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# Toxicity and Bioconcentration of Hexachlorocyclohexane (HCH) in an Air-Breathing Catfish, *Saccobranchus fossilis* (Bloch)

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The organochlorine insecticide, hexachlorocyclohexane (HCH) popularly known as BHC, is widely used for the control of insects, pests and vectors of malaria, in agricultural and public health sectors, respectively. The HCH constitutes nearly 50% of the total pesticides used in India. The large scale usage of HCH has led to its near ubiquitous presence in the Indian Environment and Food Products (Krishna Murthy, 1984). Because HCH is stable, highly fat soluble, having low-water solubility, high biological potency and relatively long-lived in the environment, it presents potential hazard to non-target species (Fish and Wildlife) and ultimately to the public health. Toxicity of HCH to aquatic animals has been reported. The 96-h LC50's of lindane and technical grade HCH to several fish species were reported in the range of 30 to 104 ug/L (Murthy 1986). The 96-h LC50 value of commercial grade HCH to Saccobranchus fossilis was reported to be 2.49 mg/L (Verma et al 1982). Bioconcentration of HCH has been studied in estuarine fishes (Schimmel et al 1978), but few attempts have been made in freshwater fishes (Canton et al 1975; Murthy 1986).

The current study was undertaken to determine the sublethal toxicity of commercial grade HCH to a freshwater air-breathing catfish, Saccobranchus fossilis (Bloch) for 14 days. The bioconcentration of HCH and its distribution in gill, brain and liver was determined. This species was selected for the present study, because it is widely distribtued in ponds, lakes and rivers of India and consumed as human diet in many parts of the world.

## MATERIALS AND METHODS

Air-breathing fish, S. fossilis (body length 8-10 cm, wet weight 20-25 gm) were collected from

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the local fish pond and acclimatized to laboratory conditions in 60-L glass aquaria. Fish were fed living mosquito larvae, daphnids and goat liver five days a week during acclimatization and toxicity tests. Commercial grade hexachlorocyclohexane (Central Insecticides and Fertilizers, Indore, M.P., India, 50% wettable powder, 6.5% $\gamma$-HCH) was used in this study. Acetone:ethanol (1:1 v/v) was used as a solvent for the preparation of stock solution. The experimental test concentrations selected were log concentrations (7-10) based on the progressive bisection of intervals on a logarithmic scale. Control experiments with the solvent were also set side by side. Test water was renewed after every 24-h. A group of ten fish with three replicates for each test concentration were tested in 60-L capacity of glass aquaria. Tubewell water was used for static bioassays and it was monitored periodically for physico-chemical characteristics as procedure given in standard methods (APHA et al 1981).

Fish were sampled at 7 and 14 days exposure for residue analysis. The feeding of fish was stopped before 24 hours of sampling. Separate experiments were carried out for HCH residue analysis. Selected organs from 4-5 fish were sampled from each test aquaria. Samples were placed in preweighed glass vials, reweighed and kept at 4 C until analysed. Residues were analysed on wet weight basis. Chlorinated pesticide residues extraction from fish was similar to those of modified method of Dale et al (1965). Samples were homogenized with n-hexane and formic acid and anhydrous sodium sulphate for 3 min. The homogenized samples were subjected to acetonitrile/n-hexane partitioning to remove fat from the extract. The pesticide residues were extracted in n-hexane and passed through silcia gel column in order to remove the contamination of PCB (Polychlorinated biphenyls) (Picer et al 1978). The samples were analysed by gas-liquid chromatography using Varian Aerograph Series 2400 equipped with electron capture detector. The gas-liquid chromatography operating conditions were as follows. Carrier gas was obtained from Indian Oxygen Limited (IOLAR) AR grade I nitrogen. Temperature of injector and detector was 250$^{o}$C, while GC column temperature was 200$^{o}$C. Glass column having 2-m length and 3-mm internal diameter was used. The column was packed with gas chrome Q (80/100 mesh), coated with 1.5% OV-17+1.95% OV-210 by weight. The residues analysis were further confirmed by thin layer chromatography (TLC). Recovery of HCH residues was above 79% from the fortified samples and these results were not corrected for the percent recovery.

