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Microdetermination of parathions by thermo- and ultra-violet decomposition products*

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Abstract

By means of the thin layer chromatography (TLC) a study was carried out on the decomposition of methyl parathion, ethyl parathion and sumithion when exposed to heat or ultra-violet irradiation. The results are briefly summarized as follows. 1. Parathions, when exposed to heat, form hydrolysates and such O-analog as paraoxon as well as S-alkyl isomers. 2. When parathions are exposed to ultra-violet rays at 365 m μ and 254 m μ , the rate of decomposition is extremely slow. For example, when exposed to such rays in Petri dish for 5 hours, only a small amount of S-alkyl isomer is formed. 3. After heating parathions in a small test tube and conducting TLC, when each O-analog and S-alkyl isomer above mentioned is confirmed, it is possible to identify a minute amount of each parathion by this method, and thus this method is feasible to apply to practical poison examination as a rapid and simple qualitative examination method.

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MICRODETERMINATION OF PARATHIONS BY THERMO- AND ULTRA-VIOLET DECOMPOSITION PRODUCTS

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In order to make microdetermination and to detect the parathions with ease, parathions isomerization produced by thermo- and ultra-violet decomposition were studied. Parathions were decomposed by heating or ultra-violet irradiation, and then the decomposition products were separated by the thin-layer chromatography (TLC), extracted from the thin layer, and these extracts were further analyzed by several steps such as the colorimetric method, TLC, gas-chromatography (GC), infrared analysis (IR) and ultra-violet absorption analysis (UV) to identify individual product.

The present communication deals briefly with the results obtained.

MATERIALS AND METHODS

As the test materials methyl parathion, ethyl parathion and sumithion of over 95% purity were made into acetone solution. To get pure pesticides each of these solutions is lined on the thin layer plate, and developed by n-hexane-acetone (4:1, v/v, G.R.) and spot parts of pesticides on the Silicagel G (SG, Merk) layer were picked up, extracted into n-hexane-acetone (1:1, v/v, G.R.). Each pesticide is confirmed to contain no contaminant by the TLC with n-hexane-acetone (4:1, v/v, G.R.), and serves for the experiments. As the preparation of thin layer plates SG is coated in the thickness 0.25 mm on a 20×20 cm glass plate using an applicator of Tōyō Roshi Co. Japan. Dry the thin plates 5 hours at room temperature, and activate them in an oven at 100—115°C for 60 minutes. Keep them in a desiccator to serve for the need.

About 10 mg each of the above sample in a Petri dish is decomposed by heating on a sand bath to 150°C with thermoelectric thermometer for 5 hours or by ultra-violet rays exposure with either at 365 m μ (without filter) or at 254 m μ of UV-lamp for 5 hours.

Then each decomposed pesticide is dissolved in a minute acetone solution and again lined on the SG layer and developed with solution of n-hexane-chloroform-methanol (7:2:0.5, v/v, G.R.) or toluene-dioxane (7:1, v/v, G.

R.). Spot parts of SG layer are picked up, and extracted into n-hexane-acetone (1:1, v/v, G. R.) solution. The oily decomposed substances thus obtained are examined with coloration by 20 % methanolic solution of potassium hydroxide, 0.5 % cyclohexane solution of 2,6-dibromoquinonechlorimide (Gibbs reagent) and Diazo reagent.

Next, they are analyzed with Shimadzu Model GC-1C gas chromatograph equipped with a hydrogen flame detector (Shimadzu Model FID-1B) and a 180 cm × 0.3 cm stainless steel column containing 5 % SE-30 on Schimalite. Operating conditions are: column temperature, 195°C, detector temperature 215°C. Carrier gas helium 30 ml/min, sensitivity 10⁻², range 0.8 V., sample 1 μl. They are subjected to further analysis with infrared analysis (IR) using Hitachi Model E PI-G2 (resolution, 2 cm⁻¹/1000 cm⁻¹, gain 3, scan speed Fast-2, sample KBr tablet) and with ultra-violet absorption analysis (UV) using Shimadzu Model QB-60.

As for the detection of a minute quantity of parathions each pesticide is decomposed on the SG layer on which 10 to 20 μg of the sample in acetone solution is spotted. Thin layer plates are heated variously in an oven in the range of 110–150°C for 30–60 min or exposed to ultra-violet rays of 365 mμ or 254 mμ at the distance of about 20 cm for about 2 hours and developed with n-hexane-chloroform-methanol (7:2:0.5, v/v, G. R.), and for the identification of spots 20 % methanolic solution of potassium hydroxide as the spray reagent is used. Moreover, by exposing to ultra-violet lamp at 365 mμ, spotted sample is heated to about 60°C.

