

**Certain Properties of Methyl Parathion Examined by Tracer Technique.** Toshiro Sato and Chojiro TOMIZAWA (National Institute of Agricultural Sciences, Nishigahara, Kita-ku, Tokyo) Received May 30, 1960. *Botyu-Kagaku*, 25, 85, 1960. (in English).

**16. トレーサーテクニックにより調べた methyl parathion の性質** 佐藤敏郎, 富沢長次郎 (農林省 農業技術研究所) 35. 5. 30 受理.

$^{32}\text{P}$  および  $^{35}\text{S}$  ラベル付 methyl parathion を用い微量領域における methyl parathion の蒸発速度および分解を調べた。 $^{32}\text{P}$  および  $^{35}\text{S}$  ラベル付 methyl parathion の間で  $^{32}\text{P}$  と  $^{35}\text{S}$  の消失速度に差異が認められ、温度によつても異なつていた。Methyl parathion は蒸発の過程において酸化、異性化、加水分解を同時にうけ、残留物は主に加水分解生成物により構成されていた。珪藻土、タルク、クレー、ベントナイトからなる methyl parathion 粉剤の経時変化はキャリアーの種類および貯蔵の乾湿状態によつて異なり乾燥状態では、いづれの粉剤においても異性化が分解の第一段階であるが湿潤状態では異性化以外の反応も分解に関与していることが示唆された。

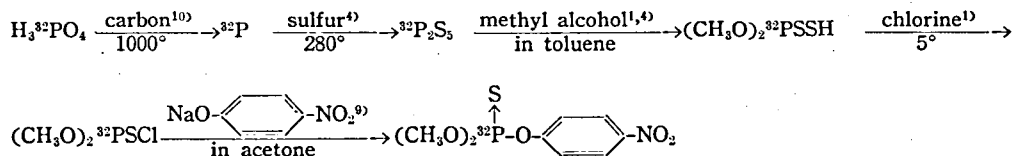
Much work concerning methyl parathion has been accomplished not only with physico-chemical properties but also with the mode of action on insects<sup>7)</sup>. Since methyl parathion is lethal to insects even in minute amounts, it has been rather difficult to examine the behavior of methyl parathion, including degradation products. Some attempts have been made by bio-assay for examining the behavior of methyl parathion in minute amounts<sup>13)</sup>, but it has not been possible to estimate the degradation products of methyl parathion which are not insecticidal. The use of compounds labeled with radioisotope is more useful than

chemical analysis or bio-assay for examining the behavior of methyl parathion, including degradation products. A study was undertaken to obtain information about the evaporation and degradation of minute amounts of methyl parathion under various conditions, and also to examine the change of methyl parathion in dust form during storage.

**Materials and Methods**

**I. Radioactive Methyl Parathion.**

1)  $^{32}\text{P}$ -labeled methyl parathion : The synthesis of  $^{32}\text{P}$ -labeled methyl parathion was carried out according to the following process ;

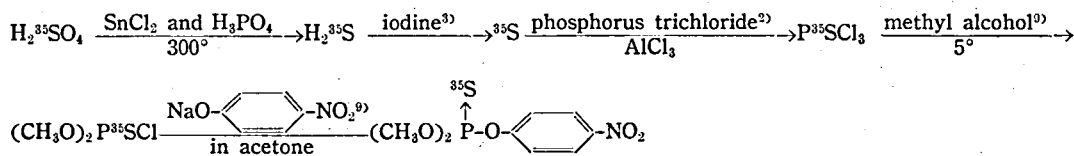


A crude methyl parathion was purified by means of a column chromatography. A mixture of silica gel, 20 g and Celite 545, 2 g was mixed with a mixture of hexane and chloroform (10 : 1), and poured into the chromatographic tube, diameter 25mm and height 400mm. Crude methyl parathion was dissolved into 8 ml of a mixture of hexane and chloroform 4 : 1 and poured onto the column. After rinsing the top of the column with three portions of each 2 ml of a mixture of

hexane and chloroform (10 : 1), the same solvent was passed into the column. The first fraction of 250 ml was passed over and the next fraction of 500ml was collected and evaporated under vacuum. Examination of the product by paper chromatography showed that the radioactive portion was more than 99% methyl parathion with the initial relative activity of 1385 cpm per  $\mu\text{g}$ . The methyl parathion degraded gradually following the formation of S-CH<sub>3</sub> isomer, dimethyl hydrogen phos-

phorothioate, and other unidentified products during the experiment, especially under the no solvent condition.

