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Behavior of Methyl Parathion in Certain Insects. Chojiro TOMIZAWA, Toshiro SATO, Jun-ichi FUKAMI, and Jun MITSUHASHI (National Institute of Agricultural Sciences, Nishigahara, Kita-ku, Tokyo) Received May 30, 1960. *Botyu-Kagaku*, 25, 91, 1960 (in English)

17. 昆虫体における methyl parathion の行動 富沢長次郎, 佐藤敏郎, 深見順一, 三橋 淳 (農林省 農業技術研究所) 35. 5. 30 受理

二化メイガ幼虫, ワモンゴキブリ, アズキゾウ成虫における ^{32}P および ^{35}S ラベル付 methyl parathion の体内への吸収移動ならびに代謝を調べた. 二化メイガ幼虫では吸収された ^{32}P ラベル付 methyl parathion の比較的大きい割合が皮膚体液中に認められ, 消化器における分布は少ない. ワモンゴキブリでは ^{32}P および ^{35}S ラベル付 methyl parathion の両者において雌雄間で体内分布が異なる. methyl parathion 中毒個体と回復個体でも体内分布に差異が認められ, 回復個体では放射性物質の消化管への活発な移動が認められる. 供試した 3 種の昆虫間およびワモンゴキブリの神経索, 脂肪体, 前腸間で methyl parathion の代謝速度および程度において差異が認められる.

Methyl parathion has been extensively used for the control of the rice stem borer, and the first instar larvae of the insect is the stage most often controlled by methyl parathion in paddy fields[5,6,7]. It is, therefore, important to examine the mode of action of methyl parathion on the rice stem borer. The fate of some insecticides has been examined so far largely with insects at an adult stage, but not at a larval stage[1,9,10,15,19]. The experiments reported here were designed to study the absorption, distribution, and metabolism of radioactive methyl parathion in the larvae of the rice stem borer (*Chilo suppressalis*), and as comparison in the adults of the American cockroach (*Periplaneta americana*), and the Azuki bean weevil (*Callosobruchus chinensis*).

Materials and Methods

Handling Radioactive Methyl Parathion: ^{32}P-labeled and ^{35}S-labeled methyl parathion used in the experiments had an initial relative activity of 1385 and 1875 cpm, respectively, per microgram when assayed on a GM counter. The procedure for the separation and radioassay of radioactive material was the same as those of a previous report[16]. The radioactivity of ^{32}P and ^{35}S was counted as forms of ammonium phosphomolybdate and barium sulphate, respectively. Counting data obtained in any one series of the experiments was corrected for decay to a standard day arbitrarily selected for each experiment, and converted to amount corresponding to methyl parathion. Solvent systems of paper chromatography were as follows: (1) filter paper impregnated with 5% Silicone 550 in hexane, mobile solvent, the upper phase from a mixture of ethyl alcohol 10 parts, chloroform 10 parts, and water 6 parts by volume, and (2) filter paper impregnated with 2.5% propylene glycol and 2.5% phenyl cellosolve 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 results are shown largely with chromatographs using the solvent system (1). Radioautographs were prepared by exposing filter paper to x-ray film. The radioautographs were examined, and density scans were prepared by means of a photoelectric densitometer.

Experimental Results

Experiment with the Rice Stem Borer: The rate of absorption of ^{32}P-labeled methyl parathion into the fifth instar larvae of the insect was examined according to poisoning symptoms. The fifth instar larvae were used in the experiment, because they were easier to handle than the first instar larvae. The larvae used in the experiment were reared on artificial medium[4], and had weights ranging from 45 to 60 mg per individual. Dosages of 3.398 μg, 0.640 μg and 0.092 μg in 1 μl of acetone solution, were applied topically on the dorsal surface of each larva with a microsyringe. The dosages were the mean values of five determinations on each dosage. In this experiment, discrimination between sexes was neglected. After treatment with ^{32}P-labeled methyl parathion, the larvae were kept in a petri dish at a temperature of 25° to 32°. At each stage of agitation, prostration and convulsion, more than five larvae which showed poisoning symptom with methyl parathion were taken out and rinsed with three portions of each 2 ml of acetone. Rinsed larvae were wet ashed with a mixture of sulfuric acid and nitric acid (5 : 1) for radioassay.