To establish most suitable conditions of the thermodecomposition, about 10–20 μg sample is put in a test tube, 7–8 cm in inside diameter and 10–12 cm in length, and heated with the heat apparatus as shown in Fig. 1, and changing the heat temperature and heating time.

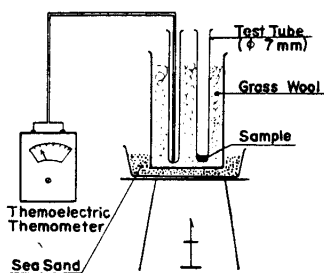


Fig. 1 Apparatus of Thermodecomposition of Thiophosphates

RESULTS

1. *Thin layer chromatography of thermo- and photo-decomposition of parathions*: In decomposing each sample by heating in a Petri dish to 150°C for 5 hours, there appear 3 spots besides parathions in the TLC as shown in Fig. 2 and Table 1. The lowest spot (shaded spots of low part in Fig. 2) is

Microdermination of Parathions

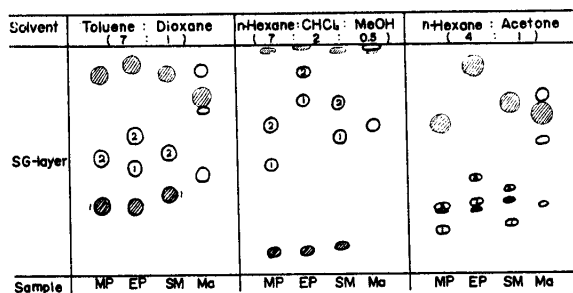


Fig. 2 Spots of Thermodecomposition Products of Thiophosphate (Heated at 150°C for 5 hrs)

Table 1 Rf-Values of 4 Thiophosphates and their Decomposition Products on SG Layers

Solvents used	Toluene : Dioxane (7 : 1)	n-Hexane : CHCl ₃ : MeOH (7 : 1 : 0.5)	n-Hexane : Acetone (4 : 1)
M. Parathion	8 7	9 6	6 5
M 1	3 0	4 8	1 9
M 2	5 1	6 5	2 7
E. Parathion	9 1	9 8	9 0
E 1	4 7	7 6	3 0
E 2	6 0	8 8	4 1
Sumithion	8 9	9 6	7 3
S 1	3 7	6 0	2 2
S 2	5 3	7 4	3 5
Marathion	7 8	9 7	6 9
Ma 1	4 4	6 5	3 0
Ma 2	7 1	9 4	5 7
Ma 3	8 9	9 8	7 9
p-Nitrophenol	3 0	1 0	2 5

identified as 3-nitrophenol (NP) or 4-methyl-3-nitrophenol (MNP) from the results of colorimetric examination and infrared analysis and its upper spots are designated in the case of methyl parathion (MP) as M₁, M₂ with ethyl parathion (EP) as E₁, E₂ and with Sumithion (SM) as S₁, S₂, and these were further studied. For the sake of convenience M₁, E₁ and S₁ are grouped as group 1, M₂, E₂ and S₂ as group 2. Even in the exposure to ultra-violet rays of either at 365 m μ and 254 m μ , the decomposition of parathion occurs only slightly (Fig. 3). Moreover in the TLC only spot 1

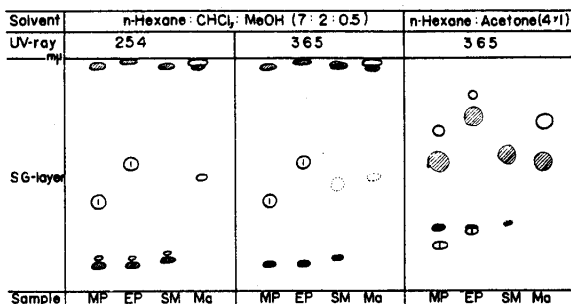


Fig. 3 Spots of Photodecomposition Products of Thiophosphate (Exposed to UV light without filter for 5 hrs)