2)  $^{35}\text{S}$ -labeled methyl parathion. The synthesis of  $^{35}\text{S}$ -labeled methyl parathion was carried out according to the following process ;



The purification of the product was carried out in the same manner as mentioned in  $^{32}\text{P}$ -labeled methyl parathion. The initial relative activity was 1875 cpm per  $\mu\text{g}$ . Like the case of  $^{32}\text{P}$ -labeled methyl parathion, the product degraded gradually during the experiment.

### II. Paper Chromatography of Methyl Parathion and Degradation Products.

The following solvent systems of paper chromatography were used for separating methyl parathion and degradation products : (1) filter paper impregnated with 5% Silicone 550 in petroleum ether, mobile solvent, the upper phase from a mixture of ethyl alcohol 10 parts, chloroform 10 and water 6 parts by volume<sup>61</sup>, and (2) filter paper impregnated with 2.5% phenyl cellosolve and 2.5% propylene glycol in acetone, mobile solvent, the upper phase from a mixture of hexane 10 parts, chloroform 2 parts, acetonitrile 1 part, and propylene glycol 1 part by volume. The ascending technique of paper chromatography was employed<sup>12</sup>, using strips of Toyo filter paper No. 51, 300 mm in length and 20 mm in width. The results are shown largely with chromatographs using the solvent system (1). Radioautographs were prepared by exposing the filter paper to x-ray film for enough time to sensitize. The radioautographs were examined, and density scans were prepared by means of a photoelectric densitometer.

### III. Radioassay.

1) Determination of specific activity. 5  $\mu\text{g}$  of  $^{32}\text{P}$ -labeled methyl parathion was transferred from an acetone solution of methyl parathion to a Kjehldahl flask, and the contents of the flask were wet ashed with a mixture of concentrated sulfuric acid and nitric acid (5 : 1). After combustion, the contents were diluted with distilled

water and the carrier of phosphoric acid was added to the solution containing  $^{32}\text{P}$ , and the precipitate of ammonium phosphomolybdate containing  $^{32}\text{P}$  was made in conventional manner.

In the case of  $^{35}\text{S}$ -labeled methyl parathion, the combustion was carried out with a mixture of concentrated nitric acid and perchloric acid (3:1). After combustion, the carrier of sulfuric acid was added, and barium sulphate containing  $^{35}\text{S}$  was precipitated<sup>17</sup>.

Both precipitates of ammonium phosphomolybdate and barium sulphate were filtered through discus of filter paper, and their radioactivities were assayed on a GM counter.

2) Determination of the rate of evaporation of methyl parathion. In this experiment, the combustion of radioactive material was not made. 4  $\mu\text{g}$  of methyl parathion in acetone solution was pipetted into a planchet for radioassay. Immediately after evaporating the acetone, the first radioassay was made, and the cpm obtained was defined as the initial activity. After keeping planchets at temperatures shown below, they were taken out at various intervals for radioassay. The experiments were carried out with  $^{32}\text{P}$ -labeled methyl parathion at temperatures of room (around 30°), and 60°, and with  $^{35}\text{S}$ -labeled methyl parathion at temperatures of room (around 30°), 60°, and 130°. On radioassay, the surface of each planchet under a GM tube was covered with a thin cellophane sheet for avoiding contamination of the GM tube by vapor of the radioactive materials. The caution of checking background of a counter was paid before and after radioassay of each sample. Counting data obtained in any one series of the experiment were corrected for decay to a standard day arbitrarily selected for each experiment, and data were expressed as the percentage decrease

of radioactivity. After radioassay, some planchets were used for examining degradation products of methyl parathion by paper chromatography. For this purpose, the contents of planchets were dissolved with a suitable volume of acetone, and chromatographed.

