Studies on Persistence of Isoxathion. I. Residue Determination of Isoxathion and Its Oxygen Analog and Preliminary Studies on Persistence of Isoxathion in Crops and Soils. Toshiie NAKAMURA, Katashi YAMAOKA and Takeshi SAITO (Sankyo Co., Ltd., Agricultural Chemicals Research Laboratories, Yasu-cho, Shiga, Japan) Received July 23, 1976. *Botyu-Kagaku*, 42, 32, 1977.

3. イソキサチオンの残留性の研究（第1報）作物および土壌中のイソキサチオンとそのオクソン体の残留分析法および残留性の予検討

中村利家，山岡 剛，斉戸猛（三共株式会社農薬研究所，滋賀県野洲町野洲1041） 51. 7. 23 受理

殺虫剤イソキサチオン［カルホス®，*O*, *O*-diethyl-*O*-(5-phenyl-3-isoxazolyl)-phosphorothioate］およびその酸化代謝物としてのオクソン体の残留分析法を確立し，植物と土壌中における残留性をメチルイソキサチオン［ダイメックス®，*O*, *O*-dimethyl-*O*-(5-phenyl-3-isoxazolyl)-phosphorothioate］およびフェニトロチオンと比較した．イソキサチオンはFPDまたはFTD-GLCにより0.2～0.3ngの検出が可能で，アセトン抽出後分配転溶法，カラムクロマトグラフィー等の組合わせにより比較的容易にクリーンアップできる．種々の作物と土壌への添加回収率は90%以上で検出限界も満足できるものであった．オクソン体のGLC最少検出量は5ngでありクリーンアップも困難になるが，添加回収率80%以上，検出限界0.05ppmを可能とした．残留性予検討は，インゲンおよび砂壌土を用い，3化合物の同時処理での比較をおこなった．本実験での消失速度を半減期で見ると，インゲンではイソキサチオン（5日）＜ダイメックス（2日）≒フェニトロチオン（1日），土壌中ではイソキサチオン（18日）＜フェニトロチオン（10日）＜メチルイソキサチオン（5日）であった．イソキサチオンは，植物でも土壌中でも有機リン剤としてはある程度安定性があり，茎葉散布剤としても土壌殺虫剤としても適度の残効性が期待できると思われる．メチルイソキサチオンは植物ではフェニトロチオン並の安定性だが，土壌中では極めて残留性の少ない化合物として興味がある．

Isoxathion, or Karphos®, *O*, *O*-diethyl-*O*-(5-phenyl-3-isoxazolyl)-phosphorothioate[1], is a registered insecticide having a broad spectrum and being expansively used for the control of many plant and soil insects[2~5].

Its acute mammalian toxicity is moderate, as shown with an acute oral LD_{50} of 112mg/kg to rats[6], and the non-effect level to rats in 90 days consecutive feeding test is approximately 1mg/kg/day. The fates of this chemical in rats[7], plants[8] and soils[9] had been reported. Isoxathion was readily metabolized to non-toxic compounds in rats and rapidly excreted mainly in the urine. It was moderately stable on plants and in soils, and the oxygen analog as an active metabolite was not detectable or occasionally detected only trace amount.

This work was initiated to know the persistence of isoxathion in various crops, soils and foodstuffs. The present paper deals with the residue determination of isoxathion and its oxygen analog by gas chromatography (GLC), and with the preliminary comparative studies on persistence of isoxathion, methylisoxathion[1] [Dymex® *O*, *O*-dimethyl-*O*-(5-phenyl-3-isoxazolyl) phosphorothioate] and fenitrothion (Sumithion®) on kidney beans and in a sandy loam soil under a laboratory condition.

