原　　著

Metabolism of ^{14}C-Labelled Sumithion, *O,O*-Dimethyl *O*-(3-methyl-4-nitrophenyl) phosphorothioate in Apples. Shunji Hosokawa and Junshi Miyamoto (Research Department, Pesticides Division, Sumitomo Chemical Co., Ltd., Hyogo, Japan) Received December 11, 1973. *Botyu-Kagaku*, 39, 49, 1974.

7. ^{14}C-標識スミチオン〔*O,O*-Dimethyl *O*-(3-methyl-4-nitrophenyl) phosphorothioate〕のリンゴにおける代謝

細川俊治，宮本純之（住友化学工業株式会社　農薬事業部研究部） 48. 12. 11 受理

フェノール側 *m*-メチル基 ^{14}C-標識スミチオンを使用し，立毛中のリンゴにおけるスミチオンの代謝および残留をしらべた．処理直後 4.5 ppm 付着していたスミチオンは急速に減少し，処理量の約60%が3日間に消失した．スミチオンの残留量は7，14，21日後にそれぞれ 1.12，0.668，0.521 ppm であった．表面における主な分解物はスミオキソンと *p*-ニトロクレゾールであり，スミオキソンは3日後に最大値約 0.02 ppm を示し，以後減少した．他に痕跡量のスミチオン *S*-異性体を認めた．内部におけるスミチオンの代謝はゆるやかであり，経時的に水溶性の高い化合物が徐々に増加してゆくのが認められた．それらの中には *p*-ニトロクレゾールの *β*-グルコシドが認められ，植物内部に侵入したスミチオンが加水分解されて *p*-ニトロクレゾールとなり，グルコース抱合を受けたものと思われる．

Sumithion 〔*O,O*-Dimethyl *O*-(3-methyl-4-nitrophenyl) phosphorothioate〕 is now being widely used to control harmful insects of rice, vegetables and fruit trees. Although metabolic studies in mammals have been extensively carried out[1-10], those in plants have been limited to rice[11], corn and bermudagrass[12]. In this study we applied carbon-14 labelled Sumithion to apple fruits in the field and examined its metabolic fate in apple fruits.

Materials and Methods

Chemicals

Radioactive Sumithion labelled with carbon-14 at *m*-methyl group had a specific activity of 3.16 mCi/mmole. The chemical purity of the preparation was more than 99% and the radioactive impurities were not detected by thin layer chromatography[13]. Desmethylsumithion 〔*O*-methyl *O*-hydrogen *O*-(3-methyl-4-nitrophenyl) phosphorothioate〕 was prepared by the reaction of Sumithion with sodium thiophenolate in absolute ethanol[14]. The sodium salt of desmethylsumioxon 〔*O*-methyl *O*-hydrogen *O*-(3-methyl-4-nitrophenyl) phosphate〕 was prepared by reacting Sumioxon with equimolar sodium iodide in acetone at 60°C for 1 hr. *S*-methyl Sumithion 〔*O,S*-dimethyl *O*-(3-methyl-4-nitrophenyl) phosphorothiolate〕 was prepared by reacting desmethylsumithion with methyl iodide. *p*-Nitrocresyl-*β*-glucoside was synthesized starting with tetraacetyl-*α*-bromoglucose and *p*-nitrocresol[15]. Other authentic compounds such as Sumithion, Sumioxon 〔*O,O*-dimethyl *O*-(3-methyl-4-nitrophenyl) phosphate〕 and *p*-nitrocresol (3-methyl-4-nitrophenol) were synthesized in this laboratory.

Application of ^{14}C-Sumithion

Apple fruits on the tree were dipped in a 0.1 % emulsion (a.i.) of ^{14}C-labelled Sumithion (3.7 *μ*Ci/mg/*ml*) and maintained under natural weather conditions. (26, Oct. 1972) Sumithion emulsion for treatment was prepared in distilled water with 0.5% v/v of sorpol 2001 (Toho Chemicals Co.) emulsifier. Weather and temperature during the experimental period are shown in table 1. The zero-time samples were collected immediately after the surface deposit was dried and other samples were harvested at 1, 3, 7, 10, 14 and 21 days after treatment.

Preparation of Samples for Radioactivity Measurement

The radioactivity on the surface of apples were collected by washing with acetone. Radioactivity inside the treated apples was extracted

Table 1. Weather conditions.
Data was presented from Nagano local meteorological observatory.