Table 2 Colorimetric Reaction of Thiophosphates

Reagent	20% KOH	Gibbs R.	Diazo R.
Sample	<chem>C1=CC=C(C=C1)P(=O)(O)O</chem>	<chem>C1=CC=C(C=C1)P(=O)(O)O</chem>	<chem>C1=CC=C(C=C1)P(=O)(O)O</chem>
MParathion	Yellow	Red. Violet	Red. Violet
M 1	*	Blue Violet	*
M 2	*	Blue Green	*
E.Parathion	*	Red. Violet	*
E 1	*	Blue Violet	*
E 2	*	Blue Green	*
Sumithion	*	Red. Violet	*
S 1	*	Blue Violet	*
S 2	*	*	*
Marathion		Brown Violet	
Ma 1		Brown	
Ma 2		Brown	
pNitrophenol	Yellow	Blue	Blue

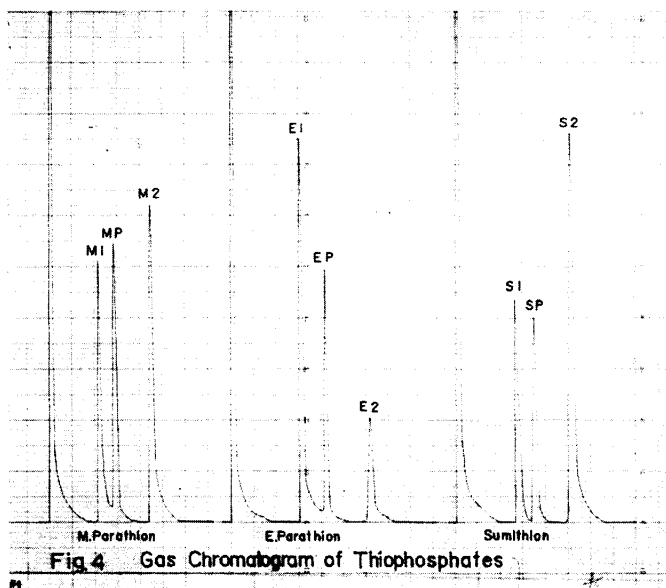


Fig 4 Gas Chromatogram of Thiophosphates

can be detected, especially difficult is to decompose by exposure to ultra-violet rays. But spots of NP and MNP (shaded spots of low part in Fig. 3) are recognized distinctly.

2. *Colorimetric reaction of thermo- and ultra-violet decomposition products of parathions*: Parathions group and their spot 1 and 2 groups colored similarly yellow as NP with 20% methanolic potassium hydroxide, but spot 1 and 2 groups do not show the color of reddish-violet based on the reaction of P=S and Gibbs reagent as shown Table 2. Diazo reaction in spot 1 and 2 groups as well as parathion groups appeared in reddish-violet.

From the above described findings it is thought that spot 1 and 2 groups have similar structure to parathion groups but no P=S combination.

3. *Gas chromatographic analysis of thermo- and ultra-violet decomposition products of parathions*: As shown in Fig. 4 and Table 3, parathion group all lie in between spot 2 group and 1 in retention time and the former is shorter and the latter longer.

Table 3 Retention Time of Thermodecomposition Products of M. Parathion, E. Parathion and Sumithion (FID)

(min)					
Heated at 150°C for 5 hrs in Vessel					
M. Parathion		E. Parathion		Sumithion	
M. P.	2.95	E. P.	4.15	S. M.	3.55
M 1	2.20	E 1	3.15	S 1	1.00
M 2	4.50	E 2	6.20	S 2	5.10
Heated at 150°C for 1 hr in test tube					
M. P.	3.15	E. P.	4.45	S. M.	3.75
M 1	2.30	E 1	3.45	S 1	1.00
M 2	trace	E 2	trace	S 2	trace

Column SE-30 (5%) 1.8 m, Column Temp. 190°C, Apparatus Shimadzu GC-1C (F1D-1B)

4. *Infrared spectroscopy of thermo- and ultra-violet decomposition products of parathion group*: In spot 1 group a strong absorption of P=O in about 7.7 μ is observed but a specific absorption of P=S in about 12.25 μ appearing in parathion group is none or minimal (Figs. 5, 6 and 7). And in spot 2 group there is a specific absorption of P=O in about 7.7–7.9 μ but scarcely one of P=S, and a new absorption to be thought as S=C (S-alkyl) in about 17 μ is observed.