Materials and Methods

I. Residue Determination Procedures

Apparatus and reagents

Solvents; all reagent grade. Florisil (FLORIDIN Co.); activated at 130°C for 3 hr and stored in a desiccator. Darco G-60 (activated charcoal, ATLAS POWDER Co.) and Celite 545 (JOHNS-MANVILLE SALES CORP.); guaranteed grade. Isoxathion; purified by the silicic acid column chromatography using 20% diethylether in hexane as an eluting solvent, approximately 99%. Isoxathion oxygen analog; prepared by the bromine oxidation of pure isoxathion and purified by the silica gel thin-layer chromatography using 20% acetone in hexane as a developing solvent,

approximately 98%. Triphenylphosphate (TPP) as an internal standard; analytical grade. Surecide® (CYP), *O*-*p*-cyanophenyl-*O*-ethyl phenylphosphonothioate, as an internal standard; recrystallized two times from methanol, mp 82.0-82.5°C.

Blendor; Universal homogenizer with 500m*l* cup (NIHON SEIKI SEISAKUSHO Co. Ltd.), or new compact Omni Mixer (IVAN SORVAL Inc.) with 1 pint Mason Jar. Shaker; V/D type KM shaker (IWAKI Co.).

Gas chromatograph; Micro Tek MT-220 equipped with a flame photometric detector (FPD) or Hewlett-Packard 7620A equipped with a flame thermionic detector (FTD, KCl tip).

Extraction and cleanup

Isoxathion

Vegetables, Fruits, Unpolished Rice Grains and Tobaccos: An appropriate amount of each sample (for example, 100g for vegetables and fruit pulps, 20-50g for fruit peels, 25-50g for rice grains and 20-25g for tobaccos) after finely chopping or grinding was blended with 100m*l* of acetone for 5 min and filter through a glass filter (17G3). The filtered cake was two more times blended with 100m*l* portions of acetone. The filtrates were combined and freed of solvent, and the residue was transferred to a 300m*l* separatory funnel with 100m*l* of 10% aq. solution of sodium chloride and 50m*l* of hexane. After shaking for 5 min, the hexane layer was separated. The aqueous layer was two more times extracted with 50m*l* portions of hexane. The hexane layers were combined and dried over anhydrous sodium sulfate and then evaporated to dryness. The residue was dissolved in 5m*l* of 15% diethyl ether in hexane and applied to the Florisil column, which was prepared by adding 5g of the activated Florisil as a slurry with 15% diethyl ether in hexane into a 15mm i.d. × 30cm column plugged with glass wool, and was eluted with 100m*l* of 15% diethyl ether in hexane as the eluting solvent. The eluate was evaporated to dryness and 1 to 5m*l* of the acetone solution containing a known amount of the internal standard was added. An aliquot of this solution was injected into the pre-conditioned gaschromatograph. The GLC operating conditions are given in Table 1, A for vegetables, fruits, tobaccos and C for unpolished rice grains.

Green Teas: Ten grams of the ground sample was macerated overnight with 100m*l* of acetone, blended in a Waring blendor for 5 min, and filtered through a glass filter (17G3). The filter cake was two more times extracted with 100m*l*

Table 1. Gas Chromatographic Conditions

Condition	A	B	C
Apparatus	Micro Tek MT-220 FPD (p-mode)	Hewlett-Packard 7620 A FTD (KCl tip)	
Column	4% XE-60 on Gas Chrom Q (80/100 mesh) 3 mm i.d. ×1.5 m	5% SE-52 on Gas Chrom Q (80/100 mesh) 3 mm i.d. ×1.2 m	3% OV-17 on Chromosorb W (AW, DMCS) (80/100 mesh) 3 mm i.d. ×1.0 m
Temperature (°C)			
column	225 (215[1])	245	225
injector	250	260	250
detector	250	265	250
Gas flow			
carrier	N_2 55 (m*l*/min)	N_2 120 (m*l*/min)	He 50 (m*l*/min)
hydrogen	130	40	40
air	45	700	400
oxygen	20	—	—
Chart speed	0.5 (cm/min)	1 (cm/min)	1 (cm/min)
Internal standard	TPP	TPP	CYP

[1] for the oxygen analog analysis

portions of acetone. The filtrates were combined and freed of solvent. The residue was transferred to a 100m*l* separatory funnel with 30m*l* of hexane saturated with acetonitrile and 30 m*l* of acetonitrile saturated with hexane. After shaking for 5 min, the acetonitrile layer was separated. To the hexane layer, 30m*l* of acetonitrile saturated with hexane was added, and the same procedure was repeated. The acetonitrile layers were combined and evaporated to dryness. The residue was dissolved in 5m*l* of 15% diethyl ether in hexane and cleaned up on the Florisil column as described above. The eluate was evaporated and dissolved in 1 to 5m*l* of the internal standard solution. This solution is ready for GLC. The operating conditions are given in Table 1, B.