Date	Days*	Temperature max.	Temperature min.	Humidity	Rainfall	Weather
Oct. 26	0	17.3 c	6.4 c	77%	0.0 mm	◐
27	1	21.6	10.6	76	3.0	◎
28	2	18.6	6.9	66	—	◐
29	3	20.9	8.7	74	—	◐
30	4	20.4	6.4	77	1.0	○
31	5	13.3	2.6	71	2.0	◎
Nov. 1	6	14.0	−0.7	68	—	○
2	7	17.7	0.9	64	—	○
3	8	14.3	10.4	80	3.5	●
4	9	14.4	9.2	87	0.5	◎
5	10	11.2	6.2	78	0.0	◎
6	11	16.1	5.3	82	5.5	●
7	12	12.2	4.0	77	0.0	●
8	13	10.0	2.2	75	0.0	◎
9	14	12.7	−0.4	78	—	◐
10	15	9.8	6.2	91	9.0	●
11	16	10.2	1.9	66	0.0	◐
12	17	13.8	−1.6	75	—	○
13	18	14.2	0.3	83	0.0	◎
14	19	13.5	5.0	49	0.0	◐
15	20	12.1	8.2	75	11.5	●
16	21	19.6	10.3	77	1.0	◎

* Days after treatment ○ fine, ◐ fair, ◎ cloudy, ● rainy

by homogenization with 70% acetone solution (2 ml/g) in a Waring blender. The homogenate was centrifuged, and the precipitate was extracted again in a similar manner. The combined supernatants were evaporated under vacuum at 40°C and the remaining aqueous layer (pH about 4.0) was extracted three times with equal volume of benzene. The aqueous fraction was acidified to pH 1.0 with hydrochloric acid and extracted three times with benzene.

Separation and Identification of the Radioactive Metabolites

The benzene layers after dehydrated with anhydrous sodium sulfate, were concentrated under vacuum at 40°C and analyzed by thin layer chromatography. Silicagel plates (HF254, Tokyo Kasei) with the thickness of 0.25mm were used and the plate was developed with the following systems: (A) benzene : ethylacetate (4 : 1), (B) toluene : ethyl formate : formic acid (5 : 7 : 1), (C) *n*-butanol : acetone : water (4 : 5 : 1). The RF values for Sumithion and its derivatives in the tlc systems used are listed in Table 2. Sumithion, Sumioxon and *p*-nitrocresol were completely extracted into benzene from aqueous layer and they were identified by solvent systems (A) and (B). Desmethylsumithion and desmethylsumioxon were extracted into benzene from the acidified aqueous fraction and identified by solvent systems (B) and (C). *p*-Nitrocresyl-β-glucoside was remained in aqueous fraction.

Table 2. Thin-layer chromatographic Rf values of Sumithion and its derivatives in different solvent systems.

compounds	(A)	(B)	(C)
Sumithion	0.95	0.91	—
p-Nitrocresol	0.59	0.73	0.91
Sumioxon	0.29	0.59	—
Desmethylsumithion	0	0.27	0.75
Desmethylsumioxon	0	0.15	0.47
p-Nitrocresyl glucoside	0	0.08	0.80

(A) benzene : ethylacetate (4:1)
(B) toluene : ethylformate : formic acid (5:7:1)
(C) butanol : acetone : water (4:5:1)

The radioactivity was localized by a radio thin layer scanner (Aloka JTC203) or by autoradiography, and identified by cochromatography with the authentic compounds, and radioassayed. The radioassay was made with a liquid scintillation spectrometer (Beckmann LS-230 or Packard Tricarb 3375). Radioactivity unextractable from the tissues was determined after combustion with an autosample oxidizer (Packard Tricarb 305).

Gaschromatographic analysis was made with a Yanagimoto Model 550F and hydrogen flame detecter. A 1-meter glass column was packed with 5% DC-200 and 10% QF-1 on 60- to 80-mesh chromosorb W. Operating temperature was 195°C with helium flow rates of 13m*l* per minute, hydrogen 17 m*l* per minute and air 0.7*l* per minute. Under these conditions, retention times were 5.3 minutes for Sumithion, 6.7 minutes for Sumioxon and 10.2 minutes for Sumithion *S*-isomer.

Results and Discussion

Sumithion initially applied (approximately 4.5 ppm) decreased rapidly during the first day, probably due to evaporation and at 24 hrs post-treatment, 1.5 ppm and 1.2 ppm of Sumithion equivalent of residues were present on the surface and in the tissues, respectively (Fig. 1, Table. 3)

Total carbon-14 on and in apple fruits were 1.4 ppm and 0.87 ppm on 7 th and 14 th day, respectively. The content of internal residue was highest on 1 st day, amounting 1.1 ppm equivalent and from 7 th day on it surpassed the residue on the surface. The possible loss by

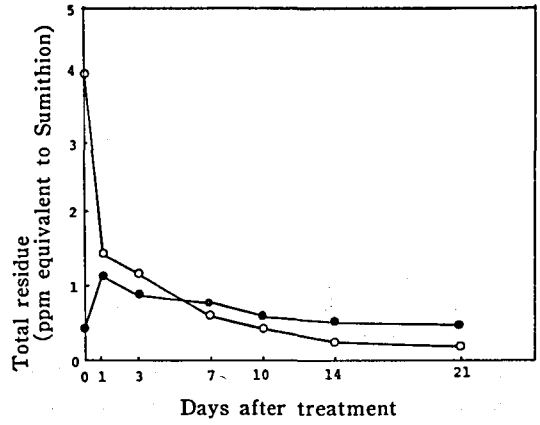

Fig. 1. Content of radiocarbon on and in apple fruits.

