原　著

Determination of Insecticide Residue in Animal and Plant Tissues. IV. Determination of Residual Amount of Sumithion and Some of Its Metabolites in Fresh Milk. Junshi Miyamoto, Yoshishige Sato and Shin-ichi Suzuki (Agricultural Chemicals Research Department, Osaka Works, Sumitomo Chemical Co., Ltd., Konohana-ku, Osaka, Japan.) Received October 19, 1967. *Botyu-Kagaku*, 32, 95, 1967.

10. 動植物組織中における残留殺虫剤の定量　4. 生ミルク中のスミチオンおよびその2，3の代謝産物の残留量測定　宮本純之，佐藤香重，鈴木信一（住友化学工業株式会社 大阪製造所 農薬研究部）

ミルクは，乳幼児や病人にとって良好な蛋白源となる食品であり，その中の農薬残留は，他の食品にもまして，公衆衛生的見地からみてのぞましいことはない．このような観点から，牧草の害虫防除に用いられたスミチオンが，その牧草を摂食した乳牛よりえたミルク中に残留するかどうかを明らかにしておくことは，きわめて重要である．それゆえ，われわれはスミチオンを体重1kgあたり1mgまたは3mg/日，1週間にわたって経口投与した乳牛よりえらた生乳牛のスミチオン，スミオキソン，アミノスミチオン，3-メチル-4-ニトロフェノールを定量した．3mg/kg/日のスミチオンを投与した牛のミルク中には投与期間中，痕跡量（0.002ppm）のスミチオンがみいだされたが，投与終了後のミルク中には検出されなかった．スミチオンの作用型たるスミオキソンは検出限界以下（0.01ppm以下），アミノスミチオンは最大0.003ppm，3-メチル-4-ニトロフェノールの含量は0.1ppmもしくはそれ以下であった．

以上の結果よりすれば，牧草の害虫防除に用いられたスミチオンが，牛体内を経てミルク中に分泌される可能性はほとんどないと考えられ，また，スミチオンの2，3の代謝産物についてもミルク中に多量に分泌されることはないと考えられる．

Introduction

With the growing interest in the potential hazard of insecticide residue in foodstuffs, no existence of the residue in animal and plant tissues has been becoming one of the most desirable properties of an insecticide. Especially in such a foodstuff as milk which is a good protein resource for new-born babies and invalids, even trace amount of insecticide residue should be undesirable. This report deals with the determination of residual amount of Sumithion* in fresh milk secreted by cows which had been administered Sumithion.

As is widely accepted, phosphorothioates such as Sumithion are converted in animal body into the toxic phosphorates (Sumioxon)** and these oxygen analogs exhibit harmful effects upon the whole animal. On the other hand, Sumithion and Sumioxon are also hydrolytically decomposed and detoxified[2,4,5,6,7]. Besides, Sumithion is presumed to undergo reduction by microorganisms in rumen fluid to form aminosumithion***[1,8]. In this paper, therefore, content of Sumioxon, aminosumithion and 3-methyl-4-nitrophenol as well as Sumithion was determined in fresh milk from cows which had been administered Sumithion.

Materials and Methods

Administration of Sumithion and sampling of milk

Four Jersey cows weghing from 390 to 450kg, which were being bred at the Agriculture and Forestry High School attached to Shimane College of Agriculture (Matsue, Shimane Pref.) were used in this experiment. One mg/kg or 3mg/kg of Sumithion was administered orally to the cows for 7 days. Half of each dosage of Sumithion (50 % emulsifiable concentrate) was mixed properly with wheat flour and given after the regular milking both in the morning and in the evening.

* *O, O*-dimethyl *O*-(3-methyl-4-nitrophenyl) phosphorothioate.

