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Sublethal Bioconcentration of Fenitrothion in the Blood and Brain of the European Eel

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One of the most important environmental transport and partitioning processes is bioaccumulation. The level at which a chemical is absorbed by fish depends on the absolute rates of the uptake and elimination process of the compound (Spacie and Hamelink 1985). Environmental toxicology studies performed on species in the laboratory provide the basis for much of the current regulation of pollutants and have allowed major improvements in the environmental quality. Organophosphate (OP) and carbamate insecticides act by inhibiting acetylcholinesterase. The inhibition of cholinesterases in brain has been used to diagnose OP and carbamate poisoning of mammals, birds and fish. The toxicity to fish (primarly the 96-hr LC_{50}) of many cholinesterase inhibiting insecticides have been determined. Such data are useful for comparing the various insecticides, but may not reflect the actual risk to an animal exposed to the insecticide. Selective toxicity of chemicals can be dominated by a series of different rate-limiting reactions and processes such as uptake kinetics, internal distribution, rates of biotransformation into active or nonactive metabolites, and the probability that the active metabolite may reach the target site of action at the concentration that is sufficient to exert toxics effects (De Bruijn et al. 1993).

Fenitrothion is used in large quantities for various purposes, mainly for elimination of rice stem bores (Lacorte and Barceló 1994). The possibility exists that agricultural use of a pesticide product could result in introduction of the agent to nearby bodies of water, leading to exposure of aquatic organisms (Van den Heuvel et al. 1996). The extent to which an organic compound may accumulate in aquatic organisms such as fish is often predicted to assess the potential of a compound to be present in the associated food chain. Sublethal levels of fenitrothion (0.02 µL/L) have been found in water-ways following spray operations (Lacorte and Barceló 1994). To assess the bioconcentration of fenitrothion in a freshwater fish, a 7-d dynamic study was undertaken using the european eel (*Anguilla anguilla*). The uptake and depuration of fenitrothion by the eel were measured to determine the corresponding rate constants and the steady-state bioconcentration factor (BCF).

MATERIALS AND METHODS

Technical grade Fenitrothion (96% purity) was purchased from AFRASA Company (Spain). The sublethal test concentration of 0.02 mg/L used for this study was based on 96-hr LC_{50} (0.2 mg/L) value of this insecticide for *Anguilla anguilla* (Ferrando et al. 1991).
Individuals of *Anguilla anguilla*, (weight, 20-30 g; length, 16-20 cm) were collected from Albufera Lake (Valencia, Spain). Animals were acclimatized to

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laboratory conditions for one week in 300 L glass tanks before the start of the experiments (Ferrando et al., 1991). The tanks were supplied with a continuous flow of tap water (temperature: 20±2°C; total hardness: 240±10 ppm as $CaCO_3$; pH: 7.9±0.2; alkalinity: 4.1±0.5 mmol/L). A 12-hr photoperiod was maintained (Ferrando et al. 1991). Eels did not respond to feeding attempts in our laboratory, but all animals were healthy (Ferrando et al. 1991). No mortality was observed during the acclimatation period.

The first experiment was done in a continuous flow-through system. Fenitrothion was dissolved in acetone (17 mg/L) and the solution was supplied to a glass mixing chamber with tap water and connected to a perfusion pump (Gilson minipulse 3) that generated a constant solution flow of 1.46 mL/min diluting to the desired pesticide concentration (0.02 mg/L) by a constant water flow of 18 L/hr and the outlet was connected to a 300-L glass test aquarium. In this way, the aqueous test solution was renewed approximately 3.6 times a day (test concentration was checked by gas chromatography). This system was connected 48 hours before the introduction of the animals in order to reach a balance of fenitrothion contaminated water in the test aquaria (untreated control eels were placed in a separate aquaria) Every 24 hours the fenitrothion mixture stock was renewed. Eels were exposed to fenitrothion for a period of 96 hours. After 2, 8, 24, 48, 56, 72 and 96 hours four eels were removed, rinsed with tap water, anaesthetized with MS222 (100 mg/L) (Van Wardee et al. 1983), weighed and dissected on an ice cold glass plate. Brain and blood samples were removed quickly for fenitrothion analyses.

In a second part of this experiment, eels previously exposed to fenitrothion for 96-hr were rapidly transferred to a 300-L glass aquarium with a flow of 300 mL/min of clean tap water (recovery period) under the above described conditions but without toxicant (untreated control eels were kept the same period of time in clean water). This system was maintained for 72 hours. Four eels were removed at 8, 12, 24, 48 and 72 hours. Sample tissues were taken out and stored (- 24°C) for further analyses of fenitrothion.

