原　著

Fate of S^{35}-labeled p-Chlorophenyl p-Chlorobenzenesulfonate in Some Organisms. Chojiro Tomizawa (National Institute of Agricultural Sciences, Nishigahara, Kita-ku, Tokyo) Received Feb. 1, 1960. *Botyu-Kagaku*, 25, 47, 1960. (in English).

9. 生物体における S^{35} 標識 p-chlorophenyl p-chlorobenzenesulfonate の消長

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ミカンハダニの卵および成虫，ワモンゴキブリ成虫，ミカン苗木，ダイズ幼苗における S^{35} 標識 p-chlorophenyl p-chlorobenzenesulfonate の吸収，体内移動，代謝を調べた．ミカンハダニでは卵，成虫共，本化合物を殆んど分解しないが，ワモンゴキブリでは腹腔内，中腸，排泄物の順序で可なり分解が進むことが認められた．ミカン苗木，ダイズ幼苗では，環境条件が両者で全く異なつていたが，前者では葉組織内への滲透は比較的少なく他葉への移動も僅かである．ダイズでは滲透も生長部分への移動も顕著であつた．いづれの場合も分解の第一段階は p-chlorobenzenesulfonic acid の生成である．

Although much work has been accomplished concerning the acaricidal action of p-chlorophenyl p-chlorobenzenesulfonate (Kenaga & Hummer[6], Barnes[1], Jeppson[5], Kirby & Read[7], Fukuda & Shinkaji[3]), the fate of the compound in organisms has hardly been examined due to the difficulty of estimating traces of the compound in organisms. It is important for the clarification of the acaricidal action to examine the fate of the compound in organisms. The experiments described below were undertaken in order to examine the behavior of p-chlorophenyl p-chlorobenzenesulfonate on and in some organisms.

Materials and Methods

Preparation of S^{35}-labeled p-chlorophenyl p-chlorobenzenesulfonate:— S^{35}-labeled p-chlorophenyl p-chlorobenzenesulfonate was synthesized from S^{35}-labeled sulfuric acid through p-chlorobenzenesulfochloride according to the description of Otto[9] and Slagh and Britton[10]. Specific activity was about 2.5 mc per mM just after the preparation. Crude product of the compound was purified through a column of activated alumina with benzene as the mobile solvent. Examination of the purified product by paper chromatography disclosed the presence of no other radioactive material except p-chlorophenyl p-chlorobenzenesulfonate. The emulsifiable concentrate consisted of p-chlorophenyl p-chlorobenzenesulfonate 25 parts, polyoxyethylene nonylphenol ether (degree of polymerization, 9.6) 25 parts, and benzene 50 parts by weight.

Test Organisms:— Citrus red mite —— Citrus leaves infested with eggs and adults of the citrus red mite, *Metatetranychus citri* McCregor were collected from citrus trees at Okitsu, Shizuoka. These leaves were used within three days after collection for determining the fate of p-chlorophenyl p-chlorobenzenesulfonate in eggs and adults of the citrus red mite.

American cockroach:—The American cockroaches, *Periplaneta americana* L. reared at 30° on a dry diet made of baker's yeast, were used.

Citrus sapling and soybean seedling:— Citrus sapling, variety of Sugiyama-unsyu, grown about three years in pot was kept at a temperature of 5° to 10° during the experiment, and soybean seedling in water culture was kept at a temperature of 20° to 25°.