Rice Straws: Twenty-five grams of the ground sample was extracted with 200 m*l* of acetone using a Soxhlet extractor for 6 hr. The extract was freed of solvent and the residue was transferred with 30m*l* of hexane saturated with acetonitrile and 30m*l* of acetonitrile saturated with hexane to a 100m*l* separatory funnel. After shaking for 5 min, the acetonitrile layer was separated. To the hexane layer, 30m*l* of acetonitrile saturated with hexane was added, and the same procedure was repeated. The acetonitrile layers were combined and evaporated to dryness. The residue was dissolved in 5m*l* of 15% diethyl ether in hexane and cleaned up on the Florisil column as described above. The eluate was evaporated, dissolved in 1 to 5m*l* of the internal standard solution, and analyzed by GLC. The operating conditions are given in Table 1, C.

Soils: Twenty grams of soil sample based on dry weight after well-mixing and removing stones and plant debris were blended with 200m*l* of acetone for 15 min and filtered through a glass filter (17G3). The filter cake was washed twice with 30m*l* portions of acetone. The filtrates were combined and freed of solvent. The residue was transferred to a 100m*l* separatory funnel with 30m*l* of 10% aq. solution of sodium chloride and 30m*l* of dichloromethane. After shaking for 5 min, the dichloromethane layer was separated. The aqueous layer was two more times extracted with 30m*l* portions of dichloromethane. The dichloromethane layers were combined, dried over anhydrous sodium sulfate, and evaporated to dryness. The residue was dissolved in 1 to 5m*l* of the internal standard solution and analyzed by GLC. The operating conditions are given in Table 1, B.

Oxygen Analog

Vegetables: One hundred grams of the finely chopped sample was blended with 100m*l* of acetone for 5 min and filtered. The filter cake was two more times extracted with 100m*l* portions of acetone. The filtrates were combined and freed of solvent. The residue was transferred to a 300m*l* separatory funnel with 100m*l* of 10% aq. solution of sodium sulfate and 50m*l* of dichloromethane. After shaking for 5 min, the dichloromethane layer was separated. The aqueous layer was two more times extracted with 50m*l* portions of dichloromethane. The dichloromethane layers were combined, dried over anhydrous sodium sulfate, and evaporated to dryness. The residue was transferred to a 200m*l* separatory funnel with 50m*l* of acetonitrile saturated with hexane and 50m*l* of hexane saturated with acetonitrile. After shaking for 5 min, the acetonitrile layer was separated. The hexane layer was two more times extracted with 50m*l* portions of acetonitrile saturated with hexane. The acetonitrile layers were combined and evaporated to dryness. The residue was dissolved in 5m*l* of acetone and applied to the charcoal column, which was prepared by adding 5g of the mixture of Darco G-60 and Celite 545 (95 : 5 by weight) as a slurry with acetone into a 15mm i.d. × 30cm column, and was eluted with 100m*l* of acetone. The eluate was evaporated to dryness, dissolved in 1 to 5m*l* of the internal standard solution and analyzed by GLC. The operating conditions are given in Table 1, A. (Operated at 215°C column temperature.)

Gas Chromatography (GLC)

The operating conditions for Micro Tek MT-220 equipped with FPD and Hewlett-Packard 7620A equipped with FTD are summarized in Table 1.

The stationary phases had been pre-conditioned for 24 hr at 250°C and further conditioning before use was achieved by repeated injections of 100ng of isoxathion and its oxygen analog until peak height became reproducible. As the oxygen analog

gave especially poor response, the repeated injections were required to obtain the good reproducibility and linear detector response.