Gas chromatographic analysis confirmed the presence of fenitrothion in the water (> 90 %) over the entire exposure period. Extraction of pesticide from water was done based on the method of Zeigh and Sherma (1972). The residues of fenitrothion in the water were measured by a Gas Chromatograph VARIAN 6000 equipped with a flame photometric detector (injector and detector operating temperatures were 280 °C, oven temperature was 200 °C, the nitrogen gas carrier had a flow rate of 24 mL/min). Pesticide recoveries from the water was 95 % (90-98). The determination of fenitrothion in the selected tissues was carried out based on the method of Richardson and Seiber (1993) (for kidney and liver tissues) and adapted to our samples as follows: Tissues were liophilized and homogenized with 50% HCl (0.01 mL) and 0.5% ethanol-ethyl acetate (20 mL). The homogenate was centrifuged at 1200 rpm (5 min). After centrifugation, a drop of 5% decanol in acetone was added to the supernatant and the mixture dried on a rotatory vacuum evaporator. The dried residue was redissolved in 3 mL of a mixture 1:1 cyclohexane-ethyl acetate and sonicated (1 min) and filtered through a nylon filter (45 pm diameter) using a Varian Vac-elut. The residue was redissolved in 3:1 ethyl acetate-cyclohexane (6 mL) and introduced in a Gas Permeation Chromatograph. The elute was dried and redissolved in ethyl-acetate (1 mL). The final volume was injected in a FISONS Instrument Gas Chromatograph equipped with a FPD 700 photometric detector and a SSL injector. The detector and injector temperatures were 280 and 240 °C, respectively. Helium was used as gas carrier with a pressure of 145 KPa. The detection limit was <0.001 µg/g. All solvents were of pesticide

residue analysis grade. Under the conditions described, fenitrothion recovery from biological samples was 87% (84-89).
A first-order one-compartment model was used to estimate the different pharmaco-kinetic parameters of fenitrothion in brain and blood of the eel (Tsuda et al. 1989). During the first part of the exposure period, elimination of the chemical can be neglected and the uptake rate constant (K_1) can be calculated from: $K_1 = C_f/(C_w \cdot t)$, where t is the time, C_f is the tissue fenitrothion content and C_w is the concentration of the pesticide in water. When the fish are transferred to clean water, the elimination rate constant (K_2) can be derived from the slope of the first-order elimination equation: $1nC_f(t) = 1n\ C_f(0) - K_2.t$, where $C_f(0)$ is the concentration of the chemical in the fish at the beginning of the elimination period. The half-life ($T_{1/2}$ of fenitrothion in the selected tissues was estimated according to the equation: $T_{1/2} = -1n\ 0.5/\ K_2$. Mean values and standard deviations were calculated for each test group based on the values obtained for each individual tissue from four fish. These values were compared by analysis of variance (ANOVA) and Duncan's multiple range test. All statistical analyses were performed on an IBM computer using SPSS+ Programme (Sancho et al. 1994). The significance level was set at 0.05.

RESULTS AND DISCUSSION

A constant sublethal concentration of 0.02 mg/L of fenitrothion in the surrounding water during 96 hours produced typical anticholinesterase response in the european eel (De Bruijn and Hermens 1993). The exposed eels showed signs of restlessness, erratic swimming, hyperactivity and loss of balance. Some of the experimental fish showed diminished motor activity but all of them survived the stipulated exposure period. These symptoms had been reported in the european eel under sublethal exposure to other organophosphate insecticides (Da Silva et al. 1993; Sancho et al. 1994).

Fenitrothion showed a rapid bioconcentration in eel tissues (blood and brain) (Fig. 1 and 2) as was already reported in other fish species (Tsuda et al. 1989; 1990; Sancho et al. 1994). This bioconcentration process is also common after exposure to other organophosphates as found by Tsuda et al. (1995) who observed that fenitrothion, diazinon and fenthion were rapidly accumulated in the whole body of the killyfish (*Oryzias latipes*) after 12 hr exposure. The high bioconcentration of 0.497 µg/g (96 hr), showed by fenitrothion in the brain in this study (Fig. 2), would be favoured by the high lipid content of this tissue (Ferrando and Andreu 1991). The peak biconcentration of fenitrothion in brain was 9.6 times of that in the blood.