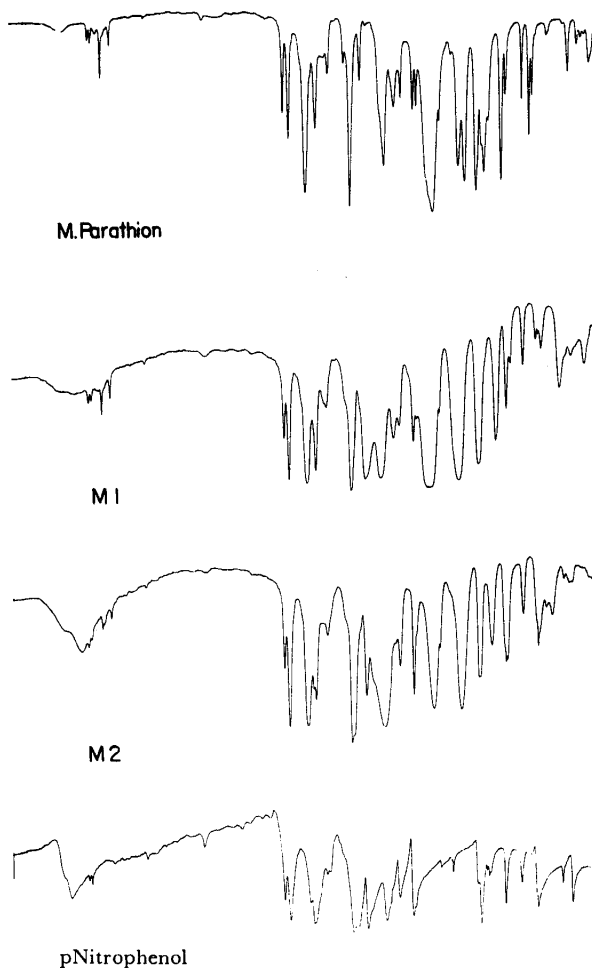


Fig. 5 IR Spectra of M. Parathion and its Decomposition Products

5. *Ultra-violet absorption analysis of thermo- and ultra-violet decomposition products of parathion group* : As shown in Fig. 8, UV-absorbance of methyl- and ethyl parathion and their decomposition products using either ethanol or ethyl-ether as solvents is resembles with one another, showing about 270 $m\mu$ in the maximum wave length.

6. *The identification of parathions by means of TLC utilizing their thermo-decomposition on Silica-gel thin layer* : As shown in Table 4, one of thermo-decomposition products of parathions, NP or MNP, on the SG-layer increases in proportion to the heat temperature and heating time and the other is of a relatively small quantity, but the spot 2 group is detected

Fig. 6 IR Spectra of
E. Parathion and its De-
composition Products

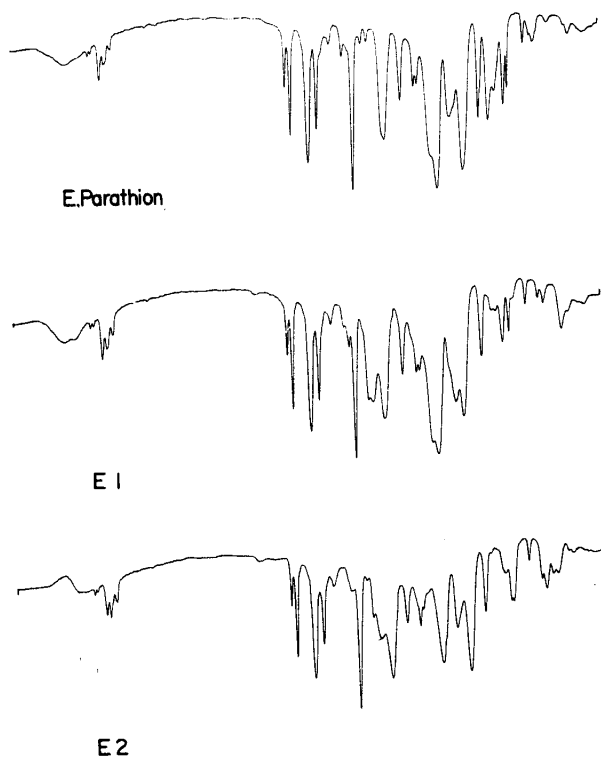


Table 4 Amount of Decomposition Products of Parathions with Heating
or Exposing to UV-light on SG-layer

Heating		M. Parathion			E. Parathion			Sumithion		
Temp. °C	Time min.	M 1	M 2	NP	E 1	E 2	NP	S 1	S 2	MNP
110	30	-	-	+	-	-	+	-	-	+
	60	±	±	±	-	-	±	±	+	±
120	30	-	+	±	-	±	±	-	+	±
	60	-	+	±	-	+	±	-	+	±
150	30	-	+	±	-	+	±	-	+	±
	60	-	+	±	-	+	±	-	+	±
Exposing to UV-light										
UV Wave Length m μ	Expos. Time min.	M. Parathion			E. Parathion			Sumithion		
		M 1	M 2	NP	E 1	E 2	NP	S 1	S 2	MNP
254	60	+	-	+	+	-	+	±	-	+
365	60	+	-	+	+	-	+	±	-	±