Calibration and determination: The amounts of isoxathion or its oxygen analog in the cleaned up samples were determined by comparing their peak heights with that of the internal standard and by reference to a calibration curve prepared by plotting weight ratios against peak height ratios of isoxathion or its oxygen analog to the internal standard.

II. Preliminary Comparative Study on Persistence

Chemicals

Purified chemicals: Isoxathion; purified as above mentioned. Methylisoxathion; purified by the repeated recrystallization from hexane under refrigeration with dry-ice acetone, approximately 99%. Fenitrothion; analytical grade.

Emulsifiable concentrates (EC): Isoxathion, methylisoxathion and fenitrothion EC; prepared by dissolving 50w/w% of each chemical as active ingredient and 18w/w% of paracol SL-15 (an emulsifier, NIHON NYUKAZAI Co.) into xylene.

Plant material and treatment

One week-old seedlings of kidney beans, var. Ōtebō, were transplanted and grown in soil contained in pots in a green house. When the plants grew approximately two foliage stage, they were treated with the combined spray solution containing each 500ppm as an active ingredient of isoxathion, methylisoxathion and fenitrothion in the formulation of EC.

Duplicate plants were sampled at appropriate intervals after the treatment, cut off roots, and weighed. The residues of three chemicals were determined to the fresh weight basis of plants.

Soil material and treatment

A sandy loam soil from a field in Hiratsuka, Shizuoka prefecture, was air-dried, passed through a 2 mm sieve, and moistened to the extent of about 50% to the maximum water holding capacity of this soil. Some chemical and physical properties of the soil are given in Table 2.

One and a half kg of the soil in an enameled tray (20×30cm) was treated with 10m*l* of acetone solution containing each 15mg of isoxathion, methylisoxathion and fenitrothion. The solution was prepared by using purified chemicals. The treated soil was thoroughly mixed, covered with a transparent resin plate, and incubated in a room maintained at 25°C. A 10~20g portion of the soil was sampled at appropriate intervals after mixing well every time, and the residues of three chemicals were determined to the dry basis of the soil.

No additional moisture supplied to soil in the course of this experiment: The moisture content was approximately 30% at first, 20% at 30 days and 11% at 100 days after the treatment.

Residue determination

The extraction and cleanup procedures of isoxathion, methylisoxathion and fenitrothion from plants and soils were carried out in a manner similar to the determination of isoxathion described above.

The determination by GLC was accomplished successfully by the operating condition B in Table 1.

Results and Discussion

Residue Determination of Isoxathion and Its Oxygen Analog

The residue determination of isoxathion and its oxygen analog was forced to separately carry out, because both chemicals differed markedly in their chemical behavior and the response to gas chromatographic detectors. Either the flame photometric detector (FPD) with a phosphorous filter (526nm) or the flame thermionic detector (FTD) with a potassium chloride tip was satisfactory for the determination of isoxathion and its oxygen analog.

The GLC conditions conducted in this work are given in Table 1. In the FPD analysis, the minimum detectable amounts on the XE-60 column were 0.2ng for isoxathion and 5ng for the oxygen analog. In the FTD analysis, the minimum detectable amounts on the SE-52 column for both chemicals were 0.3ng and 5ng, respectively. The retention times of both chemicals on various GLC columns are given in Table 3. A complete separation of isoxathion from its oxygen analog

Table 2. Physico-Chemical Properties of Hiratsuka Soil.

Soil type	Total carbon (%)	pH (H_2O)	Soil texture (%) Sand	Silt	Clay	CEC (meq/100 g)	Moisture holding capacity (%)
Sandy loam (Volcanic ash soil)	1.87	5.95	69.8	19.6	10.6	18.5	63.4

Table 3. Retention Times of Isoxathion and Its Oxygen Analog on Various Gas Chromatographic Columns.

