Korean J. Environ. Agric.
Vol. 3, No. 1, June 1984

Bioconcentration of Diazinon and Fenitrothion in Carp (*Cyprinus carpio*)

Su-Rae Lee*, Byung-Sun Yoo and Hye-Kyung Chun***

(Received October 5, 1983)

잉어에 의한 Diazinon 및 Fenitrothion의 生物濃縮

李瑞來* · 柳炳善** · 金惠敬*

Abstract

A freshwater fish carp(*Cyprinus carpio*) was exposed to two organophosphorus insecticides in laboratory to investigate the potential of its bioconcentration. The results are summarized as follows:

Bioconcentration factor of diazinon and fenitrothion after 24-hour exposure at 1 ppm concentration was 31 and 57, respectively, for the whole fish. The factor varied among different tissues of the fish in the decreasing order of viscera>rests>gills>muscle. When the fish was exposed to fenitrothion for 28 days at three different concentrations of 6, 30 and 150 ppb, bioconcentration factor in the whole fish ranged from 96 to 138, with a decreasing tendency at higher water concentration. The pesticide was continuously absorbed by the fish, but reaching an equilibrium at the tissue concentration of about 3.5 ppm.

Introduction

Chemical residues in the environment and food chains are becoming a serious problem as more chemicals are used in recent years.[1,2] In particular, high levels of pesticides are used for increased productivity of agricultural crops. The use of synthetic organic chemicals in agriculture has created several problems not only in the agroecosystem but also in the aquatic environment. These include the exposure of non-target organisms to lethal and sublethal amounts of pesticides and their degradation products. It has been generally accepted that the organochlorine pesticides present a greater danger to non-target animals than do the organophosphorus pesticides, because of their persistence in the environment, bioaccumulation in the ecosystem and greater potential for chronic toxicity.[3,4]

In many countries, fish toxicity data of pesticides are mandatory for their registration and studies in the area of aquatic toxicology have been actively undertaken in recent years.[5,6] Fish toxicity problems are more concerned with organophosphorus and carbamate pesticides by several reasons. For instance, it was reported by Murphy *et al.*[7] that mala-

**Department of Food & Nutrition, Ewha Woman's University, Seoul 120* (이화여자대학교 식품영양학과)

***Environment Division, Korea Advanced Energy Research Institute, Seoul 131* (한국에너지연구소 환경연구실)

thion, a typical organophosphorus insecticide, is highly toxic to fishes while it is slightly toxic to mammals. Its entry into freshwater ecosystem and biomagnification could have a detrimental effect on fish population while they will not harm man or domestic animals. Furthermore, the use of organophosphorus pesticides should be gradually increased in near future, thus necessitating a better knowledge of their adverse effects on fishes and other aqua tic ecosystem.

This study was, therefore, undertaken to determine the possibility of bioconcentrating two organophosphorus insecticides diazinon and fenitrothion into tissues of carp, which is the common freshwater fish as well as the test species in Korea. The results after short-term and long-term experiments are reported here.

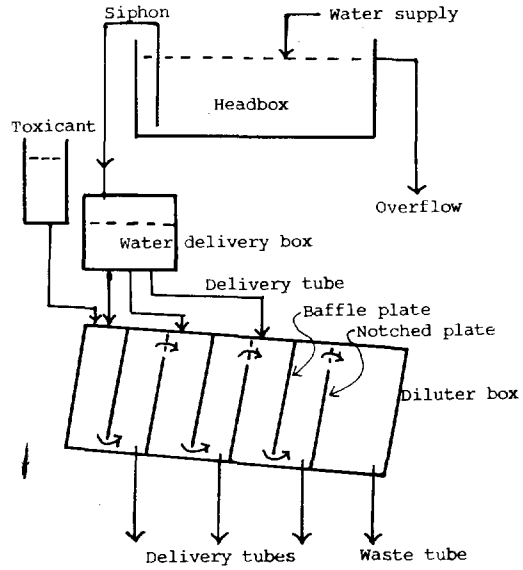

Fig. 1. Diagram of continuous-flow exposure system

Materials and Methods

1. Test pesticides and animals

Two pesticides, diazinon(dasuzine, diatone) and fenitrothion(MEP, folithion, sumithion) were obtained from some agrochemical company in Korea for toxicity testing and from US EPA Reference Standards Repository in North Carolina for authentic compounds in gas chromatographic analysis.