** *O, O*-dimethyl *O*-(3-methyl-4-nitrophenyl) phosphorate.

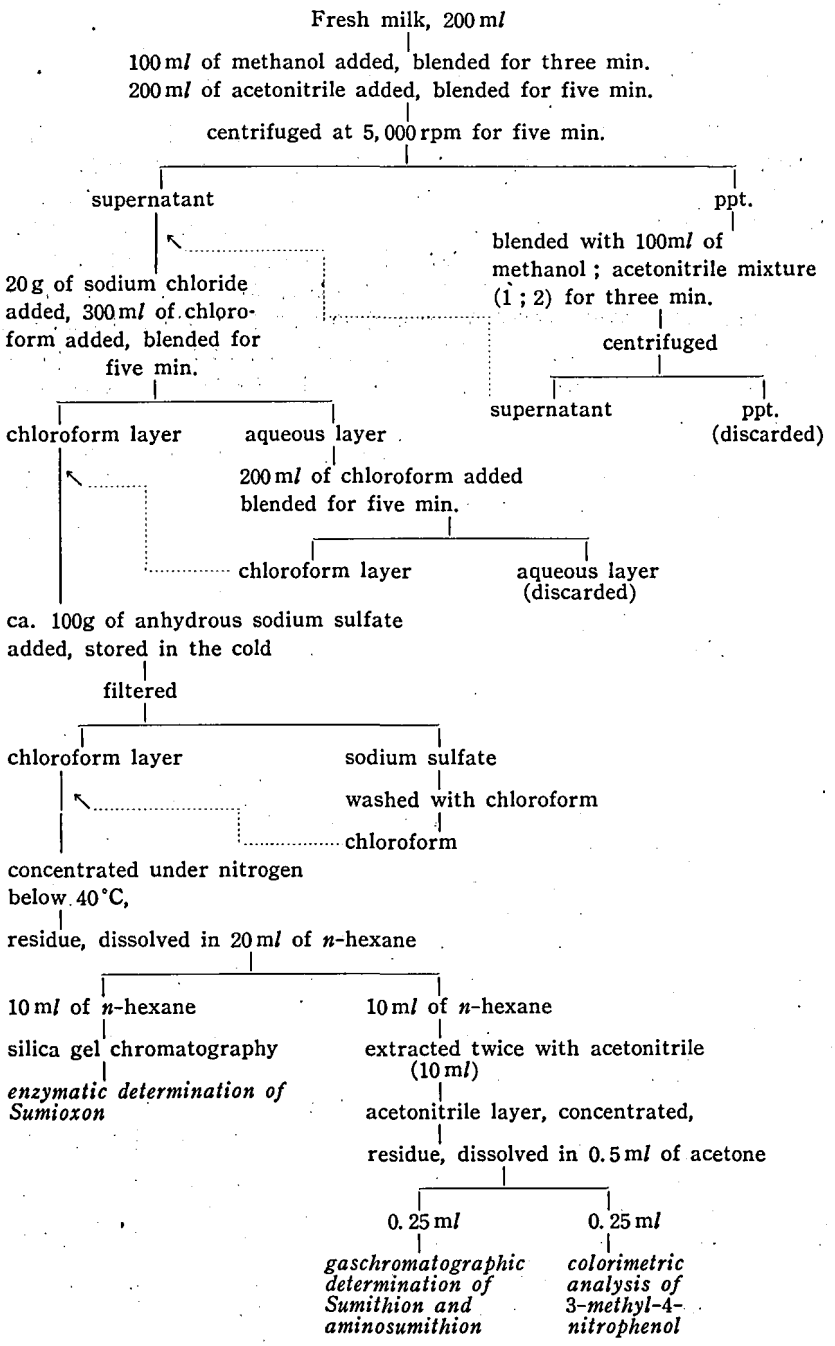
*** *O, O*-dimethyl *O*-(3-methyl-4-aminophenyl) phosphorothioate.

Each animal was supplied on an average with 8.1kg of concentrated fodder and about 70kg of green grass per day. Fresh milk obtained on suitable days during 3 days pretreatment, 7 days treatment and 7 days post-treatment periods was immediately cooled and appropriate portions were placed in a polyethylene bottle. These samples were soon transported to the laboratory.