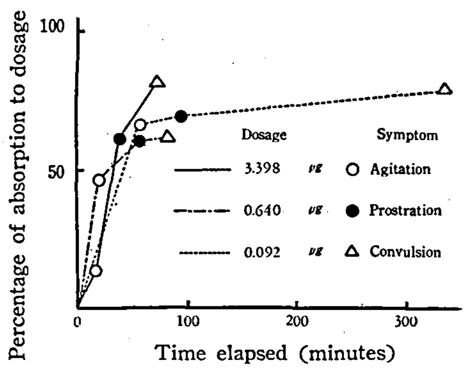

Fig. 1. Time course of absorption of ^{32}P-labeled methyl parathion in larvae of the rice stem borer.

As shown in Fig. 1, the percentage of absorption of methyl parathion to dosages was not so different between dosages at the convulsion stage, but the absolute amounts of methyl parathion absorbed into the insect body were quite different.

Distribution of methyl parathion and metabolites in the insect body was examined with the diapausing larvae having weights ranging 65 to 80 mg, collected from field. The method of topical application of ^{32}P-labeled methyl parathion on larvae was the same with that of the preceding experiment, but only the dosage of 0.755 μg per larva was applied. This dosage was high enough to estimate the radioactivity distributed in each larval organ. The diapausing larvae took some time to be killed by methyl parathion as compared with that of larvae reared on artificial medium, because of the difference in body weight and physiological condition from those of the larvae used in the preceding experiment. Although sexes were not discriminated in the preceding experiment, it was seen that the amount of methyl parathion and metabolites distributed in organs of the larvae was different between sexes. Among tissues dissected, fat body had a high content of radioactive material, and the radioactive material distributed in the tissues other than fat body was slight. The remainder, which consisted largely of integument and haemolymph showed a higher content of radioactive material than that of fat body at any stage of poisoning (Table 1).

Experiment with the American Cockroach: It is necessary for the study of the mode of action of insecticide to compare the fate of insecticide between insects being morphologically different. The American cockroach has been used generally for toxicological studies, and it is easy to compare

Table 1. Distribution of ^{32}P-labeled methyl parathion and metabolites in tissues of larvae of the rice stem borer after topical application of 0.755 μg per larva on dorsal segments. *

Sex	Male			Female		
Symptom of poisoning	Agitation	Prostration	Convulsion	Agitation	Prostration	Convulsion
Fore gut	0.003	0.002	0.004	0.004	0.002	0.003
Hind gut	0.001	0.002	0.003	0.001	0.001	0.002
Malpighian tube	0.002	0.002	——	0.002	0.001	0.002
Fat body	0.231	0.235	0.193	0.298	0.293	0.272
Nerve cord	< 0.001	0.001	< 0.001	< 0.001	< 0.001	< 0.001
Brain	< 0.001	< 0.001	0.002	< 0.001	< 0.001	< 0.001
Silk gland	0.002	0.001	0.003	0.002	0.002	0.003
Reproductive system	0.001	0.001	< 0.001	< 0.001	< 0.001	< 0.001
Remainder	0.318	0.374	0.328	0.422	0.498	0.409
Time elapsed (min.)	100	235	1225	140	310	1260

* Figures show μg weight of radioactive material expressed as methyl parathion per tissue.

the present result with those obtained with other insecticides. In this experiment, adults of the American cockroach reared on a dry diet made of baker's yeast were used.

For examining the distribution of methyl parathion and metabolites in the insect body, 2.55 μg of ^{32}P-labeled methyl parathion in 5 μl of acetone solution was topically applied on the third segment of the cockroach abdomen. Carbon dioxide anesthesia was used only during treatment with the insecticide. In the same manner as already shown in the experiment with the rice stem borer, the cockroaches were dissected following poisoning symptoms, and radioassay of ^{32}P contained in each insect organ was made (Table 2). Distribution of radioactive materials in the cockroach was

Table 2. Distribution of ^{32}P-labeled methyl parathion and metabolites in tissues of roaches after topical application of 2.55 μg per roach on abdominal segment.