The freshwater fish, carp(*Cyprinus carpio*), was obtained from 'Seol-Ak' carp farm near Chuncheon in Korea and acclimated for the laboratory conditions for a week.

2. Continuous-flow exposure system

Long-term experiments for bioconcentration of pesticides into fish were conducted with a continuous-flow toxicant delivery system after a slight modification of the method described by Garton.[8] The system consisted of headbox, small water delivery box, toxicant source bottle and diluter box as shown in Fig. 1. The diluter box was made of acryl resin and the details are shown in Fig. 2. The diluter box was divided into four compartments.

The divider plate had an upper corner cutoff as an overflow notch which was made by cutting a 45° triangle, 2.5 cm on a side, off the corner of the plate.

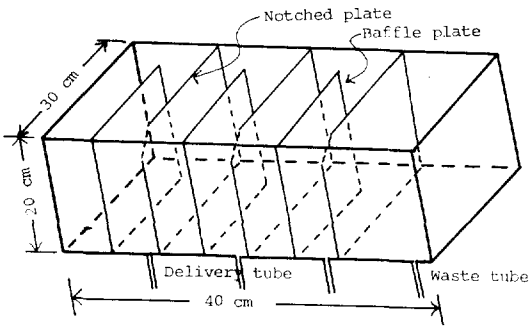

Fig. 2. Datails of diluter box in the continuous-flow exposure system

Four baffle plates of 20×30 cm size were inserted into each compartment by joining to the front wall but leaving a space between them and the back wall to provide further travel and more opportunity of mixing before the toxicant solution goes to the delivery system.

This system delivered the toxicant solution from the toxicant source bottle to the first chamber of the diluter box. This toxicant solution was then mixed with fresh water supplied from the headbox to produce the highest concentration of toxicant. A portion of the solution in the first chamber was routed to the test tank and the excess was spilled through a notch into the next diluter chamber.

The same dilution and overflow mechanism occurred in the succeeding chambers. The concentration of toxicant in the diluter chambers was calculated from the concentration of entering toxicant and flow rates of toxicant and diluting water entering into the chamber.

3. Exposure procedure

For short-term exposure of test chemicals, carps of about 15 cm length were grown in a rectangular fish culture tank (30×20×30 cm) containing 1 ppm pesticide in about 14 liters volume, under good growth conditions with continuous aeration. After 24 hours exposure, surface water of the fishes was carefully removed by blotting with tissue papers. The levels of pesticide residues in the whole fish as well as in different parts of the fish were analyzed.

Long-term experiment for bioconcentration of pesticides into fishes was conducted by the continuous-flow exposure system as described above. Concentrations of pesticides were maintained at 6, 30 and 150 μg/liter in the experimental tanks which contain about 14 liters of water per tank. The pesticide stock solution was continuously introduced at one end and drained out through a hole at the other end of the test tank. Flow rate through the chamber was maintained at 6 liters/hour so that more than five tank volumes a day were delivered to each tank. Ten test fishesof about 5 cm length were introduced in each tank and maintained for 4 weeks. Fish samples were taken out at proper intervals of exposure and analyzed for the level of the pesticide. Routine analyses of water quality for dissolved oxygen, temperature, pH, alkalinity and total hardness were carried out on a weekly basis for test solutions, according to the Standard Methods[9]. The results are shown in Table 1.

