0.33

* Manufactured by Toho Chemical Co.

** 3-methyl-4-nitrophenol.

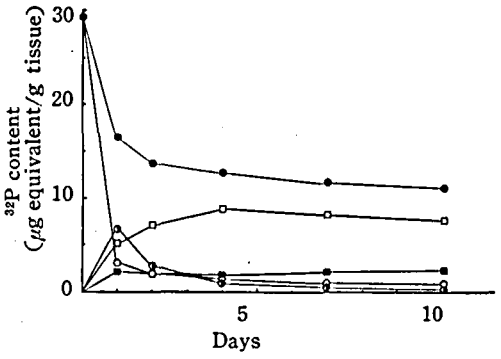

Fig. 1. ^{32}P content in leaf blade after spraying ^{32}P-Sumithion.
● Total ^{32}P, ○ Acetone washing, ◑ Chloroform soluble, □ Water soluble, ■ Precipitate.

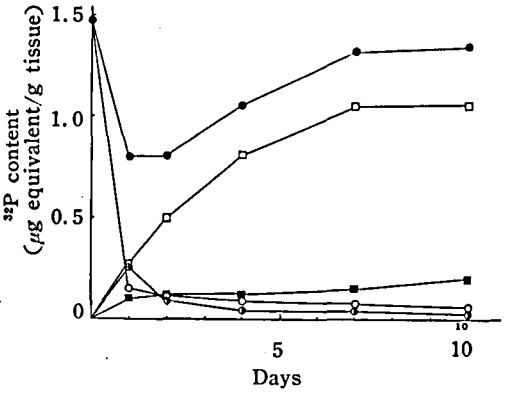

Fig. 2. ^{32}P content in leaf sheath after spraying ^{32}P-Sumithion.
● Total ^{32}P, ○ Acetone washing, ◑ Chloroform soluble, □ Water soluble, ■ Precipitate.

mained on the surface after one day. The rest was lost, presumably by evaporation. Further decrease of Sumithion on the surface was rather gradual. Sumithion adhered on the leaf blade about 20 times more than on the leaf sheath per gram. Decrease of the attached Sumithion proceeded nearly at the same rate in both parts. The contents of Sumithion and Sumioxon in leaf blade and leaf sheath are indicated in Table 2. As is clear, approximately 4.5 μg and 0.2 μg of Sumithion (33% and 30% of the total radioactivity that had penetrated) per g of leaf blade and leaf sheath respectively were present one day after application and then decreased rather rapidly. On the seventh day the contents reduced to 5% of that on the first day. Sumioxon, an oxygen analog formed from Sumithion, was also identified in both leaf blade and leaf sheath. This result confirms the previous one that rice plant tissues can oxidize Sumithion to Sumioxon[1].

Table 2. Contents of Sumithion and Sumioxon* in chloroform extract of leaf. (μg equivalent/g tissue)

Days after treatment	Sumithion	Sumioxon
	Leaf sheath	
1	0.19	0.03
2	0.06	0.02
4	0.02	<0.01
7	0.01	0.01
10	0.01	<0.01
	Leaf blade	
1	4.46	0.83
2	1.46	0.86
4	0.33	0.27
7	0.23	0.15
10	0.16	0.08

* Oxygen analog of Sumithion, 0, 0-dimethyl 0-(3-methyl-4-nitrophenyl) phosphorate.

Rice plant tissues seem to be capable of metabolizing Sumithion rapidly under the experimental conditions since twenty-four hours after spray an appreciable amount of water soluble radioactivity was already demonstrated. This radioactivity in aqueous layer increased in accordance with the decrease of the active ingredient and one week afterward the amount reached 80% of the total radioactivity in leaf blade. In leaf sheath the increment of water soluble radioactivity surpassed the decrease of radioactivity in chloroform layer plus acetone washing. Therefore, translocation of water soluble metabolites from leaf blade was suspected. Those water soluble metabolites from Sumithion were separated and identified. The results are shown in Table 3. In leaf blade dimethylphosphorothioic acid, phosphorothionic acid and phosphoric acid were considered to be the major degradation products. Besides, desmethylsumithion** was identified in the samples obtained within two days after application. In the case of leaf sheath dimethylphosphorothioic acid, phosphorothionic

** 0-methyl 0-hydrogen 0-(3-methyl-4-nitrophenyl) phosphorothioate.

Table 3. Water soluble metabolites of Sumithion in leaf blade.
(μg equivalent/g tissue)

Days after treatment	Total	Desmethyl-Sumithion	Dimethyl phosphoro-thioic acid	Phospho-rothionic and/or phospho-ric acid
1	5.21	0.45	1.24	3.52
2	7.22	0.02	1.75	5.47
4	8.85	—	1.76	7.09
7	8.29	—	1.53	6.76

acid and phosphoric acid were detectable. Although the presence of such compounds as dimethylphosphoric acid, monomethylphosphorothioic and monomethylphosphoric acids was presumed in consideration of the rapid decrease of Sumithion and Sumioxon, these were not detected. In both leaf blade and leaf sheath an appreciable amount of radioactivity unextractable either with chloroform or with perchloric acid was present, but has not yet been characterized.

2. Determination of Sumithion residue in rice grains.

Rice plants to which phosphorus-32 labeled Sumithion had been applied were reaped and the content of Sumithion remaining in rice grains was determined. 0.26μg and 0.17μg of Sumithion were found in 27 g of rice bran and 570 g of polished rice respectively. No Sumioxon was detected.