Extraction and clean-up

Extraction and separation of Sumithion, Sumioxon, aminosumithion and 3-methyl-4-nitrophenol was carried out, as depicted briefly in the following scheme.

Analytical methods



A) Gaschromatographic determination of Sumithion and aminosumithion

Gaschromatographic analysis was conducted with the following operational parameters:

apparatus	Shimadzu Seisakusho, Model GC-3AF, equipped with sodium thermoionic detector (STD).
column	glass 1.5m in length, 4mm in inner diameter (on column injection).
stationary phase	DC-200 4%(w/w), QF-1 8%(w/w) were coated simultaneously on 60/80 mesh acid-washed chromosorb W.
carrier gas	He 1.6kg/cm² inlet press. (ca. 100ml/min).
hydrogen	0.8kg/cm² inlet press. (ca. 40ml/min).
air	0.5kg/cm².
temperature of column and detector	195°C.

The detector used here was a hydrogen flame ionization detector modified to install a source of an alkali metal salt just upon the detector nozzle. This sodium thermoionic detector was found to be highly sensitive to phosphorus-containing compounds. Figs. 1 and 2 show respectively a typical chromatogram of 1μg each of Sumithion and aminosumithion with standard flame detector and of 0.01μg of each with the modified detector. Thus, Sumithion and aminosumithion can be detected in nanogram order by STD. Therefore, when 5μl of the final acetone solution (1g fresh milk equivalent) is injected onto the column, it is possible to determine 0.001ppm each of Sumithion and aminosumithion in fresh milk. None of the control milk extracts gave peaks that interfered seriously with the peaks of Sumithion and aminosumithion (Figs. 3 and 4). The calibration curve of Sumithion and of aminosumithion is reproduced in Fig. 5.

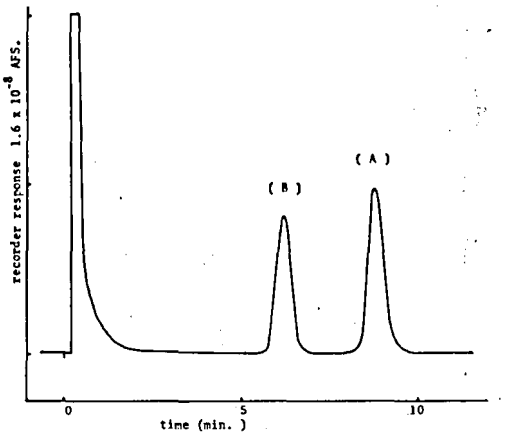


Fig. 1. STD-Gaschromatogram of Sumithion (1×10^{-8}g.) and aminosumithion (1×10^{-8}g.)
(A) Sumithion (B) aminosumithion

Sumioxon was also tried to be analyzed by gaschromatography, but every trial was found unsuccessful, due to its far lower sensitivity to STD. Therefore, this compound was analyzed

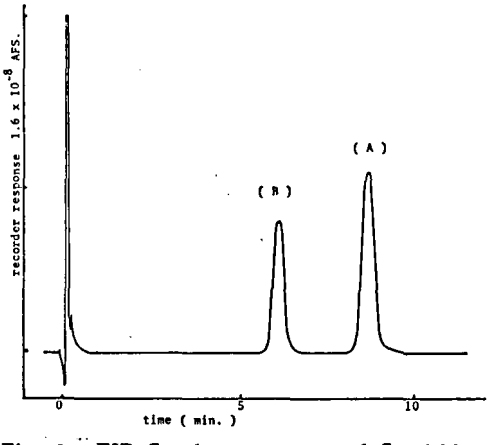


Fig. 2. FID-Gaschromatogram of Sumithion (1×10^{-6}g.) and aminosumithion (1×10^{-6}g.)
(A) Sumithion (B) aminosumithion