Sex	Male			Female			
Symptom of poisoning	Agitation	Prostration	Convulsion	Agitation	Prostration	Convulsion	Recovery
Fore gut	0.120[1)] 0.007	0.229 0.009	0.445 0.015	0.788 0.025	1.039 0.029	1.074 0.046	1.054 0.041
Crop	0.080 0.011	0.115 0.015	0.094 0.010	0.071 0.010	0.062 0.006	0.049 0.005	0.108 0.006
Caeca	0.029 0.005	0.054 0.008	0.094 0.010	0.076 0.008	0.111 0.010	0.150 0.019	0.131 0.015
Mid gut	0.093 0.015	0.191 0.038	0.252 0.023	0.082 0.009	0.082 0.007	0.070 0.006	0.084 0.007
Malpighian tube	0.046 0.013	0.021 0.012	0.004 0.002	0.019 0.009	0.029 0.010	0.018 0.010	0.029 0.008
Hind gut	0.216 0.014	0.522 0.033	0.258 0.022	0.236 0.008	0.152 0.006	0.196 0.020	0.406 0.025
Rectum	0.030 0.013	0.045 0.017	0.029 0.012	0.024 0.011	0.039 0.013	0.006 0.002	0.043 0.017
Nerve cord	0.086 0.027	0.068 0.021	0.051 0.011	0.023 0.005	0.023 0.005	0.016 0.005	0.008 0.002
Fat body	0.389 0.005	0.307 0.005	0.137 0.002	0.171 0.002	0.209 0.002	0.119 0.003	0.117 0.001
Reproductive system	0.039 0.005	0.016 0.004	0.038 0.006	0.031 0.004	0.015 0.005	0.021 0.002	0.034 0.007
Femoral muscle	0.043 0.002	0.058 0.003	0.048 0.002	0.018 0.001	0.021 0.001	0.013 0.001	0.013 0.001
Brain	0.003 ——[2)]	0.004 ——	0.007 ——	0.002 ——	0.005 ——	0.005 ——	0.001 ——
Remainder	0.474 ——	0.330 ——	0.313 ——	0.439 ——	0.392 ——	0.357 ——	0.173 ——
Time elapsed (min.)	105	205	435	195	365	1425	1445

1) Upper figures show μg weight of radioactive material expressed as methyl parathion per tissue; lower figures show μg weight per mg of tissue.
2) These weights were not measured.

quite different from that of the rice stem borer. Although fat body had a considerable amount of radioactive material, digestive tract, especially fore gut, showed a higher content of radioactive material at the convulsion stage than that of fat body. It seemed that with advance of poisoning, the radioactive material moved from fore gut to mid and hind guts. The accumulation of radioactive material in the fore gut was faster in females than in males. The amount of radioactive material accumulated in the fore gut was also greater in females than in males. The contents of radioactive material in nervous system, femoral muscle, and fat body were higher in males than in females at any stage of the poisoning. As shown in the experiment with ethyl parathion and other organophosphorus insecticides by Fernando et al[3], the percentage of radioactivity distributed in the nervous system to the radioactivity of dosage was very low. At the end of the experiment, all males died, but some females survived, and showed recovery symptoms from insecticide poisoning. The survived females had more radioactive material in the digestive tract, especially hind gut, than that of the dead females. This fact seemed to suggest the route of excretion of the insecticide in the insect. In the rice stem borer, the difference of tolerance to methyl parathion among individuals seemed to depend on the body weight which could be attributed largely to the fat body content. The highest retention of radioactive material in the insect body was seen at the prostration stage. The decrease of radioactive material contained in the insect body at the convulsion stage was probably due to the excretion and vomit which were seen during insecticide poisoning (Fig. 2).

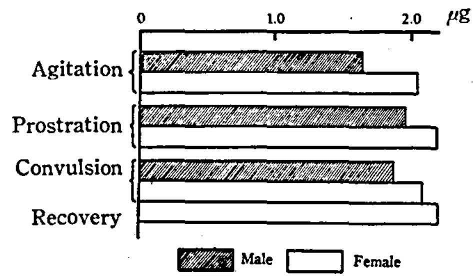

Fig. 2. Amount of ^{32}P-labeled methyl parathion absorbed by the American cockroach at various stage of poisoning.

In the above experiment, only phosphorus in methyl parathion molecule was traced. It is also interesting to examine the fate of sulfur in methyl parathion molecule in comparison with that of phosphorus. When the cockroaches were treated with 5.85 μg of ^{35}S-labeled methyl parathion in 5 μl of acetone solution, the distribution of radioactive material in the insect was different from that of ^{32}P (Table 3). In this case, no recovery from insecticide poisoning was observed in either sex of the cockroaches.