Table1. 1. Water quality of test solutions used for bioconcentration test

Quality parameter	Range	Mean
Temperature(°C)	23.5~27.0	25.5
pH	5.9~7.0	6.5
Dissolved oxygen (mg/liter)	17~23	20.0
Alkalinity (mg $CaCO_3$/liter	21~32	27.9
Total hardness (mg $CaCO_3$/liter)	46~58	52.1

4. Residue analysis

1) Extraction and clean-up

Pesticides in fish samples were extracted according to Abbott *et al.*[10] as follows. Chopped fish samples weighing about 1~5 g were mixed with a sufficient amount of anhydrous sodium sulfate and macerated with three separate 50 m*l* portions of acetonitrile in a top-driven macerator. The combined acetonitrile extracts were poured into 500 m*l* of 2.5% sodium sulfate solution and the mixture was extracted with three 50 m*l* portions of chloroform. The chloroform extracts were concentrated to a small volume in Kuderna-Danish evaporator and finally evaporated to dryness using a micro-Snyder column. The residue was redissolved to a suitable volume with acetone for gas chromatographic analysis.

Water samples were extracted according to the official method of US EPA[11] as follows. An appropriate volume of water was shaken with two 100 m*l* portions of 15% methylene chloride/hexane and with additional 100 m*l* of hexane. The extracts were dried by passing through a short column of anhydrous sodium sulfate and concentrated as described above.

2) Gas chromatographic analysis

Pesticide residues after extraction and clean-up were analyzed with Varian Aerograph Series 2000 Gas Chromatograph attached with alkali FID detector. The glass column was 180 cm×3 mm i.d., packed with 10% DC-200 on 80~100 mesh GasChrom Q. The column, injector port and detector temperatures were 210, 225 and 225°C, respectively. Flow rates of nitrogen, air and hydrogen gases were 46, 235 and 35 m*l*/min, respectively. Injection volume of sample was usually 3 μ*l*. Identification and quantitative measurement were carried out by comparison with authentic compounds.

Results and Discussion

1. Bioconcentration of pesticides by short-term exposure

When the test fishes were exposed to test chemi-

cals at the initial concentrations of 1 ppm for 24 hours, the relative concentrations of diazinon and fenitrothion in the whole fish and various tissues are shown in Tables 2 and 3. The pesticide concentration in water was obtained from the average value of the initial and final concentrations of the corresponding chemical in the medium. Bioconcentration factors were calculated according to the following equation:

$$\text{Bioconcentration factor} = \frac{\text{pesticide concentration in 1 g of tissue}}{\text{pesticide concentration in 1 m}l\text{ of water}}$$

The two organophosphorus pesticides were accumulated in the carp fish even after 24-hour exposure. Although the concentration of tested chemicals in water was somewhat lower than their LC_{50} values ranging in the vicinity of 4~5 mg/liter for carp at 48 hours, the bioconcentration factor was in the magnitude of 30~60, fenitrothion being a little higher. However, the bioconcentration phenomenon was revealed differently among different parts of the fish in both chemicals; that is, the highest in the viscera and the lowest in the muscle.

Table 2. Distribution of diazinon in carp tissues after 24-hour exposure at the initial water concentration of 1 ppm

Tissue	Residue level (μg/g fresh weight)	Biocorcentration factor
Gills	29.2	41.7
Muscle	7.4	10.6
Viscera	32.1	45.8
Rests	31.8	45.4
Whole fish	22.0	31.0

Table 3. Distribution of fenitrothion in carp tissues after 24-hour exposure at the initial water concentration of 1 ppm

Tissue	Residue level (μg/g fresh weight)	Bioconcentration factor
Gills	26.6	40.6
Muscle	5.2	8.0
Viscera	52.6	80.3
Rests	28.3	43.2
Whole fish	37.4	57.1

In general, the bioconcentration of chemicals within a given animal and biomagnification along a food chain are determined by several factors including the persistence, availability, absorption and excretion of a chemical. It is also known that less water soluble pesticide is more concentrated in the aquatic organisms[12]. A little higher bioconcentration factor for fenitrothion as obtained in this experiment could be explained by its practical insolubility in water as compared with the slight solubility (40 mg/liter) of diazinon. The distribution pattern of pesticides in various parts of the fish can not be explained much since the test period was too short and not enough to interpret on the basis of normal metabolism.

