Journal of Health Science, **45**(5) 262—265 (1999)

Effect of Fenthion on the Level of Vitellogenin in Goldfish, *Carassius auratus*

Shigeyuki Kitamura,*,[a] Mayumi Yoshida,[a] Kazumi Sugihara,[a] Shigeru Ohta,[a] and Akihiko Hara[b]

[a]*Institute of Pharmaceutical Science, Hiroshima University School of Medicine, 1–2–3 Kasumi, Minami-ku, Hiroshima 734–8551, Japan and* [b]*Faculty of Fisheries, Hokkaido University, 3–1–1 Minato, Hakodate, Hokkaido 041–0821, Japan*

(Received May 11, 1999; Accepted June 7, 1999)

The estrogenic effect of fenthion, an insecticide, in goldfish (*Carassius auratus*) was examined in terms of the induction of vitellogenin, a biomarker of estrogens in fish. When male goldfish were kept in water containing diethylstilbestrol (0.1 mg/l) or nonylphenol (0.5 mg/l) for 5 days, a significant level of vitellogenin in the blood was observed. However, vitellogenin was not detected in the blood of male goldfish kept in water containing fenthion (3 mg/l) for 5 days. When female goldfish were kept in water containing fenthion for 5 days, the levels of vitellogenin in the blood were not enhanced. Furthermore, fenthion sulfoxide and fenthion sulfone, the oxidized products of fenthion, did not induce vitellogenin in male goldfish. These results suggest that fenthion and its oxidized products do not produce estrogenic activity in goldfish.

Key words —— fenthion, estrogenic effect, vitellogenin, goldfish, *Carassius auratus*

INTRODUCTION

Fenthion [*O*,*O*-dimethyl-*O*-(4-methylmercapto)-3-methylphenylthiophosphate], an organophosphorus pesticide, is widely used throughout the world as a relatively safe substitute for parathion and malathion. However, many organophosphorus pesticides persist in the environment, and undergo chemical, physical and biological changes.[1,2] Fenthion is known to be easily oxidized to fenthion sulfoxide and fenthion sulfone in the environment (Fig. 1), and contamination with fenthion and its oxidation products in rivers and lakes has been reported in Japan.[3–9] Furthermore, Tsuda *et al.*[10] reported that fenthion accumulates in the body of killifish (*Oryzias latipes*) exposed to fenthion. However, the estrogenic effect of fenthion has not been reported hitherto. Since it is important to assess the possible risks associated with human exposure to fenthion and its related compounds, we examined its estrogenic effect in goldfish.

Many insecticides which are carcinogenic and mutagenic also have estrogenic activity. It is postulated that these compounds mimic estrogenic hormones and bind to the estrogen receptor, exhibiting an agonistic or antagonistic effect.[11–14] The measurement of vitellogenin levels in the blood of male fish is a useful method for the detection of estrogenic activity. Vitellogenin, a precursor of egg yolk protein, is induced by estrogens.[15] Vitellogenin is detectable in the sera of female goldfish at spawning time. In male fish, vitellogenin is usually not detected,

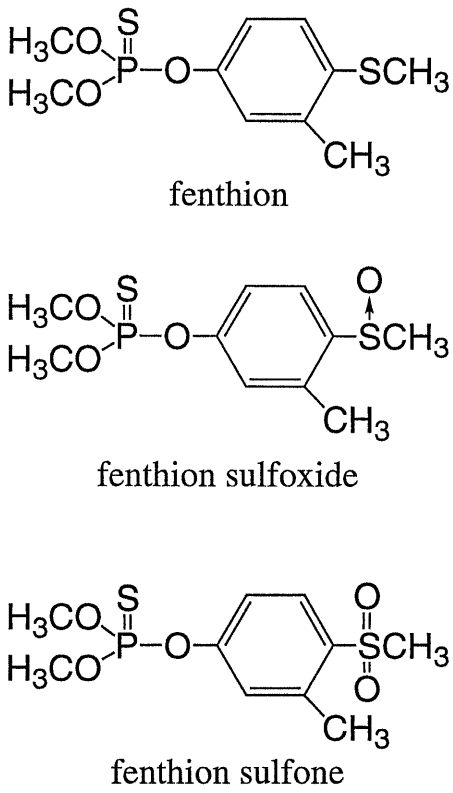

Fig. 1. Structures of Fenthion and Its Oxidation Products

*To whom correspondence should be addressed: Dr. S. Kitamura, Institute of Pharmaceutical Science, Hiroshima University School of Medicine, 1–2–3 Kasumi, Minami-ku, Hiroshima 734–8551, Japan. Tel.: +81-82-257-5328; Fax: +81-82-257-5329; E-mail: kitamura@pharm.hiroshima-u.ac.jp

but the fish have the ability to synthesize vitellogenin in the liver if they are exposed to estrogens, including environmental estrogens.[16-18]

In the present study, the estrogenic effect of fenthion, fenthion sulfoxide and fenthion sulfone in goldfish was estimated by measurement of the vitellogenin levels, in comparison with those induced by diethylstilbestrol and nonylphenol.

