their appreciation to Dr. T. Suzuki and Mr. A. Sugimoto, Agricultural Chemicals Inspection Station, for their valuable suggestions and criticisms.

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Determination of Insecticide Residue in Animal and Plant Tissues. VI. Determination of Sumithion Residue in Cattle Tissues. Junshi Miyamoto and Yoshishige Sato (Agricultural Chemicals Research Department, Osaka Works, Sumitomo Chemical Co., Ltd. Osaka) Received December 27, 1968, *Botyu-Kagaku* 34, 3, 1969.

2. 動植物組織中における残留殺虫剤の定量. VI 牛の組織中におけるスミチオン残留量の測定

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） 42. 12. 27 受理.

牧草の害虫防除に用いられたスミチオンの牛体内における残留をしらべるため，125 g/ha（通常の使用量）および 375 g/ha の割合でスミチオンを牧草地に散布し，その直後に放牧した牛の筋肉および脂肪組織中のスミチオンを経時的に定量した．

散布後24時間では，筋肉中に約 0.01ppm のスミチオンが残留するが，72時間以降では，両散布区とも筋肉中のスミチオン含量は 0.001 ppm もしくはそれ以下である．また高濃度散布区に放牧した牛の脂肪組織中には 72 時間後でごくわずかのスミチオンが見出されたが，7 日以後では，その量はほとんど無視できるくらいであった．

これらの結果からすれば，牧草とともに牛体内にとり入れられたスミチオンは，すみやかに分解されて消失し，その残留量は何ら問題とするに足りないと考えられる．

Introduction

Sumithion®, fenitrothion or *O,O*-dimethyl *O*-(3-methyl-4-nitrophenyl) phosphorothioate, is an organophosphorus insecticide characterized by the broad spectra as well as by low toxicity to warm-blooded animals and is now being widely used in the world to control harmful insects of various crops. This compound was found recently to be tremendously effective against locusts of pasture grass in Argentina and now it is put into practical use in the country. In order to assure safe usage of Sumithion from the view-point of public health, its residue in cattle tissues was determined after cattle had been kept on the pasture sprayed with Sumithion.

Materials and Methods

Sumithion spray and sampling[1]

Sumithion emulsion up to 125g/ha (usual application condition) and 375 g/ha in terms of the active ingredient was sprayed respectively to 5 ha (area A) and 8 ha (area B) of the test field in the suburbs of Buenos Aires, Argentina on July 23, 1968 and immediately thereafter each 10 head of cattle of about 300 kg in body weight were confined to the enclosures. One, three, seven and ten days after spraying, each 2 head of cattle were withdrawn from the area A and B and sent to a slaughter house. The next morning the animals were sacrificed, meat (breast muscle) and tallow (omental fat) dissected out and immediately transported to the laboratory.

Extraction of Sumithion and analysis.

i) Meat[2]

Fifty grams of minced meat was homogenized with 50 m*l* of water and 110 m*l* of ethanol, together with 5×10^{-6}g of ethylparathion as internal standard of gaschromatography, in a mixer for 30 sec., and then for further 1 min. after addition of 240 m*l* of benzene. The mixture was centrifuged at 2,500 rpm for 15 min. with an International Centrifuge, size 2, type V. The upper, benzene layer was separated and the lower layer as well as the precipitate was homogenized with 150 m*l* of benzene. Benzene layer was separated as above. Combined benzene layers were dehydrated over 100 g of anhydrous sodium sulfate overnight at room temperature. Sodium sulfate was separated by filtration, washed with 100 m*l* of benzene and

discarded. Benzene was evaporated *in vacuo* at 40～43°C and the residue was dissolved in 35 m*l* of *n*-hexane. *n*-Hexane was shaken with 15 m*l*, then 10 m*l* of acetonitrile saturated with *n*-hexane and acetonitrile layers separated was concentrated under the stream of air at 40～43°C. The residue was dissolved in 1 m*l* of acetone.

ii) Tallow.

Fifty grams of omental fat was homogenized with 250 m*l* of *n*-hexane and 5×10^{-6}g of ethyl parathion in a mixer for 1 min., then 200 m*l* of acetonitrile and ca. 20 g of Hyflo Supercel were added and the mixture was further homogenized for 1 min. Acetonitrile layer was separated with a Buchner funnel. *n*-Hexane and precipitate were blended again with 150 m*l* of acetonitrile and acetonitrile was separated. After kept stand overnight over 30 g of anhydrous sodium sulfate, acetonitrile layer was separated by filtration, sodium sulfate washed with 50 m*l* of acetonitrile. Acetonitrile layers were concentrated *in vacuo* at 40～43°C to a volume of about 25 m*l* and swirled with 30 m*l* of *n*-hexane saturated with acetonitrile. Acetonitrile layer was transferred to a smaller round-bottled flask and evaporated under the stream of air. The residue was dissolved in 1 m*l* of acetone. The method described above seems useful in extracting organophosphorus insecticides from fatty tissues in that no further elaborate clean-up processes are required to remove lipid substances contaminated which often encounters in the case of other extraction methods.

iii) Gaschromatographic determination of Sumithion

Varian Aerograph type 2100 gaschromatograph (on column injection) equipped with phosphorus detector (KBr single crystal[3]) was used. Operational parameters were as follows.