Paper chromatography:— For separating radioactive p-chlorophenyl p-chlorobenzenesulfonate and metabolites, the ascending technique of paper chromatography was employed, using strips of Toyo filter paper No. 51, 250mm in length, 25mm in width. A mixture of isopropyl alcohol 10 parts, distilled water 2 parts and acetic acid 0.5 part was used as the developing solvent. In a preliminary experiment using nonradioactive compound, p-chlorophenyl p-chlorobenzenesulfonate and metabolites were detected upon the reaction of chlo-

rine atom according to the method of Mitchell[5]. In a tracer experiment, radioautographs of S^{35} were prepared by exposing the filter paper to x-ray film for about 7 months. The radioautographs were examined, and density scans were prepared by means of a photoelectric densitometer.

p-Chlorophenyl p-chlorobenzenesulfonate is soluble in benzene, but some probable metabolites such as p-chlorobenzenesulfonic acid have a great affinity to water. The extraction of S^{35}-labeled materials in organisms was carried out as follows; samples containing S^{35} were macerated in a mortar with 10 times benzene by weight, and centrifuged. After the benzene extraction was carried out three times, the benzene extracts were pooled, and evaporated under vacuum. The residue after benzene extraction was extracted with water. Benzene and water extracts were made up to a definite volume with each solvent, and chromatographed together on a strip of filter paper, or individually on two strips.

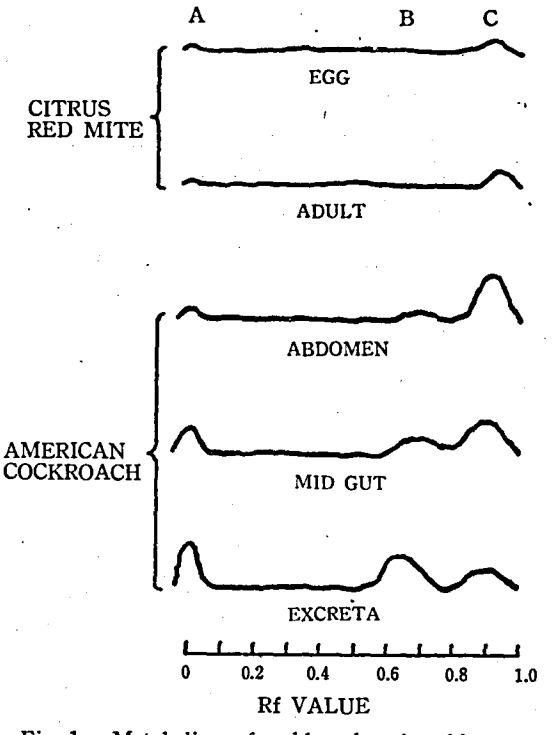

Fig. 1. Metabolism of p-chlorophenyl p-chlorobenzenesulfonate by the citrus red mite and the American cockroach, based on density scans of radioautographs prepared from paper chromatograms of benzene and water extracts of insects.

Experimental Results

Experiment with Citrus Red Mite —— 15 citrus leaves infested with eggs and adults of the citrus red mite were sprayed with 10 ml of the water emulsion containing 2.5 mg of p-chlorophenyl p-chlorobenzenesulfonate by means of a small glass sprayer. Each of three sprayed leaves was transferred to a Petri dish, and kept at a temperature of 25°C under humidity condition controlled by a saturated solution of sodium chloride. Three days after spraying, 95 adults consisting of both sexes were taken out, and washed once with benzene in a mortar, and macerated. 1451 eggs were scraped from the citrus leaves and transferred to a glass plate, and macerated with a spatula. The extracts of adults and eggs were prepared in the same manner mentioned above. It was found as a result of paper chromatography that the major part of radioactive materials in both extracts of adults and eggs contained p-chlorophenyl p-chlorobenzenesulfonate (C), and that traces of radioactive materials were located at the starting point of paper chromatographs (A) (Fig. 1).

Experiment with American cockroach :— By means of a microsyringe, 5 μl of acetone solution containing 200 μg of S^{35}-labeled p-chlorophenyl p-chlorobenzenesulfonate was injected into the third segment of the cockroach abdomen. After the injection, 10 cockroaches of both sexes were kept in a Petri dish with filter paper in the bottom, at a temperature of 30°, for 2 days. After opening the abdominal part of the cockroach, the inside of the abdomen was washed with 1 ml of benzene and water, three times. The combined extracts were chromatographed. The extracts of mid gut and excreta dropped on filter paper were also prepared, and chromatographed. As shown in Fig. 1, it is clear that the decomposition of p-chlorophenyl p-chlorobenzenesulfonate advanced in the order of abdomen, mid gut and excreta. In excreta, p-chlorophenyl p-chlorobenzenesulfonate was considerably decomposed, and p-chlorobenzenesulfonic acid (B) accumulated remarkably. The materials on the starting point of the paper chromatograph also increased with the advance of