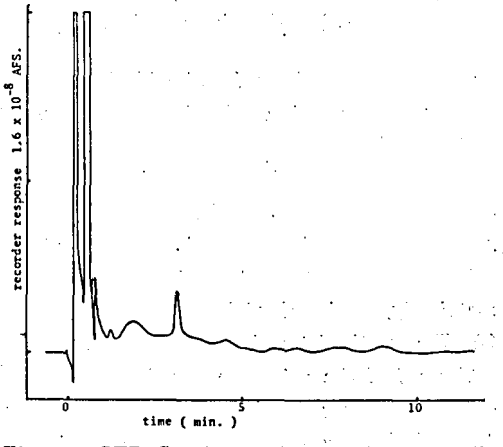


Fig. 3. STD-Gaschromatogram of whole milk control. sample size; 5μl acetone/0.5ml of the final extract (1g. milk eq.)

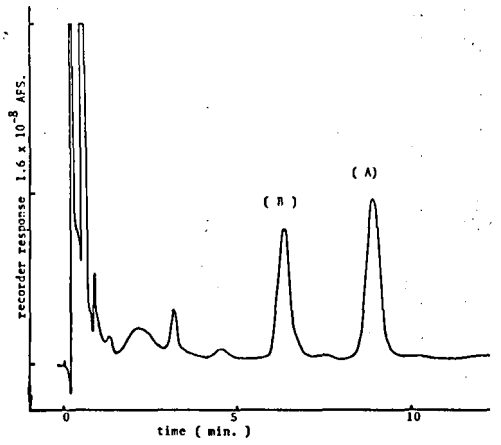


Fig. 4. STD-Gaschromatogram of Sumithion (A) and aminosumithion (B) added to whole milk. sample size; 1g. milk eq. plus Sumithion 1×10^{-8} (0.01 ppm) and aminosumithion 1×10^{-8}g. (0.01 ppm.)

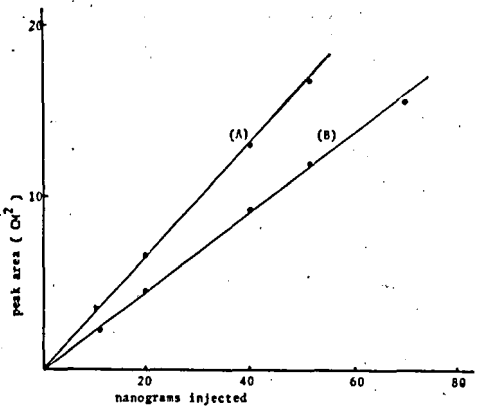


Fig. 5. Calibration curve of Sumithion (A) and aminosumithion (B)

by another method, as described below.

B) Colorimetric determination of 3-methyl-4-nitrophenol

One-quarter m*l* of the acetone solution (50g milk equivalent) was evaporated in vacuo to dryness. 3-methyl-4-nitrophenol therein was separated by thin-layer chromatography and determined colorimetrically, as described in a previous paper[9].

C) Enzymatic determination of Sumioxon

Ten m*l* of the *n*-hexane solution was poured onto the silica gel column (6g of silica gel and 4g of Hyflo Supercel) of 1.8cm in diameter and 15cm in length. The column was further washed with approximately 40m*l* of chloroform. The first 20m*l* of chloroform effluent was discarded and the next 20m*l* of eluted chloroform was collected. Chloroform was evaporated in vacuo to dryness. The residue was dissolved in 0.5m*l* of ethanol, 19.5m*l* of distilled water was added, and the mixture was shaken vigorously. The ethanol-water mixture was diluted with 4 times its volume of distilled water. This solution was used as an inhibitor against fly head cholinesterase.

Inhibition of fly head cholinesterase by Sumioxon was measured by means of conventional Warburg techniques, as in the following.

Reaction mixture:

enzyme solution (fly head 20mg/m*l* 0.15M of sodium chloride)*	1.0m*l*
sodium chloride (1.5M)	0.2
sodium bicarbonate (7.2×10^{-2}M)	1.0
acetylcholine perchlorate (5×10^{-2}M)	0.3
distilled water	0.2
inhibitor solution	0.3
total	3.0m*l*

*Four hundred mg of fly head was homogenized with 20m*l* of 0.15M of sodium chloride in a Potter-Elvehjem homogenizer under cooling.