Table 3. Distribution of ^{35}S-labeled methyl parathion and metabolites in tissues of roaches after topical application of 5.85 μg per roach on abdominal segment.*

Sex	Male		Female	
Symptom of poisoning	Agitation	Convulsion	Agitation	Convulsion
Fore gut	1.009	0.779	1.083	1.002
Crop	0.044	0.664	0.068	0.411
Caeca	0.201	0.178	0.320	0.161
Mid gut	0.391	0.253	0.307	0.161
Malpghian tube	0.201	0.104	0.175	0.080
Hind gut	0.898	0.778	0.843	1.370
Rectum	0.166	0.118	0.086	0.438
Nerve cord	0.033	0.067	0.015	0.041
Fat body	1.097	1.002	0.755	1.021
Femoral muscle	0.131	0.187	0.157	0.275
Time elapsed (min.)	85	260	125	330

* Figures show μg weight of radioactive material expressed as methyl parathion per tissue.

Although the dosage applied on the insect was different between ^{32}P-labeled and ^{35}S-labeled methyl parathion, the most distinct difference was seen in the accumulation of radioactive material in the digestive tract. ^{35}S-labeled material accumulated not only in the fore gut but also in the mid and hind guts at the agitation stage. In males, ^{32}P-labeled material accumulated gradually into the digestive tract, while ^{35}S-labeled material accumulated at the early stage of poisoning and did not increase with advance of poisoning. In females, ^{32}P-labeled material accumulated rapidly in the digestive tract at the agitation stage, and did not increase toward the later stage of poisoning, while ^{35}S-labeled material seemed to increase gradually toward the later stage of poisoning. ^{32}P-labeled material in fat body disappeared with advance of poisoning, but ^{35}S-labeled material was retained longer in fat body.

Metabolism of Radioactive Methyl Parathion : Since the size of the larva of the rice stem borer was rather small and therefore the minimum of the lethal dosage of methyl parathion for the larva was less than 0.1 μg per larva, it was impossible to examine the metabolism of methyl parathion in organs of the larva because of the low specific activity of methyl parathion used in this experiment. It was presumed, however, from the data shown in Table 1 that the haemolymph of the larvae contained a considerable amount of radioactive metabolites. The haemolymph of the larvae applied with 3.398 μg of ^{32}P-labeled methyl parathion was collected, and the paper electrophoresis of the haemolymph was made using veronal buffer (pH 8.5, 400 V). Although the haemolymph contained several protein fractions, the fraction with radioactivity remained at the origin of the paper. In addition, the electrophoretic pattern of the protein of the haemolymph separated by the paper electrophoresis was the same qualitatively between untreated and treated larvae. On the other hand, the haemolymph of the larvae applied with ^{32}P-labeled methyl parathion was precipitated with 90% ethyl alcohol. After centrifuging the protein fraction, the supernatant fluid was concentrated and chromatographed. As shown in Fig. 3, the major part of radioactivity existed at the origin of the paper chromatograph, and traces of methyl parathion (B), S-CH_3 isomer (D), and some acidic metabolites (E and F) were also detected. Peak E appeared to correspond to dimethyl hydrogen phosphorothioate.

In the experiment with cockroach applied topically with ^{35}S-labeled methyl parathion, the amount of methyl parathion and metabolites distributed in the nervous system of the insect was very small. For examining the metabolism of methyl parathion

