クルマエビにおける有機燐剤フェニトロチオンの代謝

誌名	日本水産學會誌
ISSN	00215392
著者名	小林,邦男 中村,良子 今田信良
発行元	日本水産學會
巻/号	51巻4号
掲載ページ	p. 599-603
発行年月	1985年4月

Bulletin of the Japanese Society of Scientific Fisheries 51(4), 599-603 (1985)

Metabolism of An Organophosphorus Insecticide, Fenitrothion, in Tiger Shrimp *Penaeus japonicus*[*1]

Kunio KOBAYASHI,[*2] Yoshiko NAKAMURA,[*2] and Nobuyoshi IMADA[*2]

(Accepted August 23, 1984)

A study has been made of the toxicity, absorption and metabolism of fenitrothion [*O,O*-dimethyl *O*-(3-methyl-4-nitrophenyl)phosphorothioate; Sumithion] in tiger shrimp *Penaeus japonicus*, as the first step to elucidate the cause for the occurrence of its high toxicity to the shrimp.

The 24-h LC_{50} value of fenitrothion for tiger shrimp was about 1 ppb, while that for fish was roughly 6 ppm. After 0.5–24 h exposure to 0.5 ppb [^{14}C] fenitrothion, [^{14}C]fenitrothion and its metabolites accumulated in the shrimp were extracted with benzene and separated by thin-layer co-chromatography with non-radioactive fenitrothion and its authentic metabolites. After 4-h exposure, the concentration of fenitrothion was almost equilibrated at a level of ca. 6.5 pmol/g shrimp, which corresponded to a bioconcentration ratio of 3.6, whereas the concentrations of its metabolites, especially 3-methyl-4-nitrophenol, increased with exposure time. At 24-h exposure, the amounts of fenitrothion, fenitrooxon, desmethylfenitrothion, desmethylfenitrooxon and 3-methyl-4-nitrophenol accumulated in the shrimp were 6.3, 2.9, 3.6, 6.2 and 74.3 pmol/g, respectively. The amounts of the conjugated 3-methyl-4-nitrophenol extracted with ethyl ether after benzene-extraction increased with exposure time and reached 104 pmol/g at 24-h exposure, which was almost the same level of the benzene-extract.

In general, organophosphorus insecticides display very high toxicity to crustaceans compared with that to fishes. Although tiger shrimp *Penaeus japonicus* is an important species for coastal fishery and has been cultured in quantities in Japan, the shrimp is especially susceptible to organophosphorus insecticides among crustaceans.

Fenitrothion [*O,O*-dimethyl *O*-(3-methyl-4-nitrophenyl)phosphorothioate; Sumithion] is one of the most effective organophosphorus insecticides and has been widely used in Japan amounting to approximately 3,600 t a year,[1] because of its shorter persistence in fields and its relatively low toxicity to mammals.

By the introduction of a methyl group to methyl parathion at the *m*-position in the benzene ring, the toxicity of the product, *i.e.* fenitrothion to mammals decreased by the factors of between 4.4 and 54.3 compared with that of methyl parathion in oral administration,[2-5] whereas the toxicity to fishes and shellfishes did not so differ[6,7] as well as that to insects.[4,5]

Many studies have been done on the toxicity of fenitrothion to tiger shrimp, which is higher than that to fishes by a factor of several thousands.[6,7] There are no works, however, on the metabolism of fenitrothion in the shrimp, except an accumulation study of fenitrothion without concern for its metabolites.[8] The present study deals with the toxicity, absorption and metabolism of fenitrothion in tiger shrimp, as the first step to elucidate the cause for the occurrence of its high toxicity to the shrimp.

Materials and Methods

Special Chemicals

Radioactive [ring-U-^{14}C]fenitrothion (specific activity: 20.4 μCi/μmol; 75 μCi/mg) and non-radioactive fenitrothion and its authentic metabolites (fenitrooxon, desmethylfenitrothion, desmethylfenitrooxon and 3-methyl-4-nitrophenol) were offered by the Institute for Biological Science, Sumitomo Chemical Co., Ltd.

