Uptake and Excretion of Organophosphorus and Carbamate Insecticides by Fresh Water Fish, Motsugo, *Pseudorasbora parva*

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INTRODUCTION

Recent environmental study revealed that most of pesticides applied for pest control enter to the aquatic environment through various routes. Especially in Japan, about a half amount of pesticides has been applied into paddy fields. Therefore, it is necessary to study in detail on the effect of the pesticides to aquatic living organisms. Not only evaluation of acute toxicity test against fish, but also analytical and chemical research, that is, uptake, accumulation, metabolism and excretion of pesticides by aquatic organisms exposed at the sublethal concentration of a pesticide for long term should be undertaken.

There have been several studies on the uptake, accumulation and fate of organochlorine insecticides, for example, DDT and dieldrin (CHADWICK et al. 1969, BEDFORD et al. 1973), PCB (SANDERS et al. 1972, ZITKO 1974), and evaluation of DDT and methoxychlor by the model ecosystem (METCALF et al. 1971, KAPOOR et al. 1972). However, little is known of the research about organophosphorus and carbamate insecticides. YU et al. (1974) only researched on the fate of carbofuran in the model ecosystem.

This paper describes the uptake and excretion of several organophosphorus and carbamate insecticides in fresh water fish, Motsugo (Pseudorasbora parva), and persistence of these insecticides in aquarium water tank.

MATERIALS AND METHODS

Experimental condition. Glass aquarium tank (45 × 24 × 30 cm) was used for the experiment. The tank was filled with 20 liters of tap water. The pH and total alkalinity of water were 6.4 to 6.8 and 30 to 38 ppm as $CaCO_3$, respectively. Water temperature was maintained at 23 ± 2°C and aerated with compressed air. Motsugo is the most popular fresh water fish in the surrounding area of Tokyo, which was used as a test fish, 4 to 8 cm length and 2 to 5 g body weight. Ten fishes were put in each tank, and were given commercial dry fish feed once a day.

0,0-Dimethyl S-1,2-dicarboethoxyethyl phosphorodithioate (malathion), 0,0-diethyl 0-(2-isopropyl-6-methyl-4-pyrimidinyl) phosphorothioate (diazinon), 0,0-dimethyl 0-4-nitro-3-methyl-

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phenyl phosphorothioate (fenitrothion), 1-naphthyl N-methylcarbamate (carbaryl), 2-sec-butylphenyl N-methylcarbamate (BPMC) and 3,5-dimethylphenyl N-methylcarbamate (XMC) were used as test insecticides. The initial concentration of the insecticides in water were adjusted at 0.6 to 1.2 ppm. Only water was used for dissolution of insecticides. Organic solvent was not used because some slime break out during rearing. The concentration of each insecticide in water and fish were analyzed at various intervals of day for one month.

Determination of insecticides. Ten to 300 ml of water were sampled according to the concentration of each insecticide, and extracted successively with 20, 20 and 10 ml of dichloromethane. The extracts were combined, and evaporated in vacuo. The residues were dissolved in a definit volume of hexane. Organophosphorus insecticides were determined by gas-liquid chromatography. A Microtek MT-160 gas chromatograph equipped with a flame photometric detector was used. Column employed was 1.5 m × 3 mm I.D. glass, packed with 5% OV-1 on Chromosorb W, acid washed, 60/80 mesh. Operating conditions were as follows: column temperature, 170°C and carrier gas, N_2 60 ml/min. Recovery rates in the fortified samples were above 92% for malathion at 1 ppm, 85% for diazinon at 1 ppm and 96% for fenitrothion at 0.5 ppm.

Carbamate insecticides were converted to N-trifluoroacetyl derivatives (N-TFA) with trifluoroacetic anhydride and determined by gas-liquid chromatography (UEJI et al. 1973). A Varian 1200 gas chromatograph equipped with a ^{3}H-electron capture detector was used. Column was 1.5 m × 2 mm I.D. glass, packed with 5% OV-17 on Gas Chrom-Q, 60/80 mesh. Operating conditions were as follows: column temperature, 160°C for carbaryl and 130°C for BPMC and XMC, and carrier gas, N_2 40 ml/min. Recovery rates in the fortified samples were above 86% for carbaryl at 0.5 ppm, 80% for BPMC at 0.5 ppm and 83% for XMC for at 0.5 ppm.