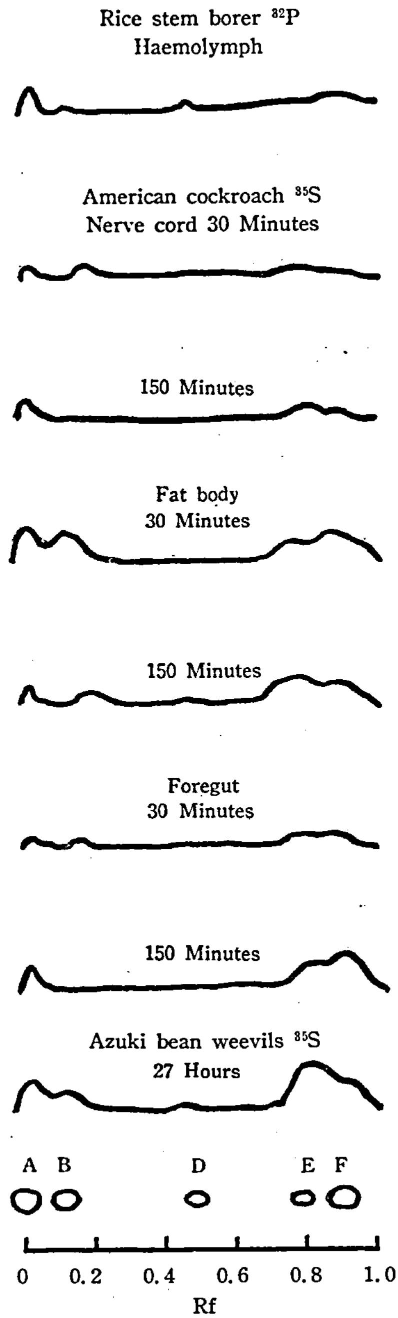

Fig. 3. Metabolism of methyl parathion in the rice stem borer, the American cockroach, and the Azuki bean weevil, based on density scans of radioautograms of acetone or alcohol extracts of the tissues or whole insect (Silicone paper).

in organs of the insect, enough radioactivity to count or to make radioautograph of paper chromatogram must be contained in each organ of the insect. It was, therefore, necessary to inject ^{35}S-labeled methyl parathion directly into the insect body. 5.85 μg of ^{35}S-labeled methyl parathion in 2 μl of acetone solution was injected into the third segment of the abdomen of male cockroach. The cockroaches were sampled twice after the injection, 30 minutes and 150 minutes. On sampling, the insects were dissected, and the nerve cord, fat body, and fore gut were taken out and homogenized with 0.2～0.5 ml of acetone in a cold mortar and centrifuged.

^{35}S-labeled material in the supernatant fluid was separated by means of paper chromatography using filter paper impregnated with Silicone 550. At the first sampling, the presence of methyl parathion in the nerve cord was obvious, but at the second sampling, methyl parathion almost disappeared, and spots, A, E and F were detected. In the fat body, methyl parathion was detected at both sampling times. In the fore gut, methyl parathion was already metabolized to other metabolites, A, E and F. In any of tissues examined, E and F increased with time, but the ratio of E to F was different between sampling times, and among tissues.

In comparison with the results of cockroach, the metabolism of methyl parathion absorbed into the Azuki bean weevil by contact with residual film of the insecticide was examined. Acetone solution of ^{35}S-labeled methyl parathion was pipetted into a petri dish having a diameter of 7.5 cm, and evaporated under air flow. Quantity put into the petri dish was 0.1 μg per square cm of dish. Fifty weevils were kept in the petri dish for 24 hours. After rinsing the insects with less than 10 ml of acetone to eliminate the contamination of methyl parathion adhering on the surface of the insects, they were homogenized with 3 ml of acetone, and centrifuged. The supernatant fluid was chromatographed in the same manner as that of cockroach. Although the chromatographic pattern detected by radioautography was the same with that of the cockroach, quantitative ratios among metabolites were different. In the Azuki bean weevil, a trace of $S\text{-}CH_3$ isomer was detected. The probability of isomerization of methyl parathion might be different among insects or organs.

Discussion

In the experiment with rice stem borer, the rate of penetration of ^{32}P-labeled methyl parathion into larvae depended on the dosages applied, and the time after treatment. The time required to induce poisoning with methyl parathion, may be controlled by concentration gradient of the insecticide from the site applied on the surface of the insect body to the target in the insect body[13]. In the levels of 3.398 μg and 0.640 μg per insect, the activity of methyl parathion metabolism in the insect body would be too low to lessen poisoning with methyl parathion. In other words, the route to the target might be saturated with active substance, and consequently the time required to induce each poisoning symptom mutually approached. In the level of 0.092 μg per insect, as soon as methyl parathion penetrated into the insect body, a part of methyl parathion degraded, and the absolute amount of methyl parathion which reached the target would be limited. It was found from the experiment of the distribution of ^{32}P-labeled methyl parathion and metabolites in the larvae, that the part of the larva which consisted largely of integument and haemolymph contained a higher concentration of radioactive material than that of any other tissues. Since the haemolymph of the larvae contains a comparatively high activity of cholinesterase[18], it was presumed that a part of radioactive material associated with the protein of the enzyme. The result of paper electrophoresis carried out with the haemolymph of the insect applied topically with methyl parathion, was obscure. On the other hand, the fraction of haemolymph which was extractable with 90% ethyl alcohol, contained several metabolites. If methyl para-oxon existed in the metabolites of the haemolymph, it should locate near peak E on the paper chromatograph. It has been shown, however, that in studies of ^{32}P-labeled parathion (presumably ethyl parathion) applied to the American cockroach, about 0.1% of the total ^{32}P was recoverable as para-oxon[2]. Since methyl parathion is more hydrolyzable in the biological system than ethyl parathion, it might be impossible to detect the presence of methyl para-oxon by radioautographic technique, because of the low specific activity of methyl parathion used in the experiment.