Toxicity Test

The approximate body weights of tiger shrimp, carp *Cyprinus carpio*, goldfish *Carassius auratus* and medaka *Oryzias latipes* were 6, 1.7, 1.1 and 0.4 g, respectively.

[*1] Studies on the Relation Between Toxicity and Metabolism of Organophosphorus Insecticides in Shrimps–I.

[*2] Laboratory of Fishery Environmental Science, Faculty of Agriculture, Kyushu University, Fukuoka 812, Japan (小林邦男・中村良子・今田信良: 九州大学農学部水産増殖環境学教室).

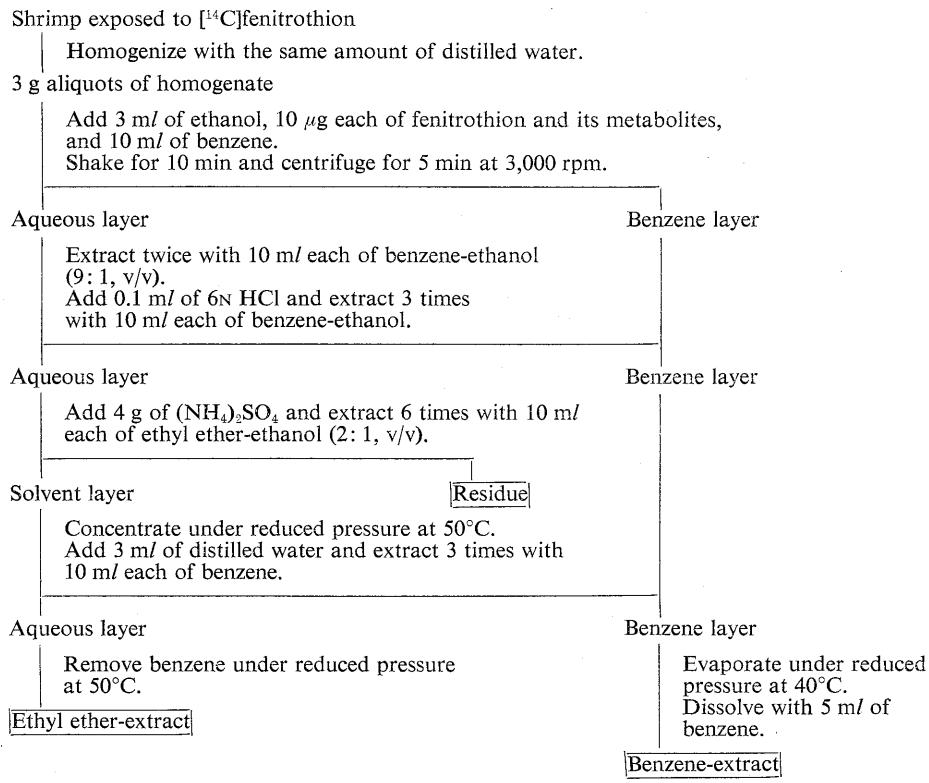

Fig. 1. Extraction procedure of [^{14}C]fenitrothion and its metabolites accumulated in tiger shrimp.

Tiger shrimp were exposed to fenitrothion-sea water ranging in concentration from 0.5 to 6 ppb at 25°C, while the test fishes were exposed to respective media containing high concentrations of fenitrothion from 2 to 10 ppm at the same temperature.

Exposure of Shrimp to [^{14}C] Fenitrothion

Five shrimps (average body weight, 2.65 g each) were placed in each of ten tanks containing 8 *l* of [ring-U-^{14}C]fenitrothion-sea water (fenitrothion, 0.5 ppb=1.8 nmol/*l*; radioactivity, 37.5 nCi/*l*= 83,000 dpm/*l*; Cl^-, 18.3‰) dispersed with 1 ppm Tween 80. Air was supplied by pump and the temperature was kept at 25±1°C. At each of 0.5-, 1-, 2-, 4-, 8-, 12- and 24-h exposure, five shrimps were taken out, rinsed with fenitrothion-free sea water, weighed, and then frozen by dipping in dry ice-ethanol at −65°C.