2. Bioconcentration of fenitrothion by long-term exposure

The test fish carp was exposed to the more accumulative pesticide fenitrothion for 28 days at three different concentrations. The results are shown in Table 4. Fenitrothion residues in carp increased with increasing pesticide concentration in water and reached a maximum of 14.5 μg/g fish at 150 ppb concentration in water. The bioconcentration factor was, however, varied from 96 to 138, with a decreasing tendency with increasing water concentration.

Table 4. Bioconcentration of fenitrothion in carp after 28-day exposure

Pesticide concentration		Bioconcentration factor
Water (μg/liter)	Fish (μg/g fresh weight)	
150	14.5	96.7
30	3.2	106.7
6	0.83	138.3

A time course study on the bioconcentration of fenitrothion into the carp was followed at 30 ppb water concentration of the pesticide. The results are shown in Fig. 3. The concentration of fenitrothion in the fish as expressed on a unit weight basis increased rapidly for the initial period and then tended to level off slowly, likely reaching an equilibrium at about 3.5 ppm, when the pesticide concentration in water was 15 ppb, probably due to

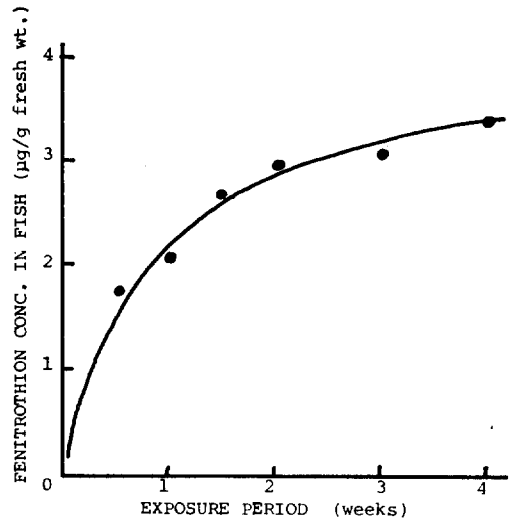

Fig. 3. Bioconcentration of fenitrothion by carp grown in water with 30 ppb pesticide

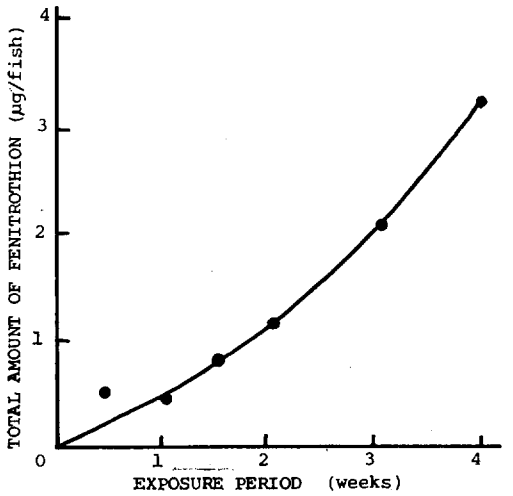

Fig. 4. Accumulation of fenitrothion into carp grown in water with 30 ppb pesticide

the degradation of the pesticide in the aqueous medium. The results of above experiment as expressed on a fish basis are shown in Fig. 4. It showed a little different pattern in that the total amount of pesticide increased as the carp grew. This means that fenitrothion is absorbed continuously by the fish, although its bioconcentrating rate falls off gradually.