○—○ on surfaces
●—● internal

a little rainfall on the initial day was denied by the supplimentary experiments in which extra apple fruits were dipped, and the treated apples were shaded with a polyethylene cover in order to prevent the effect of rainfall, and decrease of Sumithion was compared with the present trial. Degradation of Sumithion on the surface was relatively slow ; on 21 st day nearly 86% of the remaining residue on the surface was Sumithion. On the other hand, in the tissues Sumithion was more rapidly degraded and approximately 45% of ^{14}C were degradation products on 21 st day. Residue of Sumithion in both parts was 2.5 ppm on 1 st day, 1.1 ppm on 7 th day, 0.67 ppm on 14 th day and 0.52 ppm on 21 st day (Table. 3).

Table 3. Sumithion and its degradation products on and in the apple.

Days	Sumithion	Sumioxon	*p*-Nitrocresol	Unidentified[a)]
0	4.35 (3.94, 0.41)[b)]	N. D.	0.002 (N. D., 0.002)	0.101 (0.088, 0.013)
1	2.52 (1.44, 1.08)	0.014 (0.014, N. D.)	0.037 (0.018, 0.019)	0.122 (0.031, 0.091)
3	1.90 (1.15, 0.75)	0.017 (0.017, N. D.)	0.037 (0.020, 0.017)	0.190 (0.045, 0.145)
7	1.12 (0.54, 0.58)	0.014 (0.014, N. D.)	0.044 (0.018, 0.026)	0.255 (0.053, 0.202)
10	0.80 (0.38, 0.42)	0.014 (0.014, N. D.)	0.032 (0.012, 0.020)	0.197 (0.026, 0.171)
14	0.67 (0.25, 0.42)	0.008 (0.008, N. D.)	0.024 (0.006, 0.018)	0.168 (0.025, 0.143)
21	0.52 (0.21, 0.31)	0.005 (0.005, N. D.)	0.024 (0.009, 0.015)	0.277 (0.023, 0.254)

a) ppm equivalent to Sumithion

b) figures of first column in parentheses: on surface; second column: internal.

N. D.: not detected

Slow decrease of Sumithion residue as well as a meager formation of metabolites possibly resulted in part from the cool temperature under the present experimental conditions. Only on the surface Sumioxon was identified, and the amount was 0.017 ppm at its maximum. Sumioxon concentration was relatively constant from 1 st day through 10 th day and on 21 st day it decreased to 0.005 ppm. Another compound tentatively identified on the surface at 3 days was *S*-methyl isomer of Sumithion. By gaschromatographic analysis it was eluted at the retention time corresponding to the authentic *S*-methyl Sumithion.

Sumioxon and *S*-methyl isomer of Sumithion may be formed from Sumithion by radiant energy and atmospheric oxygen, as reported for parathion[16]. *p*-Nitrocresol residue was relatively constant from 1 st to 21 st day, namely 0.01～0.02 ppm either on the surface or in the tissues. Among the water soluble radioactive compounds, neither desmethylsumithion nor desmethylsumioxon was detected, as evidenced by tlc, except that desmethylsumithion was only detected in samples harvested on 21 st day, approximately 2.8% of the remaining residue in apple tissues. Presence of *p*-nitrocresyl-*β*-glucoside was demonstrated in water soluble radioactivity by cochromatography with authentic *p*-nitrocresyl-*β*-glucoside and by hydrolyzing either with 1N hydrochloric acid or with *β*-glucosidase and by identifying the liberated radioactivity as *p*-nitrocresol. That is, after acid treatment of the 21 day aqueous fraction, about 50% of the radioactivity was identified to be *p*-nitrocresol by tlc. Hydrolysis by *β*-glucosidase (emulsion from almonds) was carried out after partial purification of 21 st day aqueous layer by active carbon treatment. The radioactive sample was incubated with enzyme for 20 hrs at 30°C[17]. About 45% of the radioactivity was in the form of glucosylated *p*-nitrocresol. It seems that the absorbed Sumithion was gradually hydrolyzed to *p*-nitrocresol in the tissue and it was conjugated with glucose (Fig. 2).