Two Motsugo were sampled, and washed with running water, and weighed. After adding 10 g of anhydrous sodium sulfate and 100 ml of acetonitrile, the fishes were blended in Sorvall OMNI-mixer, for 3 minutes, and filtered on glass filter 17G3. The extract was concentrated below 50°C. The residue was dissolved in 25 ml of hexane and transfered into 100 ml separating funnel, and extracted twice with each 25 ml of acetonitrile. The acetonitrile extracts were combined, and concentrated and dissolved again in appropriate volume of acetone. Organophosphorus insecticides were determined by gas-liquid chromatography. Recovery rates in the fortified samples were above 91% for malathion at 13 ppm, 80% for diazinon at 3 ppm and 85% for fenitrothion at 13 ppm. In the case of carbamate insecticides, the acetonitrile extract was concentrated, and dissolved in hexane and cleaned up as follows. The hexane solution was passed through a column (1.5 cm × 30 cm) containing 5 g of activated Florisil 60/100 mesh with 100 ml of dichloromethane : hexane (1 : 4). This fraction was discarded, and successively eluted with 150 ml of acetonitorile : dichloromethane : hexane (0.5 : 50 : 49.5). This fraction containing carbamate insecticides was concentrated in vacuo, and the carbamate insecticides were converted to N-TFA, and determined by gas-liquid chromatography. Recovery rates in the fortified samples were above 86% for carbaryl at 0.2 to 1 ppm, 81% for BPMC at 0.5 ppm and 83% for XMC at 0.8 to 1 ppm.

RESULTS AND DISCUSSION

Organophosphorus insecticides. Results obtained on 3 organophosphorus insecticides are shown in Figure 1. Among these insecticides, diazinon was the most stable in water, 0.96 ppm of the initial concentration decreased gradually, and became to 0.27 ppm after 30 days. Subsequently, fenitrothion was decreased exponentially from 0.81 ppm of the initial concentration to 0.02 ppm after 28 days. Malathion was the most unstable among these insecticides, and decreased rapidly from 1.2 ppm of the initial concentration to less than 0.001 ppm after 7 days.

Little is known of the persistence of organophosphorus insecticides in water. ECHELBERGER et al. (1971) examined the persistence of 9 kinds of organophosphorus insecticides in Miami river water for 8 weeks at 10 ppb of the initial concentration, and reported that malathion hydrolyzed rapidly in water, and 75% of the original compound was decomposed after one week, and 90% after 2 weeks. Although the complete dissipation of diazinon was only 3 to 5 days in paddy field water of Philipine that had been previously treated with diazinon, while that in untreated paddy water was about 2 months. From these results, SETHUNATHAN (1972) found that the diazinon decomposable bacterias were enhanced in paddy field water by repeated application of diazinon.

On the uptake of the pesticides by Motsugo, there was a great difference between malathion, diazinon and fenitrothion. As shown in Figure 1, uptake of diazinon and fenitrothion by fish were higher than that of malathion. The concentration of these insecticides in fish after 3 or 4 days were higher than that of after one day, and those were 211 ppm for diazinon and 162 ppm for fenitrothion, respectively. These points are the maximum levels of the uptake, and decreased rapidly. Concentration of diazinon in fish became to 17 ppm after 30 days, and that of fenitrothion was 4.9 ppm after 28 days. As compared with diazinon and fenitrothion, malathion taken up by fish was metabolized rapidly, its concentration in fish after one day was 2.4 ppm, and became to less than 0.01 ppm after 7 days. The ratios of concentration in fishes against concentration in water were 2 for malathion after 3 days, 64 for diazinon after 30 days and 205 for fenitrothion after 28 days. Moreover, diazinon was metabolized slowly in Motsugo body, and after about 30 minutes, some fishes showed

