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Comparative Study on the Acute Toxicities of α, β, γ, and δ Isomers of Hexachlorocyclohexane to Freshwater Fishes

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Hexachlorocyclohexane (HCH), also known as BHC, is an organochlorine insecticide severely restricted or banned in most countries, but still widely used in some developing countries where insect pressures are high (e.g. India). Technical-grade HCH is synthesized from benzene and chlorine in the presence of ultra-violet light and averages 65-70% α-HCH, 7-10% β-HCH, 14-15% γ-HCH, 7% δ-HCH, 1-2% ε-HCH, and approximately 1 to 2% of other components (WHO, 1992). γ-HCH (lindane) is the insecticidally active constituent of the technical product. In Brazil, the general use of HCH in agriculture was forbidden in 1985, but its use to control insect-borne diseases is still allowed (Ministry of Agriculture - Brazil, 1985).

The α, β, δ and γ-HCH isomers are chemically stable and slowly biodegradable compounds, and have similar *n*-octanol-water partition coefficients (log K_{ow} ranging from 3.2 to 3.8) (WHO, 1991, 1992). The isomers, however, differ regarding their persistence in soil, water and living organisms (Deo *et al*, 1994). It has been reported, for instance, that β-HCH presents the highest bioaccumulation ratio and the slowest elimination rate in *P. reticulata* (Yamato *et al*, 1983).

All of the four HCH isomers have been found in soil, water and vegetables in contaminated areas. In “Cidade dos Meninos”, a highly contaminated area on the outskirts of Rio de Janeiro city, very high levels of α, β, δ and γ-HCH have been found in soil, pastures and groundwater (Brilhante & Oliveira, 1996).

It has been reported that there are marked differences between HCH isomers in terms of their toxicity to non-target species. Lindane is by far the most potent insecticide and the most studied isomer. Data on the toxicity of the other isomers to non-target organisms are scarce, and comparative toxicity studies have seldom been performed. The present study was undertaken to provide data on the comparative acute toxicity of α, β, δ and γ-HCH isomers to three freshwater fish species.

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MATERIALS AND METHODS

The fish species tested and their respective taxonomic families were as follows: 'guppy' (*Poecilia reticulata;* Poeciliidae), 'zebra fish' (*Brachydanio rerio;* Cyprinidae) and 'neon' (*Paracheirodon axelrodi;* Characidae). The fishes were purchased from a commercial supplier in Rio de Janeiro, weighed 0.2 to 0.4 g and had an average length of 2.0 to 3.0 cm.

α-HCH (purity 98,%), β-HCH (purity 98%), γ-HCH (purity 99%) and δ-HCH (purity 96%) were purchased from Riedel-de Haën, Seelze - Germany. Owing to their low water solubility, HCH isomers were first dissolved in acetone (*Pro Analysis* grade, Merck KGaA) and then diluted in a synthetic soft-water (hardness: 40 - 48 mg/L as $CaCO_3$; pH 7.2 ± 0.1; dissolved oxygen 98%; acetone final concentration < 0.1% v/v) by uning a ultrasonic device.

Acute toxicity tests (static method) were carried out as standardized by the 'Associação Brasileira de Normas Técnicas' (ABNT, 1993). Briefly, 10 adult fishes per concentration were placed in 3000 ml glass beakers, and mortality was evaluated after 24, 48, 72 and 96 hr of exposure. Negative control (synthetic soft water) and positive control (potassium dichromate) beakers were included. Room temperature (23 ± l°C) and light / dark cycle (16 hr light / 8 hr dark) were kept constant. LC_{50}s values and their 95%fcontidence limits were calculated from dosage-mortality curves using probit analysis (Finney, 1971; USEPA Probit Analysis Program Used for Calculating EC Values, version 1.4).

RESULTS AND DISCUSSION

LC_{50} values and 95% confidence limits determined for α-, β-, δ- and γ-HCH are shown in Table 1. γ-HCH was by far the most toxic isomer to the three freshwater fish species tested. The acute toxicity of α– and β-HCH isomers was nearly the same and, in most cases, LC_{50}s for δ-HCH were slightly higher than the LC_{50} values found for the α- and β-HCH isomers.

It is of note that the LC_{50} for γ-HCH does not change as the duration of exposure increases, *i.e.* all deaths were recorded during the first twenty-four hours of exposure. On the other hand, LC_{50}s for α-, β-, δ-HCH isomers tend to decrease as exposure time increases, *i.e.*, deaths also occurred in the second, third and fourth days of exposure. These results suggest that differences between γ-HCH and other isomers regarding the LC_{50} values tend to be less pronounced as the exposure time becomes longer.