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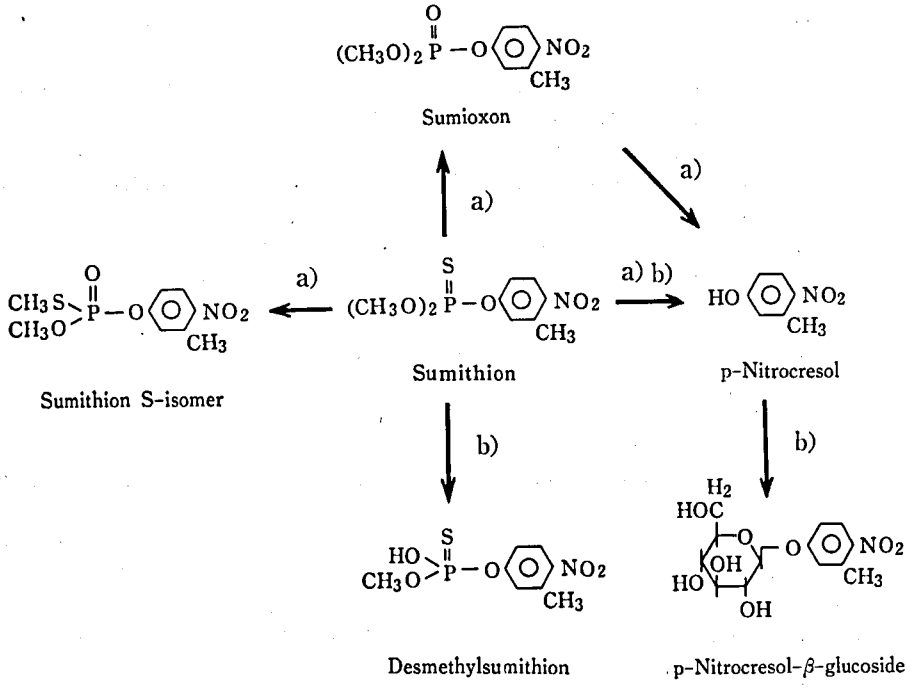

Fig. 2. Metabolic pathway of Sumithion in the apple
a) surface b) internal

carrying out this work. They are indebted to Mr. M. Endo, Mr. M. Hazue, Mr. K. Kawahara and Mr. Y. Takimoto for their skilled technical assistances. Thanks are given to Sumitomo Chemical Co., Ltd. for permission to publish this work.

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Summary

To assess biodegradability and metabolism of Sumithion in apples, radioactive Sumithion labelled with carbon-14 at *m*-methyl group was applied to apple fruits in the field. The rate of decrease of Sumithion was very rapid during 3 days after treatment, approximately 60% of the applied dosage disappeared during the period. The amounts of Sumithion residue on and in apples were 1.12 ppm on 7th day, 0.67 ppm on 14th day and 0.52 ppm on 21th day. The major degradation products on the surface were Sumioxon and *p*-nitrocresol. Sumioxon residue was highest after 3 days, and the amount was approximately 0.02 ppm. Traces of *S*-methyl Sumithion were also found at 3 days. Metabolism of Sumithion in the apple proceeded slowly and water soluble metabolites were gradually increased with time. The glucoside of *p*-nitrocresol was found among the metabolites, which suggests that *p*-nitrocresol derived from Sumithion was conjugated with glucose.

抄　録

ミクロカプセル封入 disparlure 製剤によるマイマイガの交尾抑制について

Disruption of Gypsy Moth Mating with Microencapsulated Disparlure. E. Alan Cameron, C. P. Schwalbe, M. Beroza and E. F. Knipling, *Science*, 183, 972～973 (1974).

マイマイガ (*Porthetria dispar* L.) の性フェロモンは，*cis*-7,8-epoxy-2-methyloctadecane と同定され，disparlure と名付けられている．マイマイガ防除に対する disparlure の利用について検討した．使用した disparlure 製剤の組成は次の如くである．2.2% disparlure キシレン溶液を含有するゼラチンにプラスチック被覆したミクロカプセル（直径 100～250 μm) 17.6%，ラテックス 2%，1%ミニドリフト溶液 29%，1%苛性ソーダ水溶液 1.7%，水 49.7%を混合したもの．

16ha の地域に disparlure を散布し，一定の場所に野外で採集した蛹をズック袋に入れ木の幹にとりつけた．その後，この蛹から羽化した雌が交尾したか，あるいはその産卵がふ化したかをしらべた．同様に，フェロモン処理をしない区域でも行なった．

もっとも効力あったのは，15g/ha を散布した地域で 16対/700m² の蛹をとりつけた時 9.8%の雌が受精卵を産下したにすぎず，コントロール区の47.9%に比べると低く (χ^2 18.08 $p<0.001$)，15g/ha の散布で，32対/700m² という蛹の密度でも 5.4%の受精率でその時のコントロール 53.2% に比べるとずっと低く (χ^2 71.01 $p<0.001$) なっている．使用した製剤は，残効性もよい．（高橋正三）