MATERIALS AND METHODS

Materials —— Fenthion, fenthion sulfoxide and fenthion sulfone were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Goldfish (*C. auratus*, a kind of red crucian carp, 9—12 cm length, 12—15 g) obtained commercially, were used. The antibody reacted with carp vitellogenin (anti-carp lipovitellin) was prepared by the reported method.[19]

Treatment of Fish with Chemicals —— Goldfish were kept in 5 l of water containing fenthion, β-estradiol, diethylstilbestrol or nonylphenol at concentrations of 3, 0.1, 0.1 or 0.5 mg/l, respectively, at a water temperature of 20—23℃. These solutions were prepared by the addition of an acetone solution of the chemicals (0.1—3 mg/ml) to distilled water. After 3—5 days, about 0.2 ml of blood was collected from the caudal blood vessel of the goldfish.

Western Blot Analysis —— The sera (10 μl) of goldfish were separated by 7.5% SDS–polyacrylamide gel electrophoresis according to the method of Laemmli[20], and proteins were transferred to a PVDF membrane (0.2 mm, Bio-Rad Laboratories, Hercules, CA, U.S.A.) using an Atto AE-6675 semi-dry electrotransfer apparatus (Atto Co., Tokyo, Japan). The transfer buffer contained 25mM Tris base, 192 mM glycine, SDS (0.1%) and methanol (5%). The membrane was blocked for 1h in a blot buffer (20 mM Tris-HCl buffer (pH 8.0) containing 5% nonfat milk) at room temperature, then briefly washed with the blot buffer and incubated overnight after adding anti-carp lipovitellin rabbit sera (1 in 5000 dilution). It was washed four times with the blot buffer, then incubated with goat anti-rabbit IgG horseradish peroxidase (Daiichi Pure Chemicals Co., Ltd., Tokyo, Japan) (1 in 2000 dilution) for 1 h at room temperature and washed four times with Tris–buffered saline (TBS; 25 mM Tris–HCl, 0.5 M NaCl, pH 7.5). The development was performed in TBS containing 0.15% diaminobenzidine hydrochloride and 0.06% H_2O_2 for about 5 min, then stopped by adding TBS containing 0.1% Tween 20.

RESULTS AND DISCUSSION

Vitellogenin Levels in Male and Female Goldfish

Vitellogenin levels in the sera of male and female goldfish were measured by Western blot analysis using anti-carp lipovitellin. High vitellogenin levels in the sera of female goldfish were