Column	Column temperature (°C)	Isoxathion (min)	Oxygen Analog (min)
XE-60 (4%)[1]	215	2.84	3.76
	225	2.19	2.36
SE-52 (5%)[1]	245	0.72	0.80
OV-17 (3%)[1]	225	2.05	1.99
DC-200 (5%)[2]	220	2.48	2.10

1) The operating conditions are given in Table 1.
2) Micro Tek MT-220, FPD; 5% DC-200 on Gaschrom Q (80/100 mesh), 3mm i.d. ×1.8m; column 220°C, injector 205°C, detector 195°C; carier N_2 80 ml/min, hydrogen 120 ml/min, oxygen 20 ml/min, air 40 ml/min.

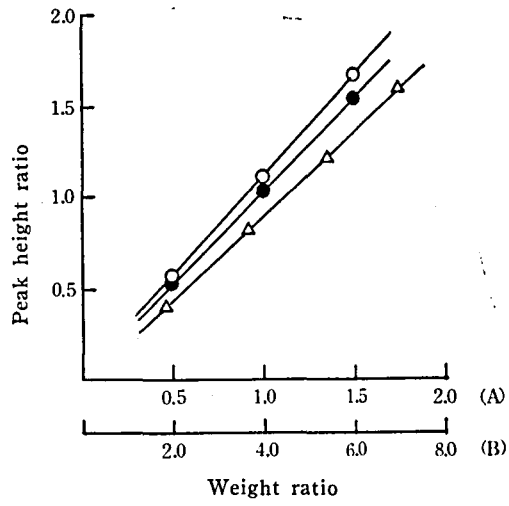

Fig. 1. Typical Calibration Curves of Isoxathion and Its Oxygen Analog.
○—○ Isoxathion (GLC condition A in Table 1)
●—● Isoxathion (GLC condition B in Table 1)
△—△ Oxygen Analog (GLC condition A in Table 1)
(A) Weight ratio for isoxathion
(B) Weight ratio for oxygen analog

was successful by using the XE-60 column at 215°C of the column temperature as shown in Table 3.

Triphenylphosphate (TPP) or Surecide® (CYP) was selected for an internal standard substance in this study, since the retention times were appropriate to isoxathion or the oxygen analog and the separation from the interfering peaks was satisfactory. Typical calibration curves and gaschromatograms are shown in Fig. 1 and 2, respectively.

Isoxathion and its oxygen analog in the sample materials were extracted with acetone by means of blending or soxhlet extraction. Both chemicals in the extracts freed of solvent were successfully transfered into hexane or dichloromethane in the presence of 10% aqueous solution of sodium chloride.

The fats, waxes and pigments coextracted were removed by the partitioning and the Florisil or alumina column chromatography. The partition coefficient of isoxathion was approximately 16 in acetonitrile/hexane and 3 in methanol/hexane, and that of the oxygen analog was approximately 32 in acetonitrile/hexane. Florisil activated at 130°C and alumina deactivated with 5% w/w amount of water were the usefull adsorbents for the cleanup of isoxathion on column chromatography. Isoxathion was thoroughly eluted with first 100ml of 15% diethylether in hexane from both the Florisil and alumina columns. The oxygen analog was strongly adsorbed in Florisil and alumina. However, it was quantitatively recovered from the mixture column of charcoal (Darco G-60) and Celite 545 with 100ml of acetone as an eluting solvent.