The distribution of radioactive methyl parathion and metabolites in the American cockroach was quite different from that of the rice stem borer. As shown in Tables 2 and 3, radioactive material appeared to excrete actively through the digestive tract of the insect. Although Roan et al.[14] did not discriminate the amount of three tetra-alkyl pyrophosphate and ethyl para-oxon absorbed into the insect body between sexes, the difference of absorption of radioactive methyl parathion was clearly seen between sexes. The difference seemed to depend in part on the size of males and females, but it was highly probable that there was a qualitative difference of excretion system between sexes. Fernando et al.[3] pointed out that the rate of movement and excretion of organophosphorus insecticides mentioned above through the body closely paralleled the susceptibility of insecticides to aqueous hydrolysis. It was presumed from the result of the present experiment that the percentage of radioactivity of the excreted metabolites to the radioactivity of the dosage was low as compared with those of tetra-alkyl pyrophosphate. No serious discrepancy existed between the present result and that reported by Fernando et al. Lockau and Ludicke[9] also pointed out the possibility of excretion of ^{32}P-labeled ethyl parathion in the American cockroach. In the experiment with ^{32}P-labeled methyl parathion, the digestive tract, especially caeca, hind gut and rectum in the surviving females contained a higher concentration of radioactive material that of the dead females, while a lower concentration was found in the nerve cord and brain of the surviving females than those of the dead females. The dead females were apparently unable to remove the toxicant from the nerve cord and brain at a sufficient rate[14]. Between sexes, there was a difference in the accumulation of radioactive material into each region of the digestive tract, and the accumulation of radioactive material in the nervous system was higher in males than in females.

The difference in the transfer rate of radioactive material into the digestive tract could be seen between ^{32}P-labeled and ^{35}S-labeled methyl parathion. Although this fact might be in part attributed to the difference of dosage applied to the insects between both of radioactive insecticides, it seemed to depend largely on the difference of the route and the rate of excretion of radioactive material. When methyl parathion was absorbed into the insect body, a part of methyl parathion underwent oxidation to methyl para-oxon following the release of sulfur[12], and another part of methyl parathion might be directly hydrolyzed to ionic products. The probability of isomerization of methyl parathion in the insect body could not be excluded. It is, therefore, natural that a clear difference in the accumulation of radioactive material into some tissues of the insects was found between ^{32}P-labeled and ^{35}S-labeled methyl parathion. In the injection experiment with ^{35}S-labeled methyl parathion, although the nerve cord and fat body contained methyl parathion itself, it might have resulted from the proximity of the tissues to the site of injection. As shown in Fig. 3, the presence of methyl parathion (B) in the nerve cord was seen 30 minutes after injection, but 150 minutes after injection the peak of methyl parathion almost disappeared. In fat body, the fact that the percentage of methyl parathion to the other metabolites was rather small, might suggest the active metabolism of methyl parathion in this tissue. In fore gut, the presence of methyl parathion was detected at the first sampling time, and it might have resulted from the diffusion of acetone solution containing ^{35}S-labeled methyl parathion injected. At the second sampling time, the radioactive material in fore gut consisted of only degradation products.

Summary

1. The penetration and the distribution of ^{32}P-labeled methyl parathion in the larvae of the rice stem borer was examined in connection with poisoning symptoms. The penetration rate of methyl parathion was different among dosages. With a decrease of dosage, the percentage of methyl parathion absorbed into the larvae to

dosage in each stage of agitation, prostration and convulsion mutually approached, and the time required to induce each poisoning symptom became prolonged. More than half of the methyl parathion absorbed into larvae existed in the integument and haemolymph. Among the tissues dissected, the fat body contained the highest concentration of radioactive material, and the distribution of radioactive material in the digestive tract was very low.