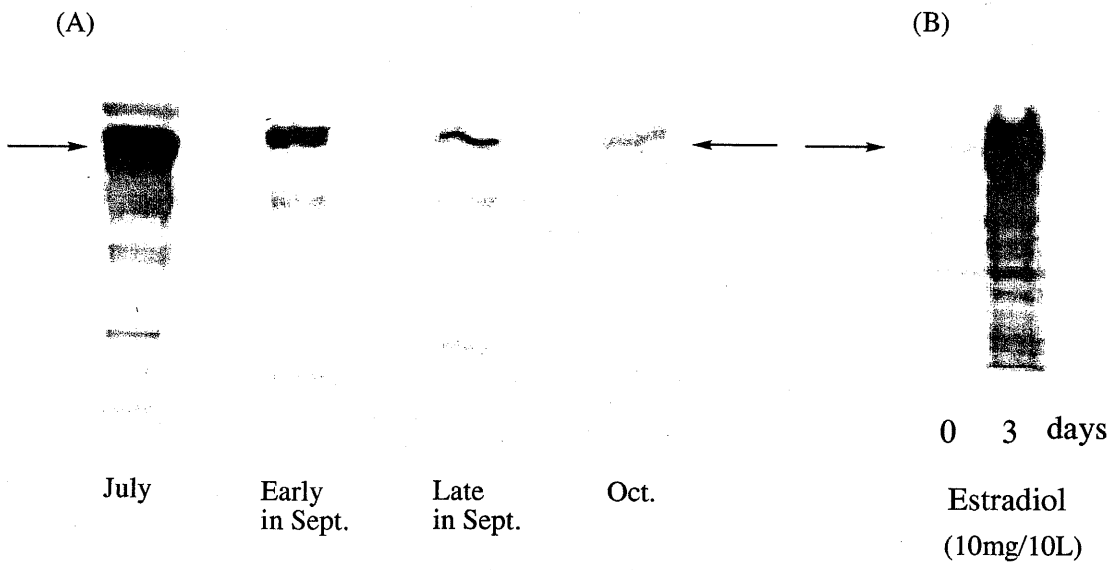

Fig. 2. Vitellogenin Levels in Sera of Male and Female Goldfish

(A) Seasonal changes in vitellogenin levels in sera of female goldfish, (B) vitellogenin levels in sera of untreated male goldfish and β-estradiol-exposed fish.

The vitellogenin levels in sera of goldfish were measured as described in Materials and Methods. In the β-estradiol experiment, male goldfish were kept in water containing β-estradiol at the concentration of 0.1 mg/l for 3 days.

detected during July—October, which is the spawning time for fish in Japan. The highest level was observed in July. However, vitellogenin could not be detected in male fish. When male fish were kept in water containing β-estradiol (0.1 mg/l) for 3 d, significant amounts of vitellogenin were detected in their sera (Fig. 2).

Effect of Fenthion on the Vitellogenin Level

The effect of fenthion and its oxidation products on the vitellogenin levels in the sera of goldfish was examined by Western blot analysis. When male goldfish were kept in water containing diethylstilbestrol (0.1 mg/l) or nonylphenol (0.5 mg/l) for 5 d, vitellogenin was strongly induced, as expected. In contrast, vitellogenin was not detected in the blood of male goldfish after exposure to fenthion. When female goldfish were kept in water containing fenthion (3 mg/l) for 5 d, their vitellogenin levels were not enhanced (Fig. 3). Furthermore, fenthion sulfoxide and fenthion sulfone, the oxidized products of fenthion, failed to induce vitellogenin in male goldfish (data not shown). These results suggest that fenthion and its oxidation products do not act as environmental estrogens in fish.

Estrogenic Effect of Fenthion in Fish

Fenthion was developed as a safe pesticide, which is not easily converted to the oxon form. However, it has been demonstrated that fenthion is accumulated in fish at a high level, and also that it is converted to fenthion oxon, which is likely to be highly toxic, in the environment.[10,21] Thus, it is necessary to reevaluate the toxicity of fenthion, including its degradation products. Furthermore, fenthion is easily converted to oxidized compounds, fenthion sulfoxide and fenthion sulfone, in the environment. Fenthion is also oxidized to fenthion sulfoxide, and the sulfoxide formed is reduced back to fenthion in fish.[22] In this study, we examined the estrogenic activity of fenthion, fenthion sulfoxide and fenthion sulfone by measurement of vitellogenin levels in goldfish, and found that fenthion and its oxidation products do not have an estrogenic effect in these fish.

The vitellogenin test using goldfish is a well-proven method for the detection of estrogenic chemicals *in vivo*. There are some advantages to using goldfish in the vitellogenin test. The fish are easily obtained in all seasons, and the effects of chemicals can be separately evaluated in male and female fish. Furthermore, it is possible to test in a small scale, and blood can easily be collected at intervals from the caudal blood vessel. We are using this system to conduct further screening tests of environmental chemicals with estrogenic effects.