Vapor phase of the vessel was replaced by a mixture of 95% of nitrogen and 5% of carbon dioxide. After the vessel had been kept at 37.5°C for thirty min., acetylcholine was tipped in from the side arm and carbon dioxide evolved due to the liberated acetic acid was measured. Inhibition (%) of fly head cholinesterase versus Sumioxon

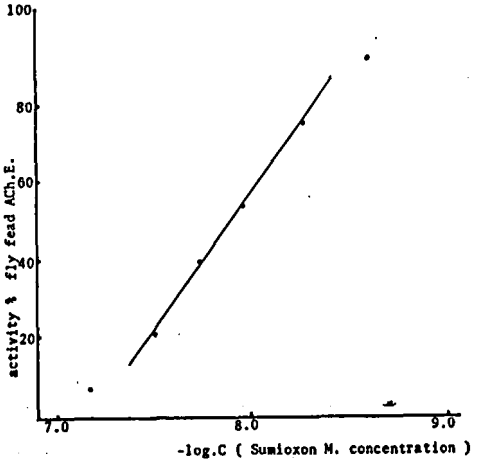


Fig. 6. Fly head cholinesterase inhibition versus Sumioxon concentration.

concentration is shown in Fig. 6. Although aminosumithion was eluted from the silica gel column in the same fraction as Sumioxon under the above conditions, aminosumithion was not inhibitory upon the cholinesterase. None of the control milk extracts were found inhibitory on the cholinesterase. Under the experimental condition 4.35×10^{-9}M of Sumioxon caused 15% of inhibition. This quantity of Sumioxon was calculated to be approximately 0.003μg/3ml reaction mixture (0.3g milk equivalent), that is, 0.01ppm of Sumioxon could be determined.

Results

1) Recovery of Sumithion and its metabolites from fresh milk

The specified amount of Sumithion or its metabolites was added to fresh milk and their recovery was tested. The results in Table 1 demonstrate that the method can be satisfactorily used for the analysis of these compounds.

Table 1. Recovery of Sumithion and its metabolites from fresh milk.

Compound added	Amount added	Recovery
Sumithion	0.1ppm	104%
	0.1	100
Aminosumithion	0.1	83
	0.2	100
Sumioxon	0.2	102
	0.2	97
3-methyl-4-nitrophenol	0.1	101
	0.1	98

2) Sumithion and its metabolites residue in milk

The residual amount in fresh milk of Sumithion

Table 2. Residual amount of Sumithion and its metabolites in fresh milk (ppm).

No. of[1] cows	Control[2] period	Treatment[3] period (day) 1	2	3	5	7	Post-treatment[3] period (day) 1	3	5	7
Sumithion										
1	0.001	—[4]	0.002	—	—	—	—	—	—	—
2	0.001	—	—	0.001	—	0.001	0.002	—	—	—
3	0.001	—	—	—	—	—	—	—	—	—
4	0.001	—	—	—	—	—	—	—	—	—
Aminosumithion										
1	0.001>	0.002	0.003	0.003	—[4]	—	—	—	—	—
2	0.001>	—	0.002	0.002	0.002	—	—	—	—	—
3	0.001>	—	0.002	—	—	0.003	—	—	—	—
4	0.001>	—	—	—	—	—	—	—	—	—
Sumioxon										
1										
2	No inhibition (0.01>)			No inhibition (0.01>)						
3										
4										
3-Methyl-4-nitrophenol										
1	0.09	0.09		0.08		0.08		—[5]		0.08
2	0.10	0.10		0.05		0.05		0.11		0.10
3	0.15	—		—		—		0.08		0.10
4	0.10	—		—		—		0.10		—