2. Distribution of ^{32}P-labeled or ^{35}S-labeled methyl parathion in the American cockroach was examined. In the experiment with ^{32}P-labeled methyl parathion, radioactive material accumulated rapidly into the digestive tract, especially the fore gut at the agitation stage, and the material moved from the fore gut to the mid and hind guts with advance of poisoning. When the same dosage was applied on both sexes of the cockroach, females absorbed more methyl parathion than males. Accumulation of radioactive material into the nerve cord and brain was smaller in females than in males, and larger transfer of radioactive material into the digestive tract was observed in surviving females than in dead females. The accumulation rate of radioactive material into each region of the digestive tract was different between ^{32}P-labeled and ^{35}S-labeled methyl parathion.

3. Metabolism of methyl parathion in the larvae of the rice stem borer, and in the adults of the American cockroach and the Azuki bean weevil was examined. When the haemolymph of the larvae of the rice stem borer treated with ^{32}P-labeled methyl parathion was fractionated with 90 % ethyl alcohol, radioactive material distributed in both of the precipitate and the supernatant fluid. It was found that the radioactive material in the alcohol soluble supernatant of the haemolymph was occupied by methyl parathion and several metabolites.

Metabolism of ^{35}S-labeled methyl parathion injected into the abdomen of the American cockroach was examined in the nerve cord fat body, and fore gut. The same pattern of metabolites was detected in the tissues examined, but the time course of rise and fall of metabolites was different between tissues. This fact suggested active metabolism and rapid excretion of methyl parathion in the insect. In the homogenate of the Azuki bean weevil applied with ^{35}S-labeled methyl parathion, similar metabolites to those of the American cockroach were detected, but the formation of S-CH_3 isomer was obvious in the Azuki bean weevil though it was questionable in the American cockroach.

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Fate of Organophosphorus Insecticides Sprayed on Rice Plant. Chojiro Tomizawa, Toshiro Sato (National Institute of Agricultural Sciences, Nishigahara, Kita-ku, Tokyo). Hiroo Yamashina*, Hiroshi Kubo** (Kyushu Agricultural Experiment Station, Chikugo, Fukuoka). Received June 11, 1960. *Botyu-Kagaku*, **25**, 99, 1960 (in English)

18. 水稲に散布した有機燐殺虫剤の動態

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水稲に散布した ^{32}P ラベル付 malathion および ^{35}S ラベル付 methyl parathion の行動を追跡した. ^{32}P ラベル付 malathion を動力噴霧機で散布し, 散布後の空気中および水中の経時変化を測定した. 水稲葉に附着した ^{32}P ラベル付 malathion の ^{32}P は葉面から直接蒸発するほか, 葉組織内へ滲透した後, 葉鞘, 根を経て他の茎へ移行してゆく. 一枚の葉では葉鞘から葉身へ向う傾向が認められる. ^{35}S ラベル付 methyl parathion の水稲葉における行動は ^{32}P ラベル付 malathion に類似した傾向を示す. 水稲葉組織内へ滲透した malathion および methyl parathion は極めて短時間に分散される.

Organophosphorus insecticides have been extensively used for the control of rice plant pests. Parathion and malathion are representative of organophosphorus insecticides against rice stem borer (*Chilo suppressalis* Walker) and green rice leafhopper (*Nephotettix bipunctatus cincticeps* Uhler), respectively. Many works on the application of organophosphorus insecticides in paddy fields have been carried out, but the results of these works do not always agree, because of the difficulty in examining the behavior of insecticides in minute amounts[3,7,9]. For this purpose, the use of a radioactive insecticide will give more reliable results for the fate of insecticides than chemical analysis or bioassay. The present experiments were undertaken to examine the fate of radioactive malathion and methyl parathion under rice plant environment with particular reference to the insecticide in dynamic state.

Materials and Methods

Radioactive Insecticides : ^{32}P-labeled malathion was prepared according to the description of March et al.,[2] starting from ^{32}P-labeled phosphoric acid (100mc). Radioactive red phosphorus was prepared by heating ^{32}P-labeled phosphoric acid with charcoal at 1000°[4]. Chromatographic analysis showed that the radioactive portion was 96.5% malathion, with an initial relative activity, 3928 cpm.

The preparation of ^{35}S-labeled methyl parathion has been described previously[6]. Chromatographic analysis showed that the radioactive portion was more than 99% methyl parathion, with an initial relative activity, 1385 cpm. ^{35}S-labeled methyl parathion was used on a small scale as compared with ^{32}P-labeled malathion. The methods employed for the investigation of radio-tracer preparations of the insecticides have been described in detail.[6]

Paper Chromatography : The following solvent systems of paper chromatography were used for

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