In addition to the experiment mentioned above, the rate of evaporation at room temperature was examined with various amounts of  $^{35}\text{S}$ -labeled methyl parathion ranging from  $1\ \mu\text{g}$  to  $10\ \text{mg}$  per planchet.

#### IV. Change of Methyl Parathion in Dust Form during Storage.

$^{35}\text{S}$ -labeled methyl parathion was more suitable than  $^{32}\text{P}$ -labeled methyl parathion for examining

the change of methyl parathion throughout a long period because of a longer half life of  $^{35}\text{S}$ . The acetone solution of  $^{35}\text{S}$ -labeled methyl parathion was added to a mixture of dust carriers in a mortar up to 1.5% of methyl parathion, and under air flow the acetone was evaporated while mixing the methyl parathion and dust carrier with a pestle. After evaporating the acetone, the dust was divided into two portions, and one portion was stored in a glass container with calcium chloride, and the other portion in a glass container with water saturated with sodium chloride (relative humidity, 76%). The constituent of dust containing  $^{35}\text{S}$ -labeled methyl parathion used in the experiment is shown in Table 1. Two samples

Table 1. Constituents of methyl parathion dusts.

(a)		(b)		(c)		(d)	
Methyl parathion	1.5%	Methyl parathion	1.5%	Methyl parathion	1.5%	Methyl parathion	1.5%
Diatomaceous earth	68.5	Clay A	67.5	Clay B	67.5	Bentonite	68.5
Talc	30.0	Talc	30.0	Talc	30.0	Clay A	30.0
		Silica gel	1.0	Silica gel	1.0		

were taken, 43 and 225 days after the initiation of the experiment. The dust was suspended in acetone, and the acetone layer was used for paper chromatography.

### Results and Discussion

Evaporation and Degradation of Methyl parathion as Residual Film. The rate of evaporation of  $^{32}\text{P}$ -labeled and  $^{35}\text{S}$ -labeled methyl parathion is shown in Figs. 1 and 2, respectively. In both

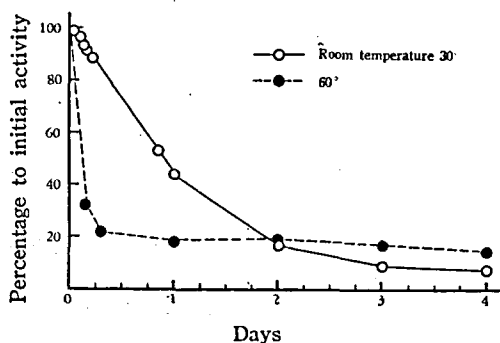


Fig. 1. Evaporation of  $^{32}\text{P}$ -labeled methyl parathion

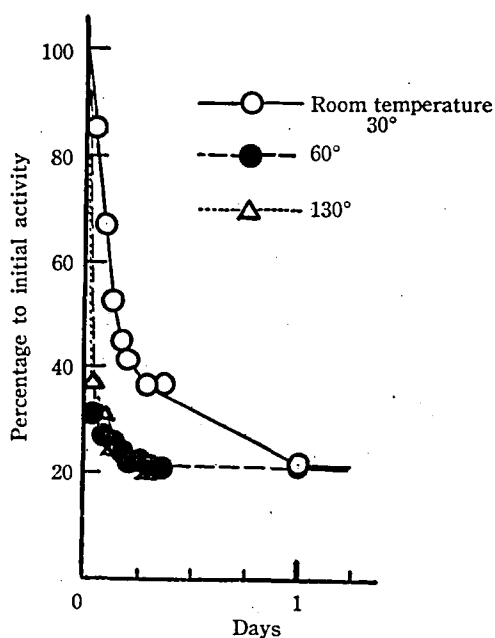


Fig. 2. Evaporation of  $^{35}\text{S}$ -labeled methyl parathion cases of  $^{32}\text{P}$ -labeled and  $^{35}\text{S}$ -labeled methyl parathion, the first portions of evaporation curves