Extraction of [^{14}C] Fenitrothion and Its Metabolites Accumulated in Shrimp

Five shrimps taken out at each exposure time were homogenized with the same amount of distilled water by a Waring blender. The homogenates were subjected to extraction of [^{14}C]-fenitrothion and its metabolites according to the procedure shown in Fig. 1.

Determination of [^{14}C] Fenitrothion and Its Metabolites Extracted with Benzene

[^{14}C]Fenitrothion and its metabolites extracted with a benzene-ethanol mixture (9: 1, v/v) were separated by thin-layer co-chromatography (TLC) on silicagel plates (Merck 60 F_{254}) with non-radioactive fenitrothion and its authentic metabolites, using three solvent systems.

The spots of fenitrothion (FS), fenitrooxon (FO), desmethylfenitrothion (DMFS), desmethyl-fenitrooxon (DMFO) and 3-methyl-4-nitrophenol (3-M-4-NP) were detected under UV-light, scraped off from the plates, and then subjected to the measurement of their radioactivities by a liquid scintillation counter (Aloka LSC-900; LSC), using toluene scintillator (PPO, 5 g+POPOP, 0.25 g/*l*).

The amounts of [^{14}C]FS and its metabolites accumulated in shrimp were calculated from their radioactivities on the basis of the specific activity of [^{14}C]FS (20.4 μCi/μmol; 75 μCi/mg) used in this experiment.

Determination of [^{14}C] Metabolites Extracted with Ethyl Ether

[^{14}C]Metabolites extracted with an ethyl ether-ethanol mixture (2:1, v/v) from shrimp after benzene-extraction are water soluble and non-extractable with benzene from their aqueous solutions. The radioactivities of these [^{14}C]metabolites were directly assayed by LSC, using ACS-II aqueous counting scintillant (Amersham).

Determination of [^{14}C] Residue

After benzene- and ethyl ether-extraction, [^{14}C]-residues in shrimp were dissolved with Hyamine 10-X (Packard) and subjected to the measurement of radioactivity by LSC, using toluene scintillator.

Results and Discussion

Toxicity of Fenitrothion to Shrimp and Fishes

As shown in Table 1, FS displays high toxicity to tiger shrimp more than one thousand times of that to fishes, in spite of the rather high FS-resistance of the shrimp tested in this experiment as compared with that of the same species in a previous paper.[8]

Accumulation of [^{14}C] Fenitrothion and Its Metabolites in Shrimp

The loss of FS in the media was approximately 10% of the initial concentration at 24-h exposure. During FS exposure, no mortality was observed in the tested shrimps except one which died at 24-h.

Table 1. Toxicity of fenitrothion to tiger shrimp and fishes

Species	Av. B. W. (g)	Fenitrothion conc. in media	50% Survival time (h)
Tiger shrimp	6	1 ppb	ca. 24
		2 〃	9.7
		4 〃	4.4
		6 〃	3.8
Carp	1.7	6 ppm	ca. 30
		8 〃	5
		10 〃	4
Goldfish	1.1	6 ppm	24
		8 〃	11
		10 〃	5
Medaka	0.4	6 ppm	ca. 40
		8 〃	19
		10 〃	16

Table 2 shows the changes in the radioactivities and amounts of [^{14}C]FS and/or its metabolites in benzene- and ethyl ether-extracts and residues of shrimp, during exposure to 0.5 ppb [^{14}C]FS-sea water.

The amount of [^{14}C]FS and/or its metabolites in each extract increased with exposure time and the total [^{14}C] reached 11,550 dpm/g shrimp (250.4 pmol/g) at 24-h exposure, corresponding to the bioconcentration ratio of 139, because the radioactivity and concentration of 0.5 ppb [^{14}C]-FS-media were 83 dpm/m*l* and 1.8 pmol/m*l*, respectively.