Aqueous layers from both bran and polished rice were obtained and radioactive compounds were separated and identified. The results are shown in Table 4. Other degradation products of

Table 4. Water soluble metabolites of Sumithion in rice grains.
(expressed as μg equivalent)

	Rice bran(27g)	Polished rice(570g)
Phosphoric acid	71	102
Phosphorothionic acid	27	32
Dimethylphosphoro-thioic acid	6	15

Sumithion such as desmethylsumithion*** desmethyl sumioxon, dimethylphosphoric acid, monomethylphosphorothioic acid and monomethylphosphoric acid were not detected. Besides, rice grains contained some amount of unidentified radioactive phosphorus (about 200μg/600g of rice grains). If rapid decomposition of Sumithion in rice plant mentioned above is taken into account, most of the unidentified radioactivity is presumed to come from the normal constituents of the tissues containing the newly incorporated phosphorus-32. Contents of Sumithion and free *p*-nitrocresol were analyzed in rice grains of three different varieties to which Sumithion had been applied. The results are reproduced in Table 5.

Table 5. Contents of Sumithion and free *p*-nitrocresol in rice grains treated with Sumithion. *, **)

Variety of rice plant	Sumithion (μg/g)	free *p*-nitrocresol (μg/g)
	Rice bran	
Kin-nampu	<0.1	0.13
Ginga	<0.1	<0.1
Aichi-asahi	<0.1	0.36
	Polished rice	
Kin-nampu	<0.1	<0.1
Ginga	<0.1	<0.1
Aichi-asahi	<0.1	<0.1

* Concerning the conditions of application, see text.

** Control values were subtracted.

Residue of Sumithion contained in both rice bran and polished rice of three varieties was found to be less than 0.1 μg/g of samples. No samples of polished rice contained detectable amount of free *p*-nitrocresol, although this degradation product of Sumithion was found in the rice bran of two varieties tested.

Thus, it can be concluded from the above results that from the view-point of public health the residual amount of Sumithion and its metabolites is considered to be completely harmless to human body. This, coupled with the low toxicity to mammals,[3,4] might ensure the favorable use of Sumithion for the control of noxious insects of rice plants.

Acknowledgement: We wish to express our thanks to Sumitomo Chemical Co., Ltd. for permission to publish this work. Radioactive Sumithion used in this experiment was synthesized

*** 0-methyl 0-hydrogen 0-(3-methyl-4-nitrophenyl phosphorate.

by Mr. M. Endo, to whom we are much obliged.

Summary

1. Metabolic fate of Sumithion was studied in rice plant after the emulsion of this insecticide labeled with phosphorus-32 had been sprayed on the plant at the preheading stage.

Sumithion penetrated into the tissues was decomposed rather rapidly to desmethylsumithion, dimethylphosphrothioic acid and phosphorothionic acid. Presence of the oxygen analog (Sumioxon) was also demonstrated.

2. Residual amount of Sumithion and its metabolites in rice grains of several varieties of rice plant applied with Sumithion under the various conditions were determined. Sumithion and Sumioxon were hardly detectable. Only minute amount of such degradation products as dimethylphosphorothioic acid, phosphorothionic acid and free *p*-nitrocresol was identified.

From the results it can be concluded that the residual amount of Sumithion as well as of its metabolic products in rice grains lies within the permissible limit from the view-point of public health.

Reference

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3) Nishizawa, Y. Fujii, K. Kadota, T. Miyamoto, J. and Sakamoto, H. : *Agri. Biol. Chem.*, 25, 605 (1961).
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Determination of Insecticide Residue in Animal and Plant Tissues. III. Determination of Residual Amount of Sumithion in Cocoa Beans grown in Nigeria. Junshi Miyamoto, Yoshishige Sato and Katsutoshi Fujikawa (Agricultural Chemical Research Department, Osaka Works, Sumitomo Chemical Co., Ltd. Kasugade-cho, Konohana-ku, Osaka, Japan) Received April. 26, 1965. *Botyu-kagaku*, 30, 1965.

10. 動植物組織中における残留殺虫剤の定量 3. ナイジエリヤ産ココア豆におけるミスチオン残留量の測定 宮本純之・佐藤香重・藤川勝利（住友化学工業株式会社 大阪製造所 農薬研究部 大阪市此花区春日出町）40. 4. 26 受理

ココアの害虫防除に用いられたスミチオンのココア豆中における残留量を測定した．

スミチオン散布後2週間で，豆中のスミチオンは最大に達し，以後次第に減少するが，最大含量は 0.12 ppm と推定される．またスミチオンの分解物たる 3—メチル—4—ニトロフェノールの含量は 0.06 ppm もしくはそれ以下であつた．

これらの量は，公衆衛生的見地よりみて，問題とするに足りず，人体には全く無害であると考えられる．

Introduction

In cocoa producing countries such as Nigeria and Ghana, benzene hexachloride (γ-BHC) has mainly been used for the control of mirids, *Distantiella theobroma* and *Sahlbergella singularis*, the most noxious insects to the plant. Recently, however, these insects have become more and more resistant against the insecticide and much effort is now being exerted in search of alternative compounds. Sumithion, or 0, 0-dimethyl 0-(3-methyl-4-nitrophenyl) phosphorothioate, has proved to be very effective against the mirids[1,2] and it is now under study for practical application. One of the most pivotal points is to establish whether or not the residue of Sumithion in cocoa beans remains within the permissible limit from the medical view-point.

In this report we determined residual amount of Sumithion as well as 3-methyl-4-nitrophenol (abbreviated as *p*-nitrocresol) in cocoa beans which had been applied with Sumithion.