The main uptake of pesticides through the gills has an important role in the uptake of fenitrothion to the organs via blood. On the other hand, high accumulation rates will be find in highly irrigated organs (e.g. liver or brain) as well as a with a high pesticide "affinity", that is the case of brain tissue where the lipid content is quite big (Sancho et al. 1994). As a result, it would supply the pesticide accumulation in the fish tissues, but also would provide its availability for metabolization and/or excretion. In fact, elimination seemed to occur rapidly when the pesticide concentration in the surrounding water decreased. Animals transferred to clean water (recovery period) during 24 hr showed a fast elimination of the bioconcentrated fenitrothion in eel brain. This rapid elimination could help the animals to recover their health and normal behaviour in natural conditions (Morgan et al. 1990).

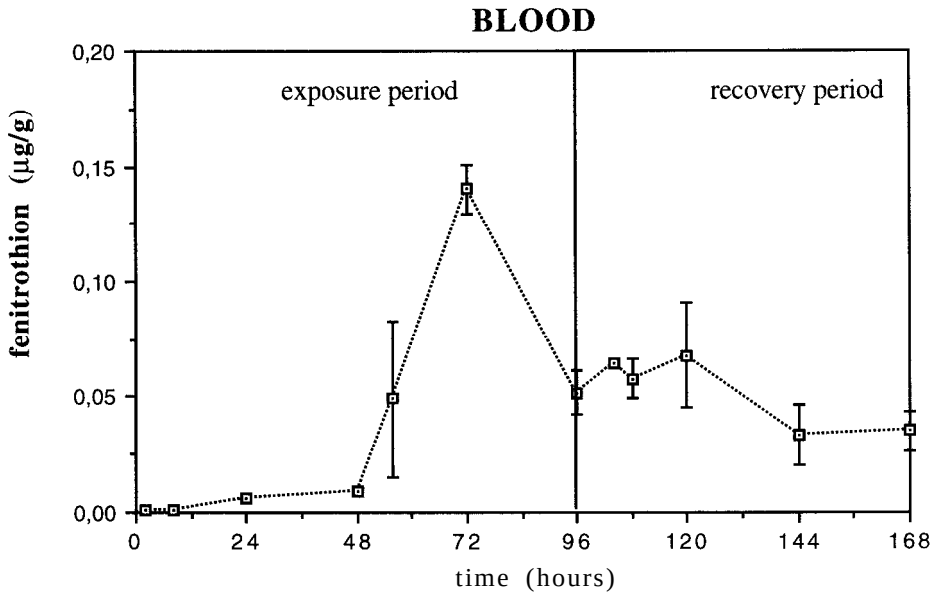

Figure 1. Fenitrothion content (µg/g of wet weight) detected in the blood of the european eel during a 96 hr exposure to 20 µg/L of fenitrothion and after transfer to clean water.

Pharmaco-kinetic parameters of fenitrothion in the selected tissues are shown in table 1. We did not observed any steady-state for fenitrothion in eel brain and blood tissues during the exposure period. Fenitrothion was incorporated in animal tissues until the end of the exposure period. At this time (96 hr) a BCF of 24.8 was calculated for brain. Lowest BCF was calculated for blood (2.6) at the same exposure time. These values indicate the high affinity of this toxicant for the brain. An uptake rate constant (K_1) for blood of 0.062 $mL \cdot g^{-1} \cdot hr^{-1}$ was calculated from the experimental data set (table 1). Highest K_1 was calculated for brain tissue in this study (2.28 $ml \cdot g^{-1} \cdot hr^{-1}$) showing the strong bioconcentration power of fenitrothion in this organ and correlating to the fast accumulation process observed.

A passive elimination may be done across the gills by the same partitioning process involved in the uptake, owing to their physical characteristics (Spacie and Hamelink 1985). This fact could explain the fenitrothion concentrations found in blood during the elimination phase proceeding from fenitrothion mobilization in other tissues together with a rapid elimination of brain residues.

Elimination could be explained by a one-compartment first-order kinetic process in both tissues (table 1). K_2 of 0.005 and 0.152 h^{r-1} were estimated for fenitrothion in blood and brain tissues, respectively. These results are related to the low half-life found in brain (4.6 hr) in this study. Fenitrothion also appears to be rapidly eliminated from brain (K_2= 0.044 hr^{-1} and $T_{1/2}$= 15.75 hr) from *A. anguilla* after a continuous exposure to 0.04 ppm fenitrothion (Sancho et al. 1997a).

