Bull. Environ. Contam. Toxicol. (1993) 50:572–577

Environmental Contamination and Toxicology

Bioaccumulation and Elimination of Endosulfan in the Fish Yellow Tetra (*Hyphessobrycon bifasciatus*)

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Endosulfan is a broad spectrum insecticide used in Brazil on cocoa, coffee, cotton and soybean crops. Although unstable in the environment, endosulfan is highly toxic to fish, with LC50 values in the range of parts per billion. Few data on the fate of endosulfan in fish tissues are available and toxicokinetic pattern of this pesticide in tropical fish is not well known. In this study, we investigated the uptake and elimination of endosulfan in the yellow tetra fish (Hyphessobrycon bifasciatus), a species widely distributed in ponds and lakes of São Paulo State and Southern Brazil.

MATERIALS AND METHODS

Adult yellow tetra of 0.78 g in average weight were collected from an unpolluted pond situated close to the University of Campinas. Fish were acclimated and maintained at room temperature ($22 \pm 1.5^{o}C$) at least 1 wk before testing. They were fed daily, 5 d a week, with commercial dry flake food (Nutral Básica, made in Brazil).

Technical Thiodan^{R} (endosulfan $\alpha + \beta$, 2:1, 97% purity) and the reference standards α endosulfan, β endosulfan, $\alpha + \beta$ endosulfan 2:1 and endosulfan sulfate were obtained from Hoechst do Brazil Ltda. Solvents and chemicals used in endosulfan analyses were of pesticide residue analytical grade.

Rectangular glass aquarium of about 20 L were used in the experiments. Water chemical characteristics were: total hardness as $CaCO_3$ 35.0 mg/L; dissolved oxygen 5.1 mg/L; chemical oxygen demand 2.4 mg/L; pH 7.1. The water temperature was maintained at $22 \pm 1.5^{o}C$ and the photoperiod was 16 hr light/8 hr darkness. Three groups

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of 23 fish each were exposed to 0.4 µg/L technical grade endosulfan during 21 d. A control tank without toxicant was included in the experiment. Fish were fed daily during the test period. Considering previously determined half-lives for α and β endosulfan in the test water (approximately 1 d), aquaria water was changed every 24 hr. Thus, the mean concentration of endosulfan during the experiment was assumed to be 0.3 µg/L (0.2 - 0.4 µg/L).

After 3, 7, 14 and 21 d, 3 groups of 2 to 4 fish each were analyzed for endosulfan residues. On day 21, the remaining fish were transferred to an aquaria set up as above without the presence of endosulfan. The water was continuosly circulated through a charcoal filter. After appropriate time intervals, 3 groups of 3 to 5 fish each were removed and analyzed.

Each group of fish was pooled, homogenized and extracted with 150 mL acetone:H_2O (100:50 v/v) in a homogenizer system. Extract was filtered under vacuum and transferred to a 500-mL separatory funnel containing 100 mL hexane-dichloromethane (50:50, v/v). After shaking, the aqueous layer was transferred to a 250-mL separatory funnel containing 5 g NaCl. Dichloromethane (50 mL) was added and mixed carefully. Organic layers from both separatory funnels were pooled, passed through a cylindrical filter funnel containing anhydrous Na_2SO_4 and concentrated under vaccum. Sample was redissolved with hexane (3 mL) and transferred to a column (id = 1 cm, h = 25 cm) of activated Florisil for clean-up.

The column was prewashed with 20 mL hexane. Sample was eluted with 20 mL hexane (Eluant 1) followed by 20 mL dichloromethane-hexane (20:80, v/v, Eluant 2) and 20 mL acetonitrile-dichloromethane-hexane (1.5:50:48.5 v/v, Eluant 3). Endosulfan residues were normally found in Eluant 3, which was concentrated, washed with 10 portions of 1 mL hexane under N_2 stream and transferred quantitatively to a graduated tube. Cleaned extract was diluted with hexane and analyzed on a Varian 3400 gas chromatograph equipped with a EC (^{63}Ni) detector and a Megabore^R DB1 column at the following conditions: injector temperature 220°C, oven initial temperature 150°C increased 3°C/min until 200°C, detector 300°C, carrier gas (N_2) flow 20 mL/min. The average recovery of endosulfan from spiked tissue samples varied between 90 to 110%.