The cleanup procedures for various samples

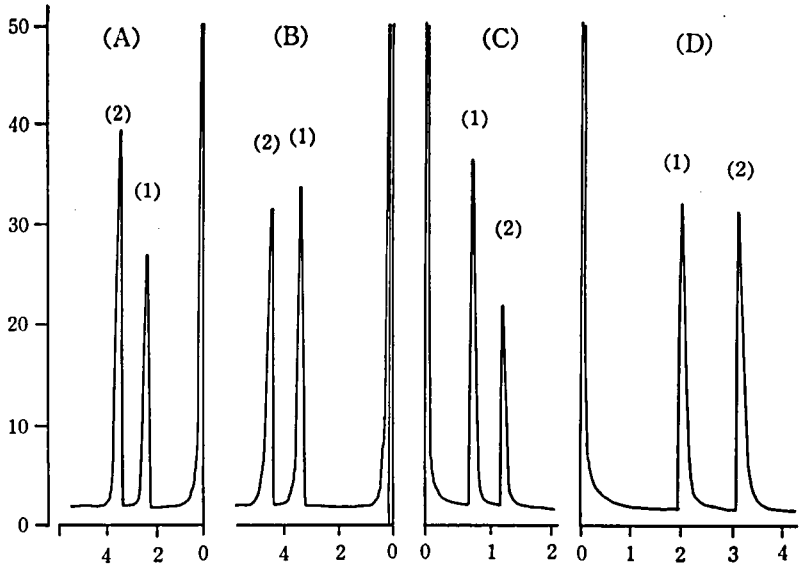

Fig. 2. Typical Gas Chromatograms' of Isoxathion and Its Oxygen Analog.

	Peak (1)	Peak (2) (Internal Standard)	GLC condition (in Table 1)
(A)	Isoxathion (2.2ng)	TPP (3.8ng)	A
(B)	Oxygen analog (13.4ng)	TPP (3.1ng)	A (operated at 215°C)
(C)	Isoxathion (2.0ng)	TPP (1.1ng)	B
(D)	Isoxathion (6.0ng)	CYP (6.0ng)	C

were constructed by combining the above mentioned techniques.

In the determination of isoxathion residues, the simplest cleanup procedure, only the transferring to dichloromethane after acetonee xtraction, was applicable to soils. The Florisil column chromatography was appropriate to low-waxy vegetables, fruits, tobaccos and rice grains. Futhermore, the partitioning followed by the Florisil column chromatography was necessary to rice straws and green teas. FPD-GLC using XE-60 column was selected for the determination of isoxathion residue in vegetables, fruits and tobaccos, and FTD-GLC using OV-17 column was applied to rice grains and straws. In the case of green teas, the removal of interfering substances was incomplete. However, the substances were less sensitive to the FTD than the FPD and the satisfactory results were obtained by the FTD analysis using SE-52 (5%) column.

The determination of the oxygen analog residues was examined to chinese cabbages. The satisfactory result was given by the charcoal-Celite 545 column chromatography after the partitioning and the FPD analysis using the XE-60 column.

The overall recoveries of isoxathion or its oxygen analog from the fortified samples are summarized in Table 4. Recoveries of isoxathion ranged from 85 to 99% at the fortification levels of 0.1 to 0.6ppm, and that of the oxygen analog was 83% at the level of 0.5ppm. All results were satisfactory and the procedures described above were not time-consuming.

The minimum detectable levels are given in Table 5, although they are variable by sampling scales and noise levels in GLC.

Preliminary Study on Persistence of Isoxathion

The purpose of this study was to know the relative persistence of isoxathion in plant and soil by comparing with that of methylisoxathion and fenitrothion under a laboratory condition. Methylisoxathion[1] is an experimental organophosphorus insecticide found in our laboratories, and has been characterized by low mammalian toxicity and high insecticidal activity, although

Table 4. Recoveries of Isoxathion and Its Oxygen Analog from Fortified Samples.

Sample		Added (ppm)	Recovered (%)	CV (%)
Isoxathion				
Apple	pulp	0.1	98.5	2.6
	peel	0.5	94.5	3.2
Cabbage		0.1	94.8	1.9
Chinese Cabbage		0.1	98.7	3.0
Cucumber		0.1	96.1	3.4
Japanese Radish	root	0.1	95.5	3.2
	leaves	0.1	94.2	4.2
Orange	pulp	0.1	97.5	2.8
	peel	0.5	93.3	4.1
	juice	0.1	95.6	2.1
Rice	straw	0.2	85.8	4.2
	grain	0.1	96.2	2.2
Tea	processed leaves	0.5	93.4	3.7
Tobacco		0.5	95.2	2.7
Kidney bean		1.0	99.5	2.0
Soil	clay loam	0.6	96.9	4.0
	loam	0.6	96.8	3.1
	loam	0.6	98.5	3.1
Oxygen Analog				
Chinese Cabbage		0.5	83.1	5.6

Table 5. Minimum Detectable Levels of Isoxathion and Its Oxygen Analog.