Column; glass column of 2 mm in inner diameter and 1.8 m in length, packed with DC-200 2.5% and QF-1 2.5% coated on acid-washed, HMDS-treated Aeropack, 80～100 mesh.

Temperature; column 180°C
detector 230°C
injector 220°C

Flow rate; carrier gas (nitrogen) 38 m*l*/min.
hydrogen 50 m*l*/min.
air 140 m*l*/min.

Under the above conditions retention time of Sumithion and ethyl parathion was 6'55" and 8'05" respectively. And at electrometer setting of 10^{-10}, attenuation x2, Sumithion and ethyl parathion at 5 ng gave peaks approximately 70% of full scale of the recorder (2 mV full scale) or 0.1 ng of Sumithion can be detectable. Control samples of

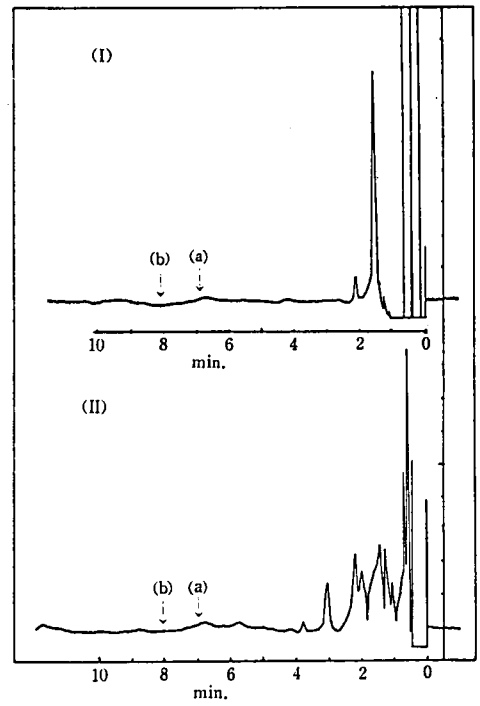

Fig. 1. Gaschromatogram of control meat (I) and tallow (II) samples.
(a) Sumithion and (b) ethyl parathion position.

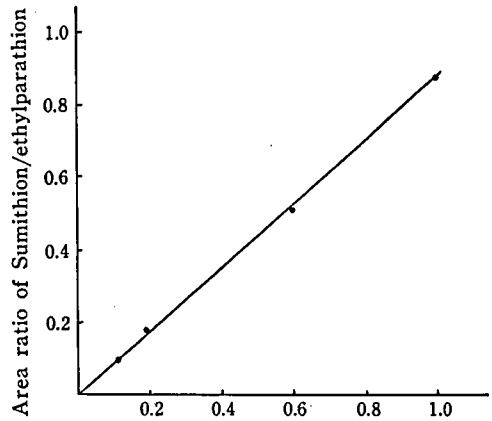

Fig. 2. Calibration curve of Sumithion.

meat and tallow contained, as indicated in Fig. 1, no appreciable amount of impurities at the retention time of either Sumithion or ethyl parathion. Calibration curve of Sumithion using ethyl parathion as internal standard is shown in Fig. 2. In determining residue in the samples, 2 μl of the final acetone solution was injected on to the column and peak areas of both Sumithion and ethyl parathion were measured on the chromatogram and content of Sumithion was calculated by using the calibration curve.

iv) Recovery experiment.

Meat or tallow sample fortified with 0.1 ppm equivalent of Sumithion at the initial step of extraction was processed as described above and recovery of Sumithion was tested. In this case ethyl parathion as internal standard was added to the sample at the final step, namely the final residue obtained was dissolved in acetone containing appropriate amount of ethyl parathion. The recovery of Sumithion from meat and tallow sample was 97% and 102%, respectively.

Results and Discussions

Two head each of cattle were withdrawn from each test field (area A and B) after the specified interval and sacrificed and content of Sumithion in both breast muscle and omental fat was determined. Typical chromatograms are reproduced in Fig. 3 and the summarized results of determination are indicated in Table 1, in which all the values are expressed after the control values shown at the bottom of the Table have been subtracted. The results in Table 1 show that from the third day after spraying, no detectable amount of Sumithion was contained in both tissues of cattle on the area A although tissues of the animals withdrawn and sacrificed on the next day of spraying contained minute amount of Sumithion. On the other hand in omental fat (but not in meat) of cattle on the area B a little amount of sumithion residue was detected on the third day, which decreased, however, nearly to the control level on around the seventh day. As is widely accepted[4], in residue analysis content exceeding double to four times of mean control value is indicative of the presence of pesticide residue, because control values are often inevitably fluctuated. Taking the considerations into account, presence of Sumithion residue can be unambiguously demonstrated only in meat of cattle on the area A and B after 0-24 hr. of exposure to