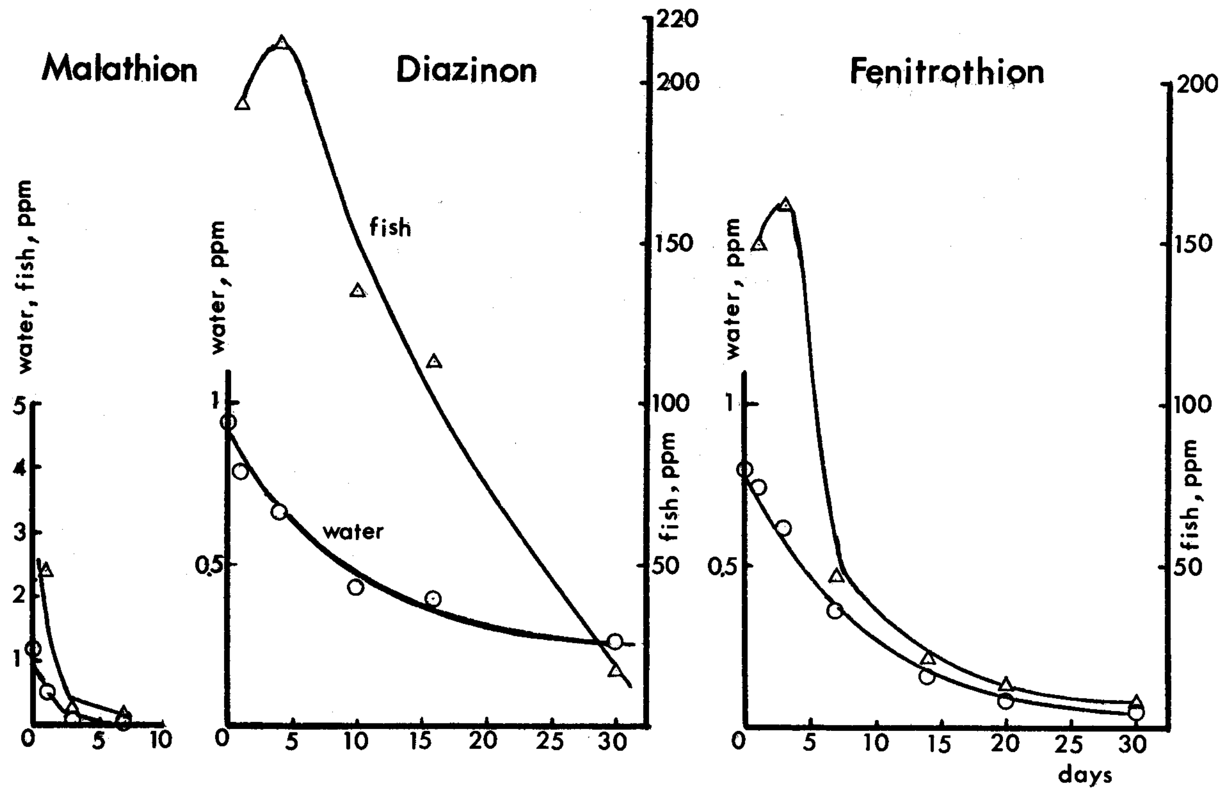

Figure 1. Uptake and fate of organophosphorus insecticides by fresh water fish, Motsugo, and its persistence in water

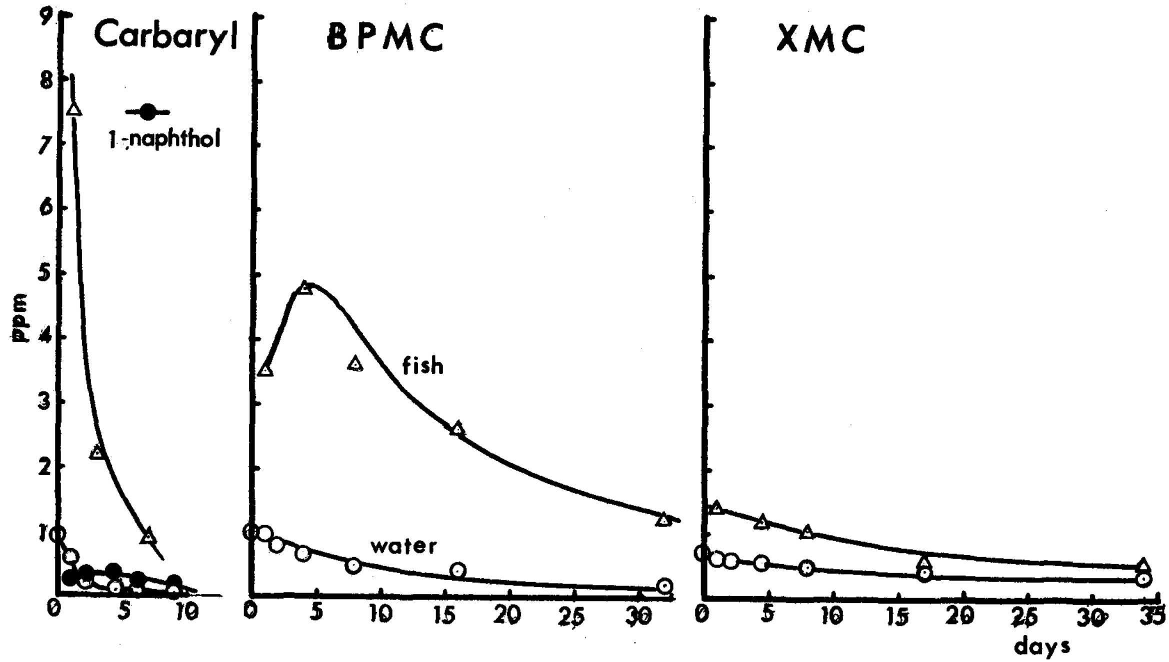

Figure 2. Uptake and fate of carbamate insecticides by fresh water fish, Motsugo, and its persistence in water

abnormal swimming, and soon about 40% of the fishes appeared bleeding in corpus vertebra of rear part of back fins. After two weeks, these fishes became permanent spinal curbature of back bone, and did not return to the original form during the experimental period. Also in the case of fenitrothion, about 10% of fishes tested showed the same symptom. Although deformity of the fish did not appeared with malathion, EATON (1970) reported that when bluegill was exposed to water containing 74 ppb of malathion for several month, similar crippled adult fish appeared. Therefore, it seems that such a deformity of fish bone is a general symptom caused by cholinesterase inhibitor. This fact is very important problem on the preservation of aquatic ecosystem.