were linear, then the rate of evaporation became slower. It was found by comparison of Figs. 1 and 2 that the decrease of  $^{35}\text{S}$  radioactivity was greater than that of  $^{32}\text{P}$  radioactivity at same temperature. The radioactivity of  $^{32}\text{P}$  remaining in the planchet approached a constant after the third day of the experiment, while that of  $^{35}\text{S}$  approached a constant at the first day. It is probable that the release of sulfur in methyl parathion molecule happened at the early stage of evapora-

tion even at room temperature, and that the bond,  $\text{P}\rightarrow\text{S}$  appeared to convert to  $\text{P}\rightarrow\text{O}$  although the degree of conversion might change with temperature<sup>5,6,9</sup>. At the early stage of evaporation, compounds such as methyl para-oxon and  $\text{S-CH}_3$  isomer (D) evaporated with methyl parathion (B), and the percentage of compounds which were difficult to evaporate increased in the planchet, and therefore the decrease of radioactivity became slower at the later stage of evaporation. The result of paper chromatography carried out with the content of planchet showed that dimethyl hydrogen phosphorothioate (E) and other unidentified materials remained in the planchet (Fig. 3).

The residual film of methyl parathion in atmosphere underwent oxidation, isomerization and hydrolysis simultaneously, and degradation products consisting largely of hydrolytic products remained. Metcalf and March<sup>9</sup> confirmed that on heating methyl parathion at  $150^\circ$  for 6 hours, methyl parathion converted to  $\text{S-CH}_3$  isomer, para-oxon and two other unknown products. McPherson and Johnson<sup>9</sup> showed that at a similar temperature, sulfur in methyl parathion molecule released as thioether and sulfite, and the remainder of molecule remained as polymerized products. The experimental conditions used by them were different from that of the present experiment. They carried out the experiments with comparatively large amounts of methyl parathion under the condition either left in an open air or sealed in ampoule, while the present experiment was carried out with the residual film of methyl parathion in the atmosphere. Under conditions used by them, reaction between methyl parathion molecules would play an important role for degradation, but under the condition of the present experiment oxidation by contact with air and removal by the evaporation of methyl parathion and other volatile products would affect the direction of degradation. The formation of polymerized products shown by McPherson and Johnson<sup>9</sup> was obscure in the present experiment. As expected, the smaller was amount of methyl parathion in planchet, the faster was the rate of evaporation (Fig. 4). Below  $10\ \mu\text{g}$  per planchet, the decrease of radioactivity was remarkable, and at  $100\ \mu\text{g}$  per planchet, the curve

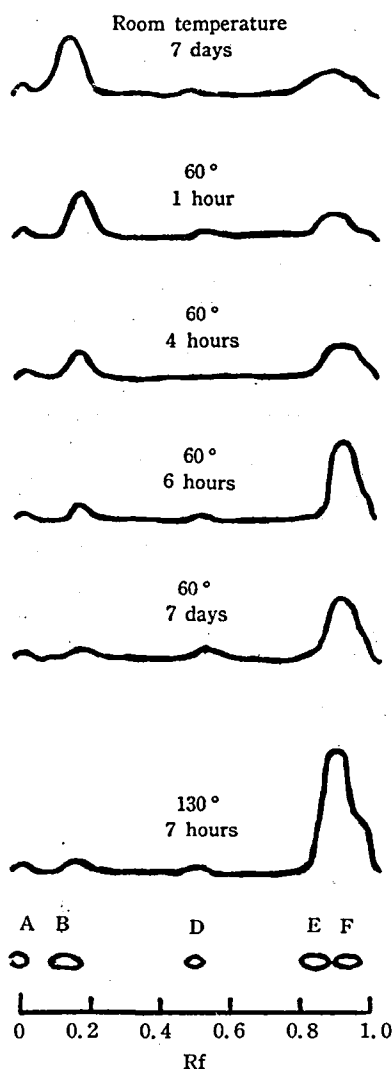


Fig. 3. Degradation of  $^{35}\text{S}$ -labeled methyl parathion as residual film, based on density scans of radioautographs prepared from chromatograms of acetone extract of the contents in planchets (Silicone paper).