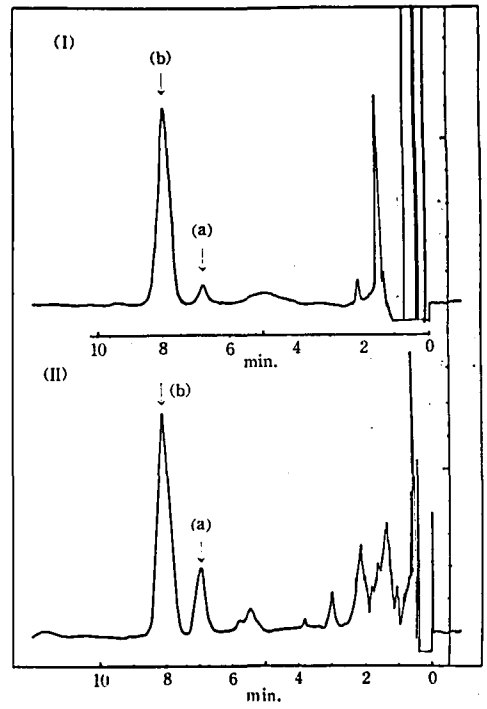

Fig. 3. Gaschromatogram of treated meat (I) (area B 48 hr exposure) and tallow (II) (area B, 24 hr exposure) samples.
(a) Sumithion and (b) ethyl parathion position.

Table 1. Content of Sumithion in Cattle Tissues.

Days*1)	Sumithion content, ppm*2)			
	125 g/ha spray (A)		375 g/ha spray (B)	
	Meat	Tallow	Meat	Tallow
1	0.007>	0.002	0.014	0.014
	0.011	0.001>	0.009	0.003
3	0.001>	0.001>	0.001	0.007
	0.001>	0.001>	0.001>	0.004
7	0.001>	0.001>	0.001>	0.005>
	0.001>	0.001>	0.001>	0.001>
10	—*3)	0.001>	—*3)	0.001
		0.001		0.004
control	0.001>	0.003	0.001>	0.004

*1) Days during which cattle kept on the pasture sprayed with Sumithion.
*2) Each value corresponding to each cattle is means of duplicated trials, after control value has been subtracted.
*3) Not tested.

Sumithion and in omental fat of cattle on the area B after 0-72 hr. of exposure. Under practical conditions (late spring or early summer), decrease of Sumithion on the pasture is expected to be faster as described elsewhere[1], so cattle take up less amount of Sumithion, which will result in less residue of Sumithion in cattle tissues.

Thus, the above results of determination of Sumithion residue in cattle tissues might imply that Sumithion undergoes rapid degradation into non-toxic compounds in animal body and they are excreted, and that accumulation, e. g. in omental fat and other adipose tissues seldom occurs, similarly to the case of other warm-blooded animals[5,6,7,8]. This organophosphorus insecticide can be, therefore, safely used for the insect control on the pasture without seriously considering any noxious residues of the compound in cattle tissues.

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Sterilizing Effect of Hempa on *Drosophila melanogaster* Meigen. Studies on the Chemosterilants of Insects. XV. Isamu NAKAYAMA, Sumio NAGASAWA and Haruko SHIMIZU (Research Laboratory, Kumiai Chem. Indust. Co., Shimizu) *Botyu-Kagaku* 34, 6, 1969. Received January 22, 1969. (with English Summary 12)

3. Hempa のキイロショウジョウバエに対する不妊作用.*

昆虫の化学不妊剤に関する研究. 第15報. 中山 勇・長沢純夫・清水春子（クミアイ化学工業株式会社研究所）44. 1. 22 受理.

Hempa をもちいて，化学不妊剤の効果を詳細に究明するときの試験用昆虫としてキイロショウジョウバエの利用価値を，イエバエのそれと比較しながら検討した．不妊効果を適正に判定しうる試験条件を定めた後，Hempa に対する両種の感受性を調べた．さらにキイロショウジョウバエの雌雄別の投与と，投与後の経過にともなう不妊効果の推移から，その作用性を推定した．

イエバエは，化学不妊剤の供試昆虫として広く利用されているが，その効果をより詳細に究明する場合は，雌の生存期間が長く，産卵が断片的であるなどの理由から，正確な効果を把握する事が困難で，さらに試験期間が長期にわたるため，多大の労力を必要とする欠点を持っている．イエバエに代わりうる試験用昆虫としてキイロショウジョウバエの利用を今回試みた．ショウジョウバエに対する化学不妊剤の投与は，注射 (Fahmy and Fahmy[4])，滴下 (Kido and Stafford[8]) 毒餌 (Cantwell and Henneberry[2])，などの方法によっておこなわれているが，ここでは種々の薬剤を選抜試験する場合にも，溶解性に左右されず，投与可能な毒餌法を採用し，hempa をもちいて，イエバエと比較しながら検討した．本文に入るに先立ち，キイロショウジョウバエをお譲りいただいた国立遺伝研究所の大島長造博士，および試料の御提供を戴いた米国農務省の A. B. Borkovec 博士に深謝の意を表する．

実験材料および方法

供試薬剤：実験にもちいた hempa は米国農務省より送られたENT-50882aの試料番号を有するresearch

* 1968年4月2日，日本応用動物昆虫学会大会(東京)において講演発表.