1) Nos. 1 and 2 cows, 3mg/kg/day of Sumithion and Nos. 3 and 4 cows, 1mg/kg/day of Sumithion, administered for a week.
2) Control value was mean of results of 3 days.
3) Value after the control values are subtracted.
4) Symbol—means amount less than 0.001ppm.
5) Symbol—means amount less than 0.05ppm.
3-Methyl-4-nitrophenol in samples at the second and fifth days during treatment period and at the first and fifth days during post-treatment period was not determined.

and some of its metabolites was determined and the results are summarized in Table 2. It is evident that Sumithion administered at the rate of 1mg/kg did not result in secreting the detectable amount of the residue, whereas in a dosis of 3mg/kg, Sumithion was found in milk obtained at the second day after the treatment had started and disappeared one day after the treatment had stopped. As to aminosumithion, only a slight amount was found during the treatment period. No detectable amount of Sumioxon (above 0.01 ppm) was found throughout the experimental periods. 3-methyl-4-nitrophenol residue amounting approximately to 0.1ppm was found.

Discussion

The above results indicate that Sumithion given at the rate of 3mg/kg resulted in secreting in milk 0.002ppm of Sumithion and 0.003ppm of aminosumithion at the maximum and trace amount of 3-methyl-4-nitrophenol. Sumioxon above 0.01 ppm was not found. The trace amount of residue of these compounds disappeared from milk in 2 days after the feeding of Sumithion had ceased. Assuming that a cow of 400kg in body weight takes 80kg of forage and that 3mg of Sumithion/kg body weight is administered per day, content of Sumithion in forage can be calculated to be 15ppm. Actual content of Sumithion in forage used for the pest control of pasture will be surmised to be far less than 15ppm, based on the results of residue determination of various vegetables tissues[9,10,11]. Thus, under practical conditions, no residue of Sumithion or its potentially harmful metabolites in milk can be reasonably expected.

The detector used for the gaschromatographic analysis of Sumithion and aminosumithion was a modified flame ionization detector. The source of alkali metal salt was prepared according to the information from Itaya (Shimadzu Seisakusho[3]), that is Pt-Ir helix wire (6 pitches, 4mm in diameter) was soaked into a solution of potassium silicate or sodium silicate and silicate was fused by heating in a gas burner. This tip was installed just upon the ceramic flame nozzle. The STD was found to be highly sensitive to organophosphorus compounds and much less sensitive to other carbon compounds. Although at present it can be satisfactorily used for about ten hrs. (sensitivity to phosphorus gradually decreases), this detector makes far easier the micro-determination of organophosphorus insecticide residue in various animal and plant tissues, if combined with the suitable clean-up procedures.

Acknowledgement: The authors are indebted to Prof. M. Kato, Laboratory of Stock Raising, Shimane College of Agriculture and Dr. N. Seo, Agriculture and Forestry High School attached to Shimane College of Agriculture for their cooperations under which this work was carried out. Technical assistance of Miss Y. Omori is appreciated.

Literatures cited

1. Ahmed, M.K., Casida, J.E. and Nichols, R.E.: *J. Agri. Food Chem.*, 6, 740 (1958).
2. Hollingworth, R.M., Metcalf, R.L. and Fukuto, T.R.: *ibid*, 15, 242 (1967).
3. Itaya, M.: private communication.
4. Miyamoto, J., Sato, Y., Kadota, T., Fujinami, A. and Endo, M.· *Agri. Biol. Chem.*, 27, 381 (1963).
5. Miyamoto, J., Sato, Y., Kadota, T. and Fujinami, A.: *ibid*, 27, 669 (1963).
6. Miyamoto, J.: *ibid*, 28, 411 (1964).
7. Miyamoto, J.: *ibid*, 28, 422 (1964).
8. Miyamoto, J., Kitagawa, K. and Sato, Y.: *Jap. J. Exp. Med.*, 36, 211 (1966).
9. Miyamoto, J., Kawaguchi, Y. and Sato, Y.: *Botyu-Kagaku*, 30, 9 (1965).
10. Miyamoto, J. and Sato, Y.: *ibid*, 30, 45 (1965).
11. Miyamoto, J.: unpublished.