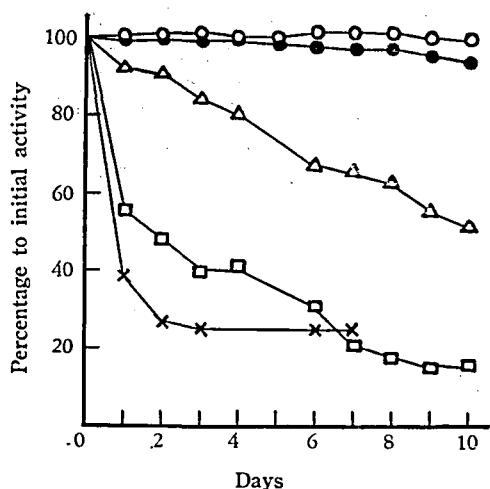


Fig. 4. Evaporation of  $^{35}\text{S}$ -labeled methyl parathion in varying amount.

—○— 10 mg, —●— 1 mg, —△— 100  $\mu\text{g}$ ,  
—□— 10  $\mu\text{g}$ , —×— 1  $\mu\text{g}$ .

of evaporation was approximately linear throughout the experiment.

#### Change of $^{35}\text{S}$ -labeled Methyl Parathion in Dust Form.

Fig. 5 shows the result of paper chromatography carried out with acetone extracts of methyl parathion dusts. The conversion of methyl parathion to S- $\text{CH}_3$  isomer (D) was remarkable under dry condition, especially in dusts (a) and (d). 43 days after the initiation of the experiment, the formation of S- $\text{CH}_3$  isomer was already found in all of the dusts under dry condition, while under wet condition it was not found. 225 days after the initiation of the experiment, the formation of S- $\text{CH}_3$  isomer was found in all of the dusts except dust (d) under wet condition. It has been recognized that methyl parathion in dust form degraded faster under dry condition than wet condition. The isomerization of methyl parathion might be main process of degradation under dry condition<sup>11)</sup>, but it was presumed from the result of paper chromatography that under wet condition, other reaction(s) than isomerization occurred in the process of degradation. Although the chromatographs of all dusts under dry condition showed five spots, A, B, D, E, and F, the chromatographs of the dusts under wet condition were different according to kinds of dust (Fig. 5).

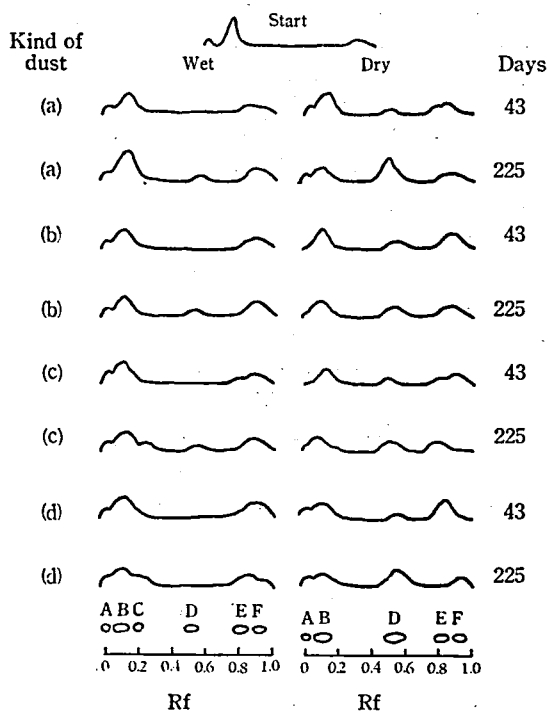


Fig. 5. Degradation of methyl parathion in dust form, based on density scans of radioautographs prepared from chromatograms of acetone extract of the dusts (Silicone paper).