Sample		Sample Size (g)	MDL[1] (ppm)
Isoxathion			
Apple	peel	20	0.01
	pulp	100	0.002
Cabbage		100	0.001
Chinese Cabbage		100	0.002
Cucumber		100	0.002
Japanese Radish	root	100	0.002
	leaves	100	0.002
Orange	pulp	100	0.002
	peel	50	0.004
	juice	100	0.002
Rice	straw	25	0.008
	grain	50	0.004
Tea	processed leaves	10	0.005[2]
Tobacco		20	0.01
Kidney bean		10	0.02
Soil		20	0.05
Oxygen Analog			
Chinese Cabbage		100	0.05

1) Minimum detectable level; based on thrice noise level, 1 μl injection and 1 m*l* of the final volume.
2) Four μl aliquot were injected.

it is rather unstable chemically. Fenitrothion was selected as one of the most popular organophosphorus insecticides.

The above three chemicals were simultaneously treated to plant or soil with the combined solution in order to simplify the experimental conditions.

The disappearance curves of chemicals on or in kidney beans are shown in Fig. 3, in which the remained amounts (ppm) are corrected to eliminate the dillution effect with plant growth and plotted against time in a semi-logarithmic co-ordinate system. The half-lives of isoxathion, methylisoxathion and fenitrothion residues were nearly 5, 2 and 1 days, and the 90% disappearance periods were nearly 18, 6 and 5 days after the treatment, respectively. Isoxathion was moderately stable, however, methylisoxathion and fenitrothion were unstable; if remotely compared, methylisoxathion was slightly more stable than fenitrothion as shown in Fig. 3.

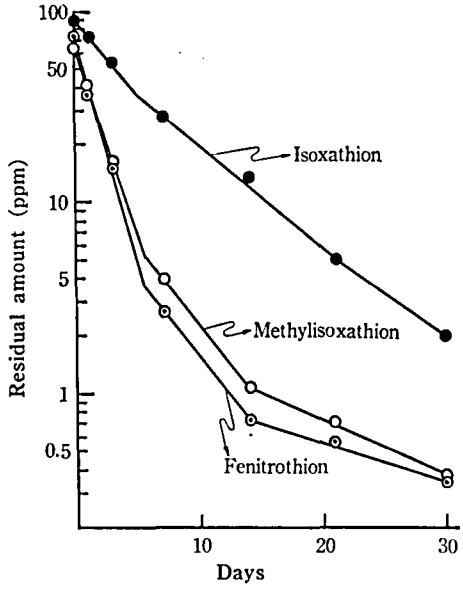

Fig. 3. Persistence of Isoxathion, Methylisoxathion, and Fenitrothion on/in Kidney Bean Plant.

The disappearance curves of chemicals in a sandy loam soil are shown in Fig. 4, in which the remained amounts (ppm) are corrected to values on the basis of dry weight of soil and plotted in a similar manner as Fig. 3. The half-lives of isoxathion, methylisoxathion and fenitrothion in the soil were nearly 18, 5 and 10 days, and the 90% disappearance periods were nearly 80, 15 and 30 days after the treatment, respectively. Isoxathion was moderately stable in the case of soil as well as in kidney beans. Although methylisoxathion and fenitrothion were also relatively unstable, however, methylisoxathion was apparently less stable than fenitrothion on the contrary to kidney beans.

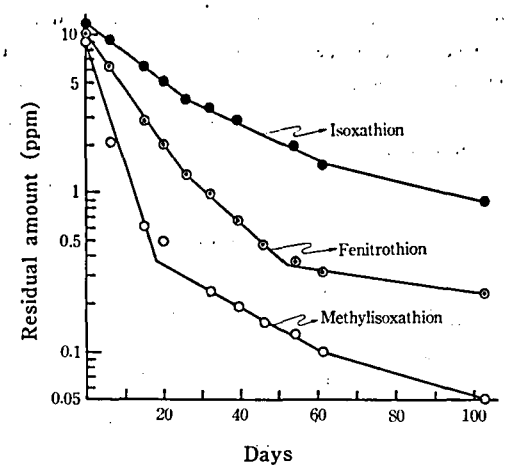

Fig. 4. Persistence of Isoxathion, Methylisoxathion, and Fenitrothion in a Sandy Loam Soil.