Bioconcentration factors (BCF) were calculated according to the method proposed by Oliver and Niimi (1985), based on the rates of uptake and clearance. Biological half-lives ($t^1/_2$) for clearance were estimated assuming a first order kinetic model.

RESULTS AND DISCUSSION

Residue data for H. bifasciatus showing uptake and loss of endosulfan are shown in Table 1. Accumulation results suggest that up to day 21 of exposure, total endosulfan residues in fish increased with increasing time. No steady state was reached during this period. Pesticide level at day 3 was similar to that found in Carassius auratus after 5 d exposure to 1 µg/L endosulfan in water (Goebel et al. 1982). Although detected during both uptake and elimination phases, the levels of endosulfan sulfate in fish tissue were very low, close to the detection limit of 20 ng/g. For this reason, no toxicokinetic parameter was derived for this metabolite.

Table 1. Endosulfan residues in H. bifasciatus, exposed to a mean concentration of 0.3 µg/L endosulfan

Time	Endosulfan Concentration (µg/g)[A] α	β	sulfate	total
(d)		uptake		
03	0.196(0.050)	0.130(0.034)	0.029(0.003)	0.355(0.087)
07	0.565(0.170)	0.280(0.093)	0.060(0.014)	0.905(0.277)
14	0.854(0.310)	0.373(0.102)	0.069(0.019)	1.296(0.424)
21	1.131(0.125)	0.516(0.042)	0.055(0.017)	1.702(0.072)
(hr)		elimination		
24	0.783(0.195)	0.299(0.054)	0.025(0.019)	1.107(0.213)
52	0.519(0.343)	0.188(0.105)	0.037(0.0002)	0.744(0.448)
79	0.511(0.144)	0.153(0.010)	0.042(0.020)	0.706(0.174)
120	0.237(0.205)	0.079(0.067)	N.D.[B]	0.316(0.273)

[A] Each value represents the mean (±SD) of 3 determinations (n=3). Based on wet weight.
[B] not detected (detection limit = 20 ng/g).

Endosulfan bioconcentration factor and half-life in H. bifasciatus are presented in Table 2. As no steady state was observed during uptake, BCF could not be calculated as the ratio of the concentration of the pesticide in fish and in water at equilibrium. The use of the kinetic parameters k_1 and k_2 to estimate the bioconcentration of pesticide in aquatic organisms has been suggested by many authors (Bishop and Maki 1980; Oliver and Niimi 1985; Niimi 1987; Shaw and Connel 1987).

Table 2. Bioconcentration factor (BCF), biological half-life ($t^1/_2$) and absorption (k_1) and elimination (k_2) constants of endosulfan in H. bifasciatus.

Endosulfan mean conc in water (µg/L)	BCF	$t^1/_2$ (d)	k_1 (d^{-1})	k_2 (d^{-1})
0.2(α)	10,994(2,749)	2.01(0.44)	3,787(947)	0.344(0.076)
0.1(β)	9,908(4,954)	1.74(0.33)	3,938(1,969)	0.397(0.067)
0.3(α+β)	11,583(2,361)	1.81(0.35)	4,436(904)	0.383(0.075)

Values in parenthesis represent one standard deviation

The BCF values determined in the present study (11,583 ± 2,361) were much higher than those calculated from data reported by Goebel et al. (1982) in Catastomus comersoni (BCF = 258-272) and in Carassius auratus (BCF = 314). Perhaps, one reason for this difference is that in these experiments only part of the fish tissues was analyzed. According to literature, endosulfan residues in fish may be found in different organs as liver, kidney, brain, gills, muscles, gut and gonads (Nowak and Ahmad 1989; Rao and Murty 1980; Rao and Murty 1982). Also, lipid content seems to determine the levels of endosulfan residue in fish. Species with high content of fat would concentrate more endosulfan in the adipose tissue (Matthiessen et al. 1982).