Dev. Soln.; n-Hexane: Chloroform: Methanol (7:2:0.5), Detection of Spot; 20%
Alcoholic KOH Solution, NP; p-Nitrophenol, MNP; 4-Methyl-3-nitrophenol

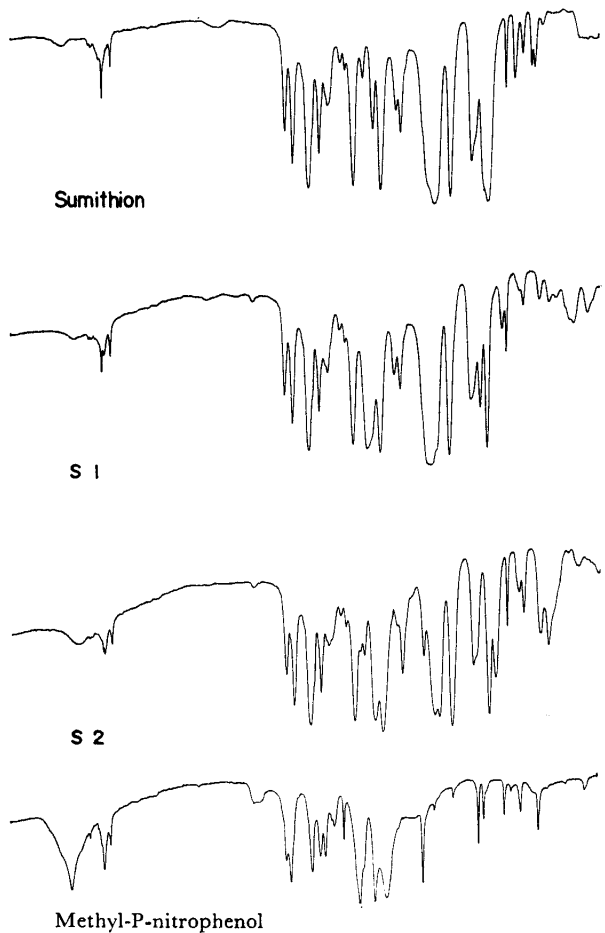


Fig. 7 IR Spectra of Sumithion and its Decomposition Products

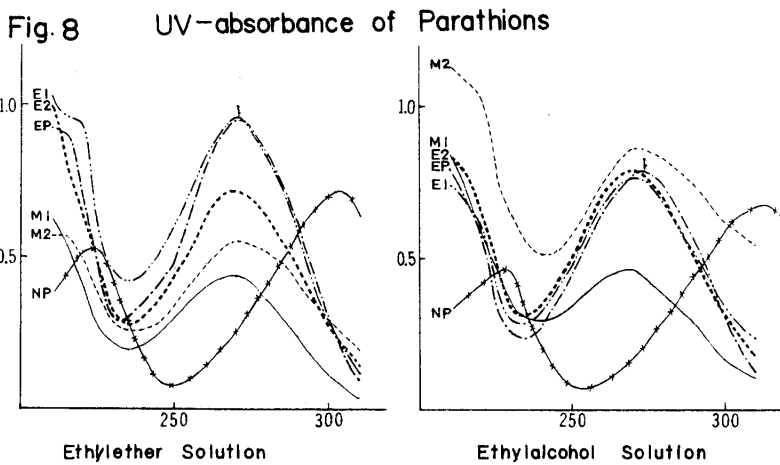


Fig. 8 UV-absorbance of Parathions

faintly, showing different results from those of Petri dish. In the UV irradiation decomposition, however, irrespective of long or short wave length, NP or MNP, and spot 1 group only are detected faintly.

As is clear from these results, the rate of decomposition of parathions on the thin layer is slow but the rate of hydrolysis of parathions to NP or MNP, and dialkyl thiophosphate (this appeared in original point on TLC) is extremely rapid. Consequently, the identification of parathions by means of the confirmation of NP or MNP by thermodecomposition is possible even in the quantity as minute as 0.5 μg .