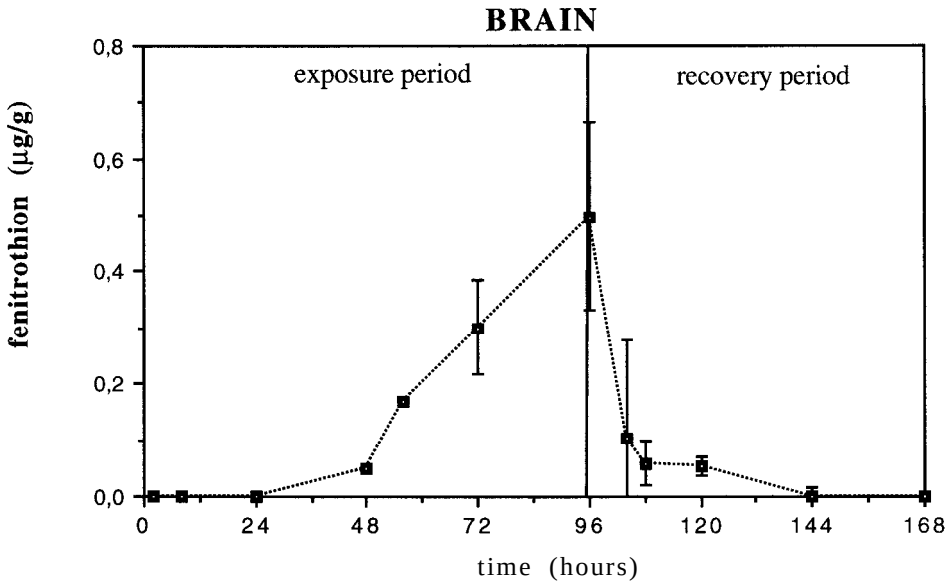

Figure 2. Fenitrothion content (µg/g of wet weight) detected in the brain of the european eel during a 96 hr exposure to 20 µg/L of fenitrothion and after transfer to clean water.

Table 1. Pharmaco-kinetic parameters of fenitrothion (20 µg/L) in blood and brain of the european eel.

tissues	steady-state (hr)	K_1 ($mL \cdot g^{-1} \cdot hr^{-1}$)	K_2 (hr^{-1})	BCF (96-hr)	$T_{1/2}$ (hr)
blood	-	0.06	0.005	2.6	128.3
brain	-	2.28	0.152	24.8	4.6

De Bruijn and Hermens (1991) adjusted the elimination of the bioconcentrated fenitrothion in the fish *Poecilia reticulata* to a first-order, one-compartment model; in that case a K_2 of 0.047 hr^{-1} was calculated for this insecticide in the full organism, indicating that a rapid elimination of fenitrothion had been done. Oxidation reactions in specific target tissues of the fish such as brain or gills could cause local inhibition of AChE activity levels and subsequent toxic effects. However, the probability that a sufficient amount of the compound will reach these target sites is dependent on the rates of uptake, elimination and all possible biotransformation reactions (De Bruijn and Hermens 1993).

Previous experiments were carried out in our laboratory in order to study the bioaccumulation and elimination of 0.04 mg/L fenitrothion in eel brain (Sancho et al. 1997a). In those experiments, fenitrothion uptake was faster (it started at 12hr

exposure) than in the present study (after 24hr), however pesticide elimination (recovery period) was very similar in both cases.

Similar studies were done by Sancho et al. (1997b) who studied the effect of 0.02 and 0.04 mg/l fenitrothion in *A. anguilla*. In this study, the authors evaluated bioaccumulation and elimination process of that pesticide in eel muscle. The results indicated that accumulation of fenitrothion in eel muscle was fast and constant during the exposure period and the pesticide elimination from muscle tissue was slower than that found in eel brain in the present study.

Considering our past work, it is important to emphasize the new knowledge that resulted from this research using a flow-through system instead of a semi-static condition, selecting a new tissue which is not very often chosen and exposing the animals to a low pesticide concentration because it is the amount of fenitrothion that usually is found in aquatic environments.

Although the physico-chemical properties of the insecticide should act allowing and supplying some detoxification routes, the rapid elimination of toxicant could be directly related with a fast response of the detoxification systems. This fact could protect the eel against many transformation products (e.g. fenitro-oxon) which are known to be even more toxic than the parent compound (Kobayashi et al. 1985).

In vivo studies will done in our laboratory to check the real concentration of the toxic compound reaching a particular organ or tissue, the elimination rates, and its functional meaning in the whole alive organism.

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