Compared to BCF determined for organochlorine compounds, the value obtained in this study for H. bifasciatus is higher than those determined for heptachlor (BCF = 3,800), endrin (BCF = 6,200), dieldrin (BCF = 2,384) and kepone (BCF = 2,593) in other fish species (Murty 1986; Anderson and De Foe 1980; Shannon 1977; Bahner and Oglesby 1979). Although not conclusive, these data indicate that even though being considered a compound not persistent in the environment, endosulfan, as many organochlorines, may accumulate in aquatic organisms continuosly exposed to sublethal concentrations of the pesticide.

Endosulfan depuration by fish (Table 1) demonstrated that the loss of α and β isomers was fast when fish were transferred to clean water. Approximately 81% total endosulfan was eliminated within 120 hr with estimated biological half-life of 1.8 d. This value, as well as those related for other fish species (Goebel et al. 1982; Niimi 1987) are in general lower than the $t^1/_2$ determined for organochlorine compounds in various teleosts (Niimi 1987). Nevertheless, they are similar or very close to the $t^1/_2$ determined for pesticides of low persistence in biological systems as the organophosphates fenitrothion ($t^1/_2$ = 1.7 d) (McLeese

et al. 1979) and diazinon ($t_{1/2}$ < 1 d) (Niimi 1987), the carbamate aminocarb ($t_{1/2}$ = 1 d) and carbofuran ($t_{1/2}$ < 2 d) (Niimi 1987).

The low persistence of endosulfan in water and its rapid elimination by biological systems seem to be peculiar characteristics of endosulfan that justify its allowance of use in agriculture.

Acknowledgments. These investigations were partially supported by Fundo de Amparo à Pesquisa da Universidade Estadual de Campinas and EMBRAPA. We also thank CNPq for scholarship support.

REFERENCES

Anderson RL, De Foe DL (1980) Toxicity and bioaccumulation of endrin and methoxychlor in aquatic invertebrates and fish. Environ Pollut (Ser A) 22:111-121

Bahner LH, Oglesby JL (1979) Test of a model for predicting kepone accumulation in selected estuarine species. In: Marking LL and Kimerle RA (eds). Aquatic toxicology, ASTM STP 667. American Society for Testing and Materials, Philadelphia, pp 221-231

Bishop WE, Maki AW (1980) A critical comparison of two bioconcentration test methods. In: Eaton JB, Parrish PR and Hendricks AC (eds). Aquatic toxicology, ASTM STP 707. American Society for Testing and Materials, Philadelphia, pp 61-77

Goebel H, Gorbach S, Knauf W, Rimpau RH, Hüttenbach H (1982) Properties, effects, residues and analytics of the insecticide endosulfan. Residue Rev 83:1-174

Matthiessen P, Fox PJ, Douthwaite RJ, Wood AB (1982) Accumulation of endosulfan residues in fish and their predators after aerial spraying for the control of tsetse fly in Botswana. Pest Sci 13:39-48

McLeese DW, Zitko V, Sergeant DB (1979) Uptake and excretion of fenitrothion by clams and mussels. Bull Environ Contam Toxicol 22:800-806

Murty AS (1986) Toxicity of pesticides to fish vol 1. CRC Press Inc., Boca Raton, p 70

Niimi AJ (1987) Biological half-lives of chemicals in fishes. Rev Environ Contam Toxicol 99:1-46

Nowak B, Ahmad N (1989) Residues in fish exposed to sublethal doses of endosulfan and fish collected from cotton growing area. J Environ Sci Health B 24:97-109

Oliver BG, Niimi AJ (1985) Bioconcentration factors of some halogenated organics for rainbow trout: limitations in their use for prediction of environmental residues. Environ Sci Technol 19:842-849

Rao DMR, Murty AS (1980) Toxicity, biotransformation and elimination of endosulfan in Anabas testudineus (Bloch). Indian J Exp Biol 18:664-666

Rao DMR, Murty AS (1982) Toxicity and metabolism of endosulfan in three freshwater catfishes. Environ Pollut (Ser A) 27:223-231

Shannon LR (1977) Equilibrium between uptake and elimination of dieldrin by channel catfish, Ictalurus punctatus. Bull Environ Contam Toxicol 17:278-284

Shaw GR, Connel DW (1987) Comparative kinetics for bioaccumulation of polychlorinated biphenyls by the polychaete (Capitella capitata) and fish (Mugil cephalus). Ecotoxicol Environ Saf 13:84-91

Received May 26, 1992; accepted November 1, 1992.