The amounts of [^{14}C]metabolites in ethyl ether-

Table 2. Changes in radioactivity and amount of [^{14}C]fenitrothion and/or its metabolites accumulated in tiger shrimp, during exposure to 0.5 ppb [^{14}C]fenitrothion-sea water

		Exposure time (h)						
		0.5	1	2	4	8	12	24
Benzene-extract	(dpm/g)	285	394	720	1,173	2,374	2,088	4,728
	(pmol/g)	6.2	8.5	15.6	25.4	51.5	45.3	102.5
	(%)	44.0	42.2	46.1	38.8	49.5	27.5	41.0
Ethyl ether-extract	(dpm/g)	110	240	420	1,202	1,471	3,423	4,798
	(pmol/g)	2.4	5.2	9.1	26.1	31.9	74.2	104.0
	(%)	17.0	25.7	26.9	39.7	30.6	45.1	41.5
Residue	(dpm/g)	252	300	421	651	957	2,075	2,024
	(pmol/g)	5.5	6.5	9.1	14.1	20.8	45.0	43.9
	(%)	39.0	32.1	27.0	21.5	19.9	27.4	17.5
Total	(dpm/g)	647	934	1,561	3,026	4,802	7,586	11,550
	(pmol/g)	14.0	20.3	33.9	65.6	104.1	164.5	250.4
	(%)	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Bioconcentration ratio of total [^{14}C]		7.8	11.3	18.8	36.4	57.8	91.4	139.1

Table 3. Rf values of fenitrothion and its metabolites in thin-layer chromatography

Compounds	Rf in solvent systems		
	I	II	III
Fenitrothion	0.91	0.95	0.89
3-Methyl-4-nitrophenol	0.76	0.91	0.50
Fenitrooxon	0.65	0.83	0.20
Desmethylfenitrothion	0.22	0.58	0
Desmethylfenitrooxon	0.11	0.27	0

TLC plate: Silicagel 60 F_{254} (Merck) 10×20 cm, 0.25 mm thick.
Solvent systems: I, toluene-ethyl formate-formic acid (5: 7: 1, v/v); II, toluene-ethyl acetate-isopropanol-acetic acid (8: 12: 5: 3, v/v); III, ethyl acetate-benzene (1: 4, v/v).

extracts increased remarkably with exposure time after 4-h and reached rather higher levels than those in the benzene-extracts. According to a similar separation procedure for FS-metabolites in rainbow trout,[6] the ethyl ether-extract contains some conjugates of 3-M-4-NP which is a hydrolyzate of FS. The increase of FS-metabolites in ethyl ether-extracts after 4-h exposure suggests a time lag due to the formation of the conjugates with 3-M-4-NP. The details of the conjugated metabolites in tiger shrimp will be reported in a subsequent paper.

The separation of [^{14}C]FS and its metabolites in benzene-extracts was performed by TLC. The Rf values of FS and its authentic metabolites in TLC using three solvent systems are shown in Table 3.

Fig. 2 shows the changes with exposure time in the amounts and bioconcentration ratios of FS, FO, DMFS, DMFO, 3-M-4-NP and other metabolites extracted with benzene from shrimp. Although FS concentration at 0.5-h and 1-h exposure was the highest among the detected [^{14}C]compounds corresponding to 54 and 56% of the total [^{14}C] in the respective benzene-extracts, after 4-h exposure it was almost equilibrated at a level of approximate 6.5 pmol/g shrimp which corresponded to a bioconcentration ratio of 3.6, resulting in a decrease of the distribution, e.g. only 6.3% of the total [^{14}C] at 24-h exposure.

On the other hand, the concentrations of FS-metabolites, *i.e.* FO, DMFS, DMFO, 3-M-4-NP and others in the shrimp increased with exposure time and reached 2.9, 3.6, 6.2, 74.3 and 6.3 pmol/g shrimp at 24-h exposure, corresponding to the molar ratios of 0.46, 0.57, 0.98, 11.79 and 1.00 to