It has been known that fenitrothion sprayed on rice plants was rapidly penetrated into the tissues, approximately one-half of the amount during the subsequent 24 hours after spraying, and was easily decomposed to water soluble compounds[11]. On the other hand, isoxathion treated on leaf surface of cabbage, chinese cabbage and bean was gradually absorbed to plant tissues, then metabolized to water soluble compounds[8]. Although no experiment has been carried out on methylisoxathion, it may be attributed to the slow penetration into the tissues from the leaf surface that methylisoxathion is relatively more stable on or in kidney beans than in the soil compared with fenitrothion.

It was supposed under experimental conditions that isoxathion should be promised not only as an insecticide for the foliage application but as an excellent soil insecticide because of the moderate persistence on plants and in soils. Methylisoxathion seems to be attractive as an

insecticide having remarkably low persistency.

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Analysis of Optically Active Allethrin in Mosquito Coils. Takenosuke TAKANO (Technical Division, Japan Insecticide Industrial Association) Received Sept. 7, 1976. *Botyu-Kagaku*, 42, 40, 1977. (with English Summary 45)

4. 蚊取線香中の光学活性アレスリンの分析

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ガスクロマトグラフ法により蚊取線香中の光学活性アレスリン (3-allyl-2-methylcyclopent-2-en-4-on-1-yl *d-cis, trans*-chrysanthemate) を定量し，あわせてその幾何ならびに光学異性体を分析した．アレスリンに対する方法を準用し，光学異性体は線香から抽出して薄層クロマトグラフ法でクリーンアップしたのち，加水分解して生ずる菊酸を *d*-2-オクタノールのジアステレオマーエステル誘導体にしてガスクロマトグラフ法で *d*-トランス，*l*-トランス，*d*-シスおよび *l*-シス型の4種の異性体を分離しピーク面積の比から異性体組成を分析した．これらの方法により線香中のアレスリン異性体の1年後の経時変化を検討し，異性体はいづれも変化がなく，定量値も平均2～3％の減少で，実用的にはほとんど変化していないことを確認した．

緒　　言

アレスリン (3-allyl-2-methylcyclopent-2-en-4-on-1-yl *dl-cis, trans*-chrysanthemate) は菊酸部位の立体配座の違いによって幾何および光学異性体があり *d*-シス，*l*-シス，*d*-トランスおよび *l*-トランスの4種の異性体が存在し，それぞれ昆虫に対する殺虫効力が異なり，*d*-型が *l*-型よりもはるかに強い効力を有する．最近アレスロロンと *d*-シス，トランス菊酸のエステルである光学活性アレスリンの工業的製造が可能となりピナミンフォルテ®なる商品名で市販され，ラセミ型のアレスリン（ピナミン®）に代って電気かとりマットや蚊取線香に使用されつつある．

日本殺虫剤工業会技術部会は，さきに蚊取線香中のアレスリンの定量法について研究し，ソックスレー抽出器を用いてメタノールで抽出後，ガスクロマトグラフ (GC) で正確に分析できることを報告した[1]が，前報に引き続きピナミンフォルテについても，その分析法を検討し，アレスリン同様に定量でき，また光学異性体も薄層クロマトグラフ (TLC) でクリーンアップしてのち，GC により分析が可能であることを確認した．さらにピナミンフォルテ線香の1年後の経時変化についても検討したので，その結果についてもあわせて報告する．

試薬器具および装置

ピナミンフォルテ基準品　ピナミンフォルテ原体を前報[1]のアレスリン原体の GC 内標準法で分析し，純度を求めたものを線香定量用基準品に使用した．(純度93.3%, *cis/trans* 比 17.4/82.6)

内標準液　(i) β-ナフトキノリン（試薬特級）約750mg を精密にはかりアセトンを加えて溶かし，正確に 50ml として原体定量用の内標準液とする．

(ii) ステアリン酸エチル (ES) またはメチル (MS)