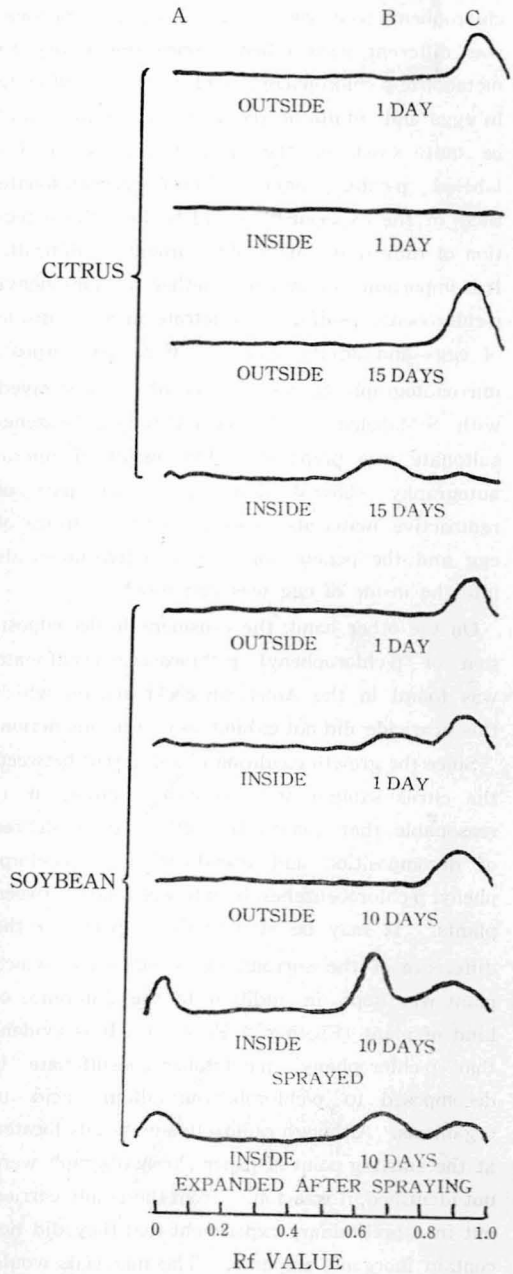

Fig. 2. Metabolism of p-chlorophenyl p-chlorobenzenesulfonate by the leaves of citrus and soybean, based on density scans of radioautographs prepared from paper chromatograms of benzene and water extracts of plants.

decomposition of p-chlorophenyl p-chlorobenzenesulfonate.

Experiment with citrus sapling and soybean seedling:— In the experiment with citrus sapling, 2 samples were taken, 1 and 15 days after spraying. After washing the surface of the leaves with benzene, they were macerated in a mortar with benzene. After mixing the macerate with benzene, the benzene extract was separated by centrifugation. Subsequently, the water extract was prepard in the same manner. As shown in Fig.2, only p-chlorophenyl p-chlorobenzenesulfonate was detected on the surface of the leaves at any sampling time. It was found that p-chlorophenyl p-chlorobenzenesulfonate penetrated very little to the inside of the leaves 1 day after spraying ; but 15 days after spraying a considerable amount of p-chlorobenzenesulfonic acid and a trace amount of p-chlorophenyl p-chlorobenzenesulfonate were found in the inside of the leaves.

In the experiment with soybean seedling, primary leaves were almost expanded but terminal buds were still small at the time of spraying.

Fig. 3. Radioautogram of twig of citrus sprayed with S^{35}-labeled p-chlorophenyl p-chlorobenzenesulfonate.