In place of the more persistent organochlorine insecticides, various organophosphorus pesticides including diazinon and fenitrothion should be used to a greater extent now and in future. However, the information on their chronic toxicity toward freshwater fishes is quite limited.[13,14] This study showed that carp could accumulate fenitrothion to concentrations up to 140 times greater at ppb levels in water. Thus, the entry of organophosphorus pesticides into water body could have detrimental effects on fish population at low concentrations that would not harm man. As this study was not sufficient and thorough, it is suggested that further investigations on the bioconcentration and potential chronic effects of presently used pesticides on economic fishes should be undertaken in future.

요 약

잉어에 의한 두 가지 有機燐系 살충제의 生物濃縮을 追求하기 위하여 실험실 조건하에서 수행한 실험결과는 다음과 같다.

1) Diazinon과 fenitrothion의 1 ppm농도에서 잉어에 의한 生物濃縮係數는 24시간후에 각각 31과 57이었다. 이 係數는 잉어의 部位에 따라 달리 나타났는 바 內臟>기타 부위>아가미>筋肉의 순서로 나타났다.

2) 잉어를 fenitrothion의 세가지 농도에 28일간 노출시킨 결과 生物濃縮係數는 96~138로 나타났는 바 水中농도가 높을수록 적게 나타났다. Fenitrothion의 生體內 濃縮은 계속적으로 일어났으며 組織內 농도가 약 3.5 ppm에서 平衡에 도달하였다.

References

1. Korte, F. (1974): Global inputs and burdens of chemical residues in the biosphere: the problem and control measures, in *Comparative Studies of Food and Environmental Contamination*, IAEA, STI/PUB/348, p. 3.
2. Guthrie, F. E. and Perry, J. J. (1980): *Introduction to Environmental Toxicology*, Elsevier North Holland, New York, 484 pp.
3. Edwards, C. D. (Ed.) (1973): *Persistent Pesticides in the Environment*, 2nd edition, CRC Press, Ohio, 158 pp.
4. Doull, J., Klaassen, C. D. and Amdur, M. O. (1980): *Toxicology*, 2nd edition, Macmillan Pub. Co., New York, Chapter 16.
5. 田中二郎(編)(1978): 水生生物と 農藥 [2] 急性毒性資料編, サイエンテイスト社, 日本, 351 pp.

6. Kanazawa, J. (1981): Measurement of bioconcentration factors of pesticides by freshwater fish and their correlation with physicochemical properties of acute toxicities, *Pestic. Sci.*, **12**, 417.
7. Murphy, S. D., Lauwerys, R. R. and Cheever, K. L. (1968): Comparative anticholinesterase action of organophosphorus insecticides in verte brates, *Toxicol. Appl. Pharmacol.*, **12**, 185.
8. Garton, R. R. (1980): A simple continuous-flow toxicant delivery system, *Water Research*, **14**, 227.
9. American Public Health Association (1976): *Standard Methods for the Examination of Water and Wastewater*, 14th edition, APHA, Washington, D.C., 1193 pp.
10. Abbott, D. C., Crisp, S., Tarrant, K. R. and Tat ton, O'G. (1970): Pesticide residues in the total diet in England and Wales, 1966～1967, *Pestic. Sci.*, **1**, 10.
11. US EPA (1974): *Manual of Analytical Methods for the Analysis of Pesticide Residues in Human and Environmental Samples*, Section 10A.
12. 西尾 建 (1978): 沿岸漁業から 見た 農薬の 問題點, 生態化學, **1**, 131.
13. Eaton, J. G. (1970): Chronic malathion toxicity to bluegill, *Water Research*, **4**, 673.
14. Hermanutz, R. O. (1978): Endrin and malathion toxicity to flagfish, *Arch. Environ. Contam. Toxicol.*, **7**, 159.

Acknowledgement——The authors express their sincere gratitude for the financial assistance from the International Atomic Energy Agency under the contract numbers of 2798/RB and 2798/RI/RB.