The dusts under wet condition showed the following spots; dust (a), A, B, trace of C, D, E, and F; dust (b), A, B, D, E, and F; dust (c), A, B, C, D, E, and F; dust (d), A, B, C, trace of D, E, and F. The dusts under dry condition were especially different from one another in the appearance of spot C. It was also interesting that dust (d) hardly contain S- $\text{CH}_3$  isomer and it might depend on faster hydrolysis of S- $\text{CH}_3$  isomer by basicity of dust (d). Both dusts of (b) and (c) contained more than 50% of clay, but the difference in distribution or proportion of degradation products was found between dust (b) and (c). Sato and Kubo<sup>11)</sup> showed that the degradation of methyl parathion in dust form was different with physico-chemical properties of clay according to source. It may be possible to elucidate the mechanism of degradation by examining the degradation products of methyl parathion in dust which contain various kinds of clay.

### Summary

1. The evaporation and degradation of methyl parathion as residual film were examined by using  $^{32}\text{P}$ -labeled and  $^{35}\text{S}$ -labeled methyl parathion. The higher was temperature tested, the faster was the rate of evaporation and degradation. It was found from the comparison of evaporation curves of  $^{32}\text{P}$ -labeled and  $^{35}\text{S}$ -labeled methyl parathion that phosphorus and sulfur in methyl parathion molecule behaved separately in the process of evaporation. Sulfur in methyl parathion molecule disappeared faster than phosphorus, and it would be due to the release of sulfur by oxidation of methyl parathion. A part of methyl parathion degraded even at room temperature, and the formation of S-CH<sub>3</sub> isomer was accelerated with an increase of temperature. It could be concluded that the residual film of methyl parathion in atmosphere underwent oxidation, isomerization, and hydrolysis simultaneously, and that degradation products consisting largely of hydrolytic products remained later.

2. The degradation products of methyl parathion in dust form which contained various kinds of carrier were examined under both wet and dry conditions. It was found that under dry condition, the isomerization of methyl parathion to S-CH<sub>3</sub> isomer was the first stage of degradation, and that the difference in the distribution of the degradation products was not found among dusts. The distribution of the degradation products, however, under wet condition was different among dusts, and it was presumed that other reaction (s) than isomerization occurred in the process of the degradation of methyl parathion.

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### References

- 1) Fletcher, J.H., Hamilton, J.C., Hechenbleikner, I., Hoegberg, E.I., Sertl, B.J. and Cassaday J.C. : J. Amer. Chem. Soc. **72**, 2461 (1950)
- 2) Fukuto, T.R. and Metcalf R.L. : J. Amer. Chem. Soc. **76**, 5103 (1954)
- 3) Kiba, T. : Bull. Chem. Soc. Japan **28**, 641 (1955)
- 4) March, R.B., Fukuto, T.R. Metcalf, R.L. and Maxon M.G. : J. Econ. Entomol. **49**, 185 (1956)
- 5) Metcalf, R.L. and March R.B. : J. Econ. Entomol. **46**, 288 (1953)
- 6) Metcalf, R.L. and March R.B. : Science **117**, 527 (1953)
- 7) Metcalf, R.L. : Organic Insecticides, Interscience Publishers, Inc., New York (1955)
- 8) McPherson, J.B. and Johnson G.A. : J. Agr. Food Chem. **4**, 42 (1956)
- 9) Murray, D.H. and Spinks W.T. : Can. J. Chem. **30**, 497 (1952)
- 10) Oosterbaan, R.A. and Van Rotterdam J. : J. Amer. Chem. Soc. **78**, 5641 (1956)
- 11) Sato, R. and Kubo H. : *Botyu-Kagaku* **24**, 93 (1959)
- 12) Shishido, T. and Suwanai M. : J. Agr. Chem. Soc. Japan **32**, 956 (1958)
- 13) Suwanai, M. : Bull. Natl. Inst. Agr. Sci., Series C, **7**, 113 (1957a); *Ibid.* **9**, 33 (1957b)
- 14) Walkenstein, S.S. and Knebel C.M. : Anal. Chem. **29**, 1516 (1957)