Even 1 day after spraying, p-chlorophenyl p-chlorobenzenesulfonate and p-chlorobenzenesulfonic acid were already detected in the inside of leaves. 10 days after spraying, p-chlorophenyl p-chlorobenzenesulfonate decreased in the inside of leaves but p-chlorobenzenesulfonic acid increased inversely. The same feature was seen in the leaves expanded after spraying though the proportion of both compounds was different from that in the sprayed leaves. Like the citrus leaves, only p-chlorophenyl p-chlorobenzenesulfonate was detected on the surface of leaves at any sampling time.

Since the translocation of S^{35}-labeled metabolites in plant is interesting from the standpoint of acaricidal action, radioautographs of the plants sprayed with S^{35}-labeled p-chlorophenyl p-chlorobenzenesulfonate were prepared. The translocation of radioactive metabolites to the parts which grew after spraying was obvious in the soybean seedling, while it was slight in the citrus sapling (Figs. 3 and 4)

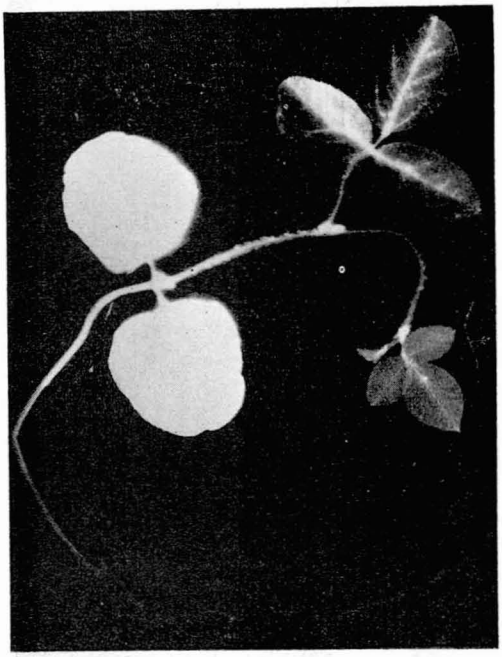

Fig. 4. Radioautogram of soybean seedling sprayed with S^{35}-labeled p-chlorophenyl p-chlorobenzenesulfonate.

Discussion

As shown in the above results, the fate of p-chlorophenyl p-chlorobenzenesulfonate in organisms was different each other. Since the ability to metabolize p-chlorophenyl p-chlorobenzenesulfonate in eggs and adults of the citrus red mite would be quite small or the specific activity of S^{35}-labeled p-chlorophenyl p-chlorobenzenesulfonate used in the experiment would be low, the detection of radioactive metabolites might be difficult. It is important to examine whether p-chlorophenyl p-chlorobenzenesulfonate penetrate into the inside of eggs and adults or not. For this purpose, microautograph of the section of eggs sprayed with S^{35}-labeled p-chlorophenyl p-chlorobenzenesulfonate was prepared. The result of microautography showed that the major part of radioactive materials existed near the surface of egg and the penetration of radioactive materials into the inside of egg was very low*.

On the other hand, the considerable decomposition of p-chlorophenyl p-chlorobenzenesulfonate was found in the American cockroach on which this acaricide did not exhibit any injurious action.

Since the growth condition was different between the citrus sapling and soybean seedling, it is reasonable that quantitative difference in degree of decomposition and translocation of p-chlorophenyl p-chlorobenzenesulfonate was found between plants. It may be attributed, in part, to the difference of the surrounding condition in which plant was kept, in addition to the difference of kind of plant (Ebeling & Pence[2]). It is evident that p-chlorophenyl p-chlorobenzenesulfonate is decomposed to p-chlorobenzenesulfonic acid in organisms. Although radioactive materials located at the starting point of paper chromatograph were not identified, it was found from the result carried out in a preliminary experiment that they did not contain inorganic sulphate. The materials would contain two or more metabolites.