The LC_{50}s of lindane (γ-HCH) obtained in the present study were higher than those previously reported by Geyer *et al.* (1993) for *P. reticulata* (48 hr LC_{50}, 0.14 mg/L) and *B. rerio* (48 hr LC_{50}, 0.09 mg/L). The 48 hr LC_{50} of α-HCH for *P. reticulata* determined in our study (1.95 mg/L) was also higher than the values previously found by Canton *et al.* in 1975 (0.8 mg/L) and in 1978 (1.38 mg/L).

Table 1. Acute toxicities of α, β, δ and γ - hexachlorocyclohexane (HCH) isomers to freshwater fish species after 24, 48, 72 and 96 hours of exposure. Data are shown as LC_{50} values (mg/L) and respective confidence limits 95%.

	Poecilia reticulata				*Brachydanio rerio*				*Paracheirodon axelrodi*			
Exposure time :	24 hr	48 hr	72 hr	96 hr	24 hr	48 hr	72 hr	96 hr	24 hr	48 hr	72 hr	96 hr
α-HCH	2.65 1.86-4.83	1.95 1.43-2.72	1.58 1.12-2.31	1.49 1.05-2.18	1.41 1.10-1.81	1.11 0.82-1.51	1.11 0.82-1.51	1.11 0.82-1.51	1.64 1.23-2.48	1.52 1.15-2.04	1.52 1.15-2.04	1.52 1.15-2.04
β-HCH	3.14 2.10-7.99	2.68 1.81-5.54	2.18 1.41-4.53	1.66 1.00-3.55	1.78 1.32-3.59	1.63 1.23-2.48	1.63 1.23-2.48	1.52 1.15-2.04	1.70 1.13-5.15	1.10 0.77-1.83	1.10 0.77-1.83	1.10 0.77-1.83
γ-HCH	0.36 0.28-0.45	0.36 0.28-0.45	0.36 0.28-0.45	0.36 0.28-0.45	0.16 0.11-0.22	0.16 0.11-0.22	0.16 0.11-0.22	0.16 0.11-0.22	0.14 0.11-0.20	0.14 0.11-0.20	0.14 0.11-0.20	0.14 0.11-0.20
δ-HCH	4.05 3.03-5.38	3.79 2.87-4.96	3.27 2.30-4.42	2.83 1.96-3.90	2.64 1.82-3.65	1.87 1.32-2.57	1.58 1.15-2.17	1.58 1.15-2.17	2.59 1.72-3.99	2.19 1.45-3.34	1.42 0.95-2.11	0.84 0.48-1.34

No deaths were observed in the negative control groups (synthetic soft water). Positive control ($K_2Cr_2O_7$) results were as follows: 96 hr-LC_{50} and CL 95% (mg/L): *P. reticulata*, 114.6 (72.7 - 180.7); *B.rerio*, 89.1 (71.9 - 110.5) and *P.axelrodi*, 97.6 (68.6 - 138.8).

While LC_{50} (48 hr) of β-HCH for *P. reticulata* was 2.68 mg/L in the present experiment, a value as low as 0.9 mgL had been obtained in a previous study (Boulekbache, 1980, cited in WHO, 1992). No previous data on the acute toxicity of δ-HCH to freshwater fishes was found in the literature. The deviations of LC_{50} values found in the present study from those reported in the literature were probably due to differences in testing conditions. LC_{50} as well as LD_{50} values depend on so many factors (e.g. age, feeding conditions, circadian and seasonal rhythms, temperature, dissolved oxygen, water quality, bioavailability, etc) that, even under standardized experimental conditions, interlaboratory deviations occur due to factors not clearly described in the test protocols (Zbinden and Flury-Roversi, 19 1; Mayer and Ellersieck, 1988).

B. rerio seemed to have been as susceptible as *P. axelrodi* to the toxic effects of HCH isomers. *P. reticulata*, however, tends to be more resistant to HCH than the other two fish species tested. Geyer *et al* (1993, 1994) found a positive linear relationship between the lipid content (% on a wet basis) of the fishes and their resistance to γ-HCH-induced toxicity *i.e.* the higher the lipid content, the higher the LC_{50} value. Since it has been reported that the lipid content of *P. reticulata* (5.4%) is higher than that of *B. rerio* (4.05%) (Geyer *et al* 1993), the lower susceptibility of the former species to the four HCH isomers could be attributed to its higher content of lipids. Lipid content of *P. axeroldi* has not been determined yet. However, according to the relationship found by Geyer *et al* (1993), it could be predicted that the lipid content of *P. axeroldi* is similar to that of *B. rerio.*

The neon fish has not been used as a testing species in aquatic toxicology. In contrast to *P. reticulata* and *B. rerio,* which are exotic species in Brazil, *P. axeroldi* is a fish native to the Amazon rivers. Since the Brazilian guidelines for evaluating ecotoxicity of chemical agents recommend the use of native fishes (SEMA, 1988), this representative of the Characidae family, easy to handle and to maintain in the laboratory, seems to be a suitable species for further testing of effects of chemicals on Amazon ecosystems.

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