7. *The identification of parathions by means of TLC utilizing their thermodecomposition in small test tube*: After the thermodecomposition of parathions in the quantity of 10—20 μg sample in small test tube using the apparatus as shown in Fig. 1 the TLC is conducted. The result, as shown in Table 5, reveal that spot 1 group is sensitive, differing from the result

Table 5 Detection of Thermodecomposition Products from Thiophosphates in Test Tube

Temperature °C	Times min	Spot No.	Methyl Parathion	Ethyl Parathion	Sumithion
145—150	1 5	1	++	—	—
		2	+	—	—
	3 0	1	+++	+++	+++
		2	+	—	±
155—160	3 0	1	+++	+++	+++
		2	+	±	++
	6 0	1	+++	+++	+++
		2	++	+	++
160—175	3 0	1	+++	+++	+++
		2	+++	++	+++

of decomposition on thin layer, and especially with one of SM. And also the higher the heat temperature, the greater is the spot 2 group product, and of course, NP or MNP can be detected similarly. But in this instance, the sample of less than 20 μg is suitable because when the sample is more than this amount, the oxidation does not take place so well, thus the production of spot 1 group goes on insufficiently.

The thermodecomposition of parathions in a small test tube is suitable for the exposure at 150—160°C for 30—60 min and by this method the identification of parathions of even 1—2 μg is possible.

DISCUSSION

There are some reports (1) of the studies on decomposition of parathions in the plants or in the pesticides themselves, and also MIYAMOTO *et al.* (2, 3) have reported at length that the decomposition of parathions in animal or plant tissues form hydrolysates and such O-analog as paraoxon as well as des-alkyl isomer freeing alkyl. The authors agreed exclusive of a part to him from the results of authors experiment. And also it has been reported by NEAL (4) that ethyl parathion was decomposed to paraoxon and its hydrolysates *in vivo*, but he does not describe about the decomposition to S-alkyl isomer. The authors have studied the decomposition of parathions by exposing to the heat and UV-irradiation in the presence of air, and have confirmed that the heat treatment produces a particularly large amount of products such O-analogs of parathions (Spot 1 in Table 6)

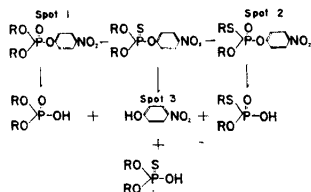


Table 6 Thermodecomposition Products of Parathions

as methyl paraoxon or sumioxon, and their S-alkyl isomer (Spot 2 in Table 6) as well as their hydrolysates. But we have not yet recognized the decomposition products such as the des-alkyl isomer in our conditions as described by MIYAMOTO *et al.*

And the O-analog of parathions is easily formed by heating to around of 150°C *in vitro*, but S-alkyl isomer is formed in a greater quantity at the temperature higher than this (170°C). In the thermodecomposition of 110—150°C on the thin layer the O-analog as above mentioned is not formed, but only small amount of S-alkyl isomer.

In the experiment, 3 different solvents have been used for developing, and each of them has characteristics of its own as solvent, and the mixture of n-hexane-chloroform-methanol (7:2:0.5) proves to separate samples tested quite distinctly in TLC.

In our tests of various color reagents for TLC, we find that 20% methanolic potassium hydroxide proves to be the most suitable reagent. However, in the identification of parathions from their O-analogs, S-methyl isomer and hydrolysate, the best results can be obtained by first spraying Gibbs reagent on the TLC and then heating.

Thus the attempts of simple qualitative examination of parathions by means of this thermodecomposition have yielded good results. By heating and decomposing parathions in a small test tube and by TLC, and then confirming each decomposition product formed, each parathion can be identified.

Therefore, it is possible to use TLC analysis, though it usually gives unstable R_f-values. We consider that this technique is a useful, simple qualitative method of identifying decomposition products of pesticides.

CONCLUSION

By means of the thin layer chromatography (TLC) a study was carried out on the decomposition of methyl parathion, ethyl parathion and sumithion when exposed to heat or ultra-violet irradiation. The results are briefly summarized as follows.

1. Parathions, when exposed to heat, form hydrolysates and such O-analog as paraoxon as well as S-alkyl isomers.
2. When parathions are exposed to ultra-violet rays at 365 m μ and 254 m μ , the rate of decomposition is extremely slow. For example, when exposed to such rays in Petri dish for 5 hours, only a small amount of S-alkyl isomer is formed.
3. After heating parathions in a small test tube and conducting TLC, when each O-analog and S-alkyl isomer above mentioned is confirmed, it is possible to identify a minute amount of each parathion by this method, and thus this method is feasible to apply to practical poison examination as a rapid and simple qualitative examination method.

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