The penetration of p-chlorophenyl p-chlorobenzenesulfonate into plant tissue was examined by Gunther and Jeppson[4] on the basis of estimation of p-chlorophenol produced by hydrolysis of the

* Because of the resignation of the coworker who carried out microautography technique, the radioautograph of egg was not ready at the time of publication.

original compound. They indicated that the largest portion of the residue of p-chlorophenyl p-chlorobenzenesulfonate on and in mature oranges and lemons at any time shortly after application is subcuticular. It is possible that some portion of the residue in Gunther and Jeppson's data might contain metabolites of p-chlorophenyl p-chlorobenzenesulfonate based on the analytical procedure used by them. There is considerable evidence concerning the penetration of p-chlorophenyl p-chlorobenzenesulfonate into plant from the standpoint of controlling mite. This evidence must be reviewed upon definite growth condition and kind of plant.

Summary

1. S^{35}-labeled p-chlorophenyl p-chlorobenzenesulfonate was hardly decomposed in eggs and adults of the citrus red mite.

2. When p-chlorophenyl p-chlorobenzenesulfonate was injected into the abdomen of the American cockroach, the decomposition of the acaricide advanced in the order of abdomen, mid gut and excreta. The appearance of p-chlorobenzenesulfonic acid increased in the same order mentioned above.

3. In citrus sapling and soybean seedling, p-chlorophenyl p-chlorobenzenesulfonate penetrated into the inside of plants and was decomposed to p-chlorobenzenesulfonic acid and other metabolites. The degree of decomposition in plants would be different due to growth condition and kind of plant. Within the experiments, p-chlorophenyl p-chlorobenzenesulfonate translocated easily to the newer leaves expanded after spraying in soybean seedling, while the translocation in citrus sapling which did not grow during the experiment was slight.

Acknowledgement

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References

1) Barnes, M. M.: J. Econ. Entomol. **44**, 672 (1951)
2) Ebeling, W. and R. J. Pence: J. Econ. Entomol. **47**, 789 (1954)
3) Fukuda, J. and N. Shinkaji: Tokai-Kinki Agri. Exp. Sta., Horticul. Div., Special Report No. 3. 23 (1956)
4) Gunther, F. A. and L. R. Jeppson: J. Econ. Entomol. **47**, 1027 (1954)
5) Jeppson, L. R.: J. Econ. Entomol. **44**, 823 (1951)
6) Kenaga, E. E. and R. W. Hummer: J. Econ. Entomol. **42**, 996 (1949)
7) Kirby, A. H. M. and R. P. Tew: Nature **171**, 474 (1953)
8) Mitchell, L. C.: Assoc. Offic. Agri. Chem. **39**, 980 (1956)
9) Otto, R.: Ann. **141**, 374 (1867); **143**, 106 (1868)
10) Slagh, H. R. and E. C. Britton: J. Am. Chem. Soc. **72**, 2808 (1950)

Release Studies on the Dispersion of the Lesser House Fly, *Fannia canicularis*, in the Residential Area of Bibai, Hokkaido. Studies on the behavior of public health important flies, III. Kazuki Ogata (Department of Medical Entomology, National Institute of Health, Tokyo) and Takeshi Suzuki (Department of Parasitology, Institute for Infectious Diseases, University of Tokyo) Received March 17, 1960. *Botyu-Kagaku*, 25, 51, 1960 (with English résumé, 56).

10. ヒメイエバエの分散飛翔に関する記号放逐実験 ハエの行動に関する研究 第3報. 緒方一喜（国立予防衛生研究所 衛生昆虫部）・鈴木 猛（東京大学伝染病研究所 寄生虫研究部）

ヒメイエバエの記号個体を放逐して，その分散を調べた．放逐個体1,000匹のうち，87匹が回収されたが，77.0% が 30 m 半径内で，97.7% が 110 m 半径内で回収された．放逐点を離れるにつれて激減するが，少数個体はかなり遠くまで飛来する傾向がうかがわれた．ハエ防除の観点からは，多数活動範囲を 300～400 m 半径と考えた．