Carbamate insecticides. Results obtained on 3 carbamate insecticides are shown in Figure 2. On the stability of these insecticides in water, carbaryl is the most unstable, and namely, 1 ppm of the initial concentration degraded to 0.575 ppm after one day, 0.193 ppm after 2 days, 0.068 ppm after 4 days and less than 0.01 ppm after 9 days. With the degradation of carbaryl, 1-naphthol appeared as a hydrolysis product, and its concentration reached to the maximum level of 0.38 ppm after 4 days, and then decreased gradually, and became to 0.16 ppm after 19 days. EICHELBERGER et al. (1971) studied on the persistence of carbaryl in Miami river water and reported that 95% of carbaryl was decomposed for 7 days. BPMC showed middle stability in water, and it was degraded gradually from 1 ppm of the initial concentration to 0.2 ppm after 32 days. XMC is the most stable in water, and namely, degradded gradually from 0.6 ppm of the initial concentration to 0.33 ppm after 34 days.

Uptake by Motsugo after one day was the most high in carbaryl (7.5 ppm), followed by BPMC (3.5 ppm) and XMC (1.4 ppm). Carbaryl taken up by fish was metabolized the most rapidly as compared with other carbamate insecticides, and decreased to 0.89 ppm after 7 days. KORN (1973) reared channel catfish in water containing 0.05 and 0.25 ppm of carbaryl and adminstered feeds containing 0.28 and 2.8 mg of carbaryl per kg for 56 days. He reported that concentration of carbaryl in fish is not more than 11 ppb, and carbaryl was metabolized and excreted very rapidly and accumulation did not occured. As menthioned above, author's results may supported his results. Concentration of BPMC in fish after 4 days was 4.8 ppm, and that is higher than after one day, and this was the maximum level of uptake, and then decreased gradually, and became to 1.2 ppm after 32 days. Metabolism of BPMC in Motsugo was very slow, and after 2 weeks, about 30% of test fishes showed permanent spinal curvature of back bone as seen in diazinon. Concentration of XMC in fish was lowest, and it was 1.4 ppm after one day, and became to 0.55 ppm after 34 days. As menthioned above, it is probable that uptake level of carbamate insecticides were generally lower than organophosphorus insecticides such as diazinon and fenitrothion. However, it should be bear in mind that there is a pesticide such as BPMC among carbamate insecticides, which caused the chronic effect to central nervous system of fish.

SUMMARY

Fresh water fish, Motsugo was reared in aquarium water tank containing about 1 ppm of 3 organophosphorus and 3 carbamate insecticides for about 30 days. The persistence of these insecticides in water and uptake and excretion of insecticides by fish were examined. Among organophosphorus insecticides, malathion is the most unstable in water, and degraded more than 99% for 7 days. Fenitrothion is moderately stable, and degraded 97% for 29 days. Diazinon is the most stable, and degraded 72% for 30 days. Among carbamates, carbaryl is the most unstable in water, and degraded more than 95% for 6 days. BPMC is moderately stable, and degraded 80% for 32 days. XMC is the most stable, and degraded 45% for 34 days.

As for the uptake of the pesticides by fish, organophosphorus insecticides were generally higher than carbamate insecticides. The concentration of diazinon in fish reached to 211 ppm of the maximum level after 3 days, and that of fenitrothion reached to 162 ppm of the maximum level after 4 days. Afterwards, the concentration of both the insecticides decreased gradually due to the metabolism and excretion of the insecticides in fish. Uptake of malathion was very low and metabolized rapidly, and its concentration became to less than 0.01 ppm after 7 days. Among carbamate insecticides, the concentration of carbaryl in fish after one day reached to 7.5 ppm which was the maximum level of uptake. On the other hand, the concentration of BPMC in fish after 4 days became to 4.8 ppm, which was the maximum level, and decreased gradually. The concentration of XMC in fish was only 1.4 ppm after one day, but the metabolism rate of XMC in fish was fairly slow. Therefore, 0.55 ppm of XMC in fish remained even after 34 days. Moreover, in the test tank of diazinon, fenitrothion and BPMC, the appearance of deformed fish with spinal curvature of back bone came out at the rate of 10 to 30%.

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