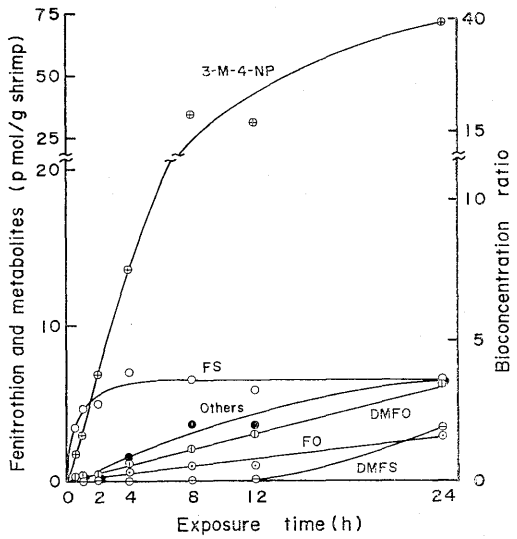

Fig. 2. Changes in the amounts and bioconcentration ratios of fenitrothion and its metabolites extracted with benzene from tiger shrimp, during exposure to 0.5 ppb [^{14}C]fenitrothion-sea water.
FS, fenitrothion; FO, fenitrooxon; DMFS, desmethylfenitrothion; DMFO, desmethylfenitrooxon; 3-M-4-NP, 3-methyl-4-nitrophenol.

FS, respectively.

The ramarkable increase of 3-M-4-NP among FS-metabolites as shown in Fig. 2 and the relatively large amounts of its conjugates as shown in Table 3 must be due to the high phosphatase activity in the mid-gut gland of shrimp, which corresponds to about 1.6 times of that in rat liver as reported by KOBAYASHI *et al.**

The results indicate that the equilibrium of the FS concentration in shrimp occurs in a balance between the absorption of FS by shrimp from surrounding water and its high decomposition *in vivo* by metabolic sequences, such as oxidative desulfurization, *O*-demethylation and hydrolysis, although much less the degradation of FS in rainbow trout as compared with that in the shrimp has been reported.[6]

It is well known that the conversion of phosphorothioate insecticides to respective oxo-forms (P=S→P=O) results usually in an increase of their toxicity.[5] Not excepting FS, the toxicity of FO to mammals is higher by approximately 9 times than that of FS.[3] Therefore, it is presumed that FO displays an important role in the occurrence of high mortality in shrimp during FS

* K. KOBAYASHI, Y. OSHIMA, and N. IMADA: Proc. of the Japan, Soc. Sci. Fish., p. 166, No. 612 (April 2nd, 1981).

exposure. On this point, another experiment has been undertaken on the relation between mortality and FO accumulation in tiger shrimp. The result will be reported in a following paper.

Acknowledgements

We wish to express our gratitude to the generosity of the Institute for Biological Science, Sumitomo Chemical Co., Ltd., for the gift of ^{14}C-labeled and non-labeld fenitrothion and its authentic metabolites.

This work was supported in part by Grant-in-Aid for Scientific Research (56560227) from the Ministry of Education.

References

1) Noyaku-Yoran: Noyaku-Yoran Editorial Committee, Japan Plant Protection Association, Tokyo, 1984, pp. 49–274.
2) Y. Nishizawa, K. Fujii, T. Kadota, J. Miyamoto, and H. Sakamoto: *Agr. Biol. Chem.*, **25**, 605–610 (1961).
3) J. Miyamoto, Y. Sato, T. Kadota, and A. Fujinami: *Agr. Biol. Chem.*, **27**, 669–676 (1983).
4) R. M. Hollingworth, T. R. Fukuto, and R. L. Metcalf: *J. Agr. Food Chem.*, **15**, 235–241 (1967).
5) C. Fest and K.-J. Schmidt: The Chemistry of Organophosphorus Pesticides, 2nd. ed., Springer-Verlag, Berlin, 1982, pp. 312–214.
6) J. Tanaka: Aquatic Organisms and Pesticides—Acute Toxicity Reviews, Scientist Inc., Tokyo, 1978, pp. 49–51.
7) J. Tanaka: Aquatic Organisms and Pesticides—Acute Toxicity Reviews, Scientist Inc., Tokyo, 1978, pp. 119–122.
8) O. Deshimaru, J. Iwata, K. Kuroki, and K. Shintani: *Kagoshima Pref. Fish. Expt. Stn. Rep. for 1976*, 39–53 (1978).
9) Y. Takimoto and J. Miyamoto: *J. Pesticide Sci.*, **1**, 261–271 (1976).