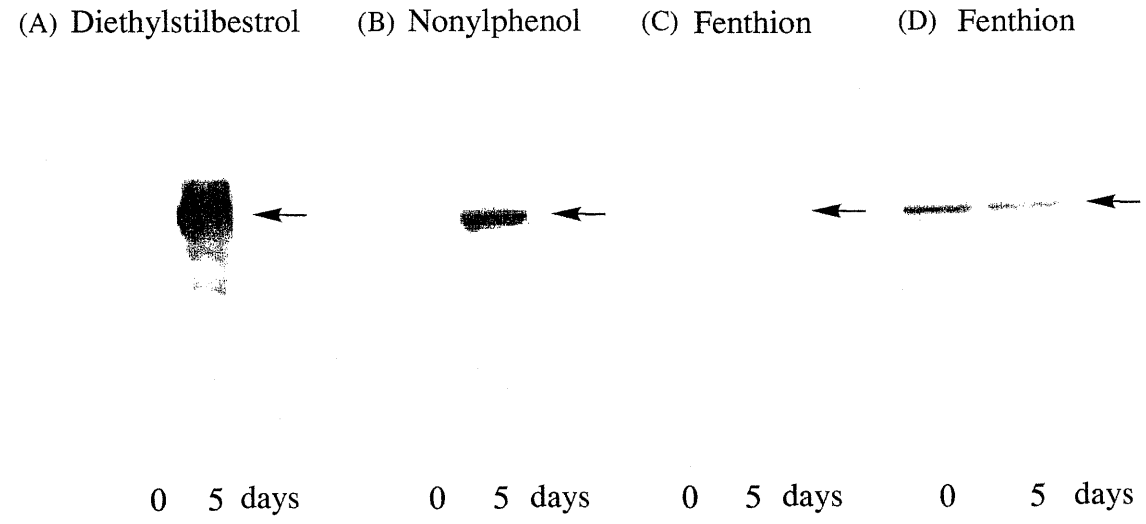

Fig. 3. Vitellogenin Levels in Sera of Goldfish Exposed to Diethylstilbestrol, Nonylphenol or Fenthion

(A) Sera of diethylstilbestrol-exposed male fish, (B) sera of nonylphenol-exposed male fish, (C) sera of fenthion-exposed male fish, (D) sera of fenthion-exposed female fish.

Goldfish were kept in water containing diethylstilbestrol, nonylphenol or fenthion at 0.1, 0.5 or 3 mg/l, respectively. Blood was collected at 0 and 5 days from the same fish. The levels of vitellogenin were measured as described in Materials and Methods.

REFERENCES

1) Eto M., "Organophosphorus Pesticides: Organic and Biochemistry," CRC Press, Boca Raton, FL, 1979.
2) Perelra W.E., Hostettler F.D., *Environ. Sci. Technol.*, **27**, 1542—1552 (1993).
3) Tsuda T., Aoki S., Kojima M., Fujita T., *Toxic. Environ. Chem.*, **34**, 39—55 (1991).
4) Fukushima M., *Jpn. J. Water Pollut. Res.*, **14**, 79—83 (1991).
5) Tsuda T., Aoki S., Kojima M., Fujita T., *Chemosphere*, **24**, 1523—1531 (1992).
6) Yamaguchi Y., Fukushima M., *Environmental Conservation Engineering*, **23**, 2—5 (1994).
7) Moriguch Y., Shindo S., Takenaka K., Fukushima M., Fujita T., Yamaguchi Y., *J. Japan Water Works Assoc.*, **63**, 28—37 (1994).
8) Tsuda T., Aoki S., Kojima M., Fujita T., *Toxic. Environ. Chem.*, **41**, 85—90 (1994).
9) Araki K., Hayakawa S., *Rept. Environ. Sci. Int. Mie Pref.*, **15**, 87—89 (1995).
10) Tsuda T., Kojima M., Harada H., Nakajima A., Aoki S., *Comp. Biochem. Physiol.*, **113C**, 45—49 (1996).
11) Nelson J. A., *Biochem. Pharmacol.*, **23**, 447—451 (1974).
12) Robinson A.K., Schmidt W.A., Stancel G.M., *J. Toxicol. Environ. Health*, **16**, 493—508 (1985).
13) Kelce W.R., Stone C.R., Laws S.C., Gray L.E., Kemppainen J.A., Wilson E.M., *Nature (London)*, **375**, 581—585 (1995).
14) Chen C.W., Hurd C., Vorojeikina D.P., Arnold S. F., Notides A.C., *Biochem. Pharmacol.*, **53**, 1161—1172 (1997).
15) Wahli W., Dawid I.B., Ryffel G.U., Weber R., *Science*, **212**, 298—304 (1981).
16) Flouriot G., Pakdel F., Ducouret B., Valotaire Y., *J. Mol. Endocrin.*, **15**, 143—151 (1995).
17) Sumpter J. P., Jobling S., *Environ. Health Perspect.*, **103**, 173—178 (1995).
18) Yamanaka S., Arizono K., Matsuda Y., Soyano K., Urushitani H., Iguchi T., Sakakibara R., *Biosci. Biotechnol. Biochem.*, **62**, 1196—1200 (1998).
19) Matsubara T., Wada T., Hara A., *Comp. Biochem. Physiol.*, **109B**, 545—555 (1994).
20) Laemmli U.K., *Nature (London)*, **227**, 680—685 (1970).
21) Lacorte S., Jeanty G., Marty J.-L., Barcelô D., *J. Chromatogr. A*, **777**, 99—114 (1997).
22) Kitamura S., Kadota T., Yoshida M., Ohta S., *J. Health Sci.*, **45**, 266—270 (1999).