Mortality results were analyzed for the calculation of the LC50 and its 95% confidence limits over the

Table 1. Physico-chemical properties of test water used for fish toxicity tests. N = 5

| Characteristics | Unit | Mean | Range |
|---|---|---|---|
| Temperature | $^{o}C$ | 25 | 24-26 |
| pH | | 7.6 | 7.5-7.7 |
| Dissolved Oxygen | mg/L | 5.7 | 5.4-6.0 |
| Total hardness | mg/L as $CaCO_3$ | 240 | 230-253 |
| Total alkalinity | mg/L as $CaCO_3$ | 394 | 385-415 |
| Calcium | mg/L | 150 | 145-160 |
| Magnesium | mg/L | 92 | 84-100 |
| Chloride | mg/L | 10 | 8-13 |

different periods during the toxicity test as described by Harris (1959).

## RESULTS AND DISCUSSION

The physioco-chemical characteristics of test water used in the present study are given in Table 1. Fluctuations in physico-chemical properties during the experimental period were small. No mortality was observed in control experiments.

When fish exposed to a series of HCH concentrations, fish showed behavioral charges, such as increased erratic and jerky body and opercular movement, surfacing, difficulty in respiration and loss of equilibrium. Before death, fish frequently swim to the surface of the water to gulp air and try to jump out of test container. More secretion of mucus, and color of the body appeared light at higher HCH concentrations. Ultimately before death, fish sank to the bottom of the test contianer and died.

The result of the 14 day toxicity study (LC50 values and their 95% confidence limits) clearly indicates that the sensitivity of fish to HCH markedly increased with exposure time. Concentrations of HCH lower than 0.1

Table 2. LC50 values (mg/L of HCH) and 95% confidence limits for S. fossilis

| Exposure time (h) | LC50 values | 95% confidence limits |
|---|---|---|
| 24 | 1.504 | 1.252 - 1.810 |
| 48 | 1.115 | 0.924 - 1.324 |
| 96 | 0.858 | 0.710 - 0.992 |
| 168 | 0.760 | 0.613 - 0.316 |
| 336 | 0.608 | 0.482 - 0.677 |

mg/L did not show fish mortality during the test period. There is a marked and progressive decline of LC50 values between 24-h and 336-h (14 day). Higher fish mortality occurred in first 48-h of exposure and thereafter death rate decreased considerably. At 3.2 mg/L of HCH, the medium survival time (LT 50) was 15-h while at 1.0 mg/L it was 86-h. The results of replicates were pooled to increase the sample size, and the LC50 values were determined on pooled data.

The calculated 96-h and 336-h (14-day) LC50 values of 0.858 and 0.608 mg/L determiend for S. fossilis (Table 2). is lower than previously estimated 96-h value of 2.49 mg/L (Verma et al 1982). The difference may be attributable to many variables such as diluent water quality, age of test organism, temperature and HCH formulation. The 96-h LC50 vlaues of technical grade HCH and lindane to several species of freshwater fish were reported to be 30 to 40 ug/L (Murthy 1986). In evaluation of acute toxicity of ten organochlorine pesticides to four species of teleosts, Henderson et al (1959) observed that endrin was the most toxic and HCH was the least toxic. For ten pesticides, the 96-h LC50 was less than 100 ug/L.

To obtain an estimate of intersample variability, we measured the contents of $\alpha$-, $\beta$- and $\gamma$-HCH in group of 4-5 fish organs from a population of fish that had been exposed to HCH in water for 7 and 14 days. Bioconcentration of total HCH and its isomers with time in various tissues of exposed and control fish are shown in Table 3. The bioconcentration was expressed as sum of total HCH ($\alpha$-HCH +$\beta$-HCH and $\gamma$-HCH). Total residue of HCH ranged from 4.65 to 30.62 ug/g in gill; from 0.828 to 7.153 ug/g in brain and from 0.243 to 11.867 ug/g in liver after 7 and 14 days of exposure under static

Table 3. Mean of $\alpha$-HCH, $\beta$-HCH, $\gamma$-HCH and total HCH residues ( ug/g wet weight) in gill, liver and brain of S. fossilis after 168-h and 336-h of exposures

| HCH conc in water (mg/L) | 168-h | | | | | 336-h | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | $\alpha$-HCH | $\beta$-HCH | $\gamma$-HCH | Total HCH | BCF | $\alpha$-HCH | $\beta$-HCH | $\gamma$-HCH | Total HCH | BCF |
| | | | | | GILL | | | | | |
| 0.32 | 10.014 | 13.140 | 6.674 | 29.828* | 93.2 | 14.014 | 9.395 | 7.214 | 30.623 | 95.7 |
| 0.1 | 3.557 | 8.556 | 2.243 | 14.357 | 143.6 | 6.587 | 8.443 | 5.242 | 20.272 | 202.7 |
| 0.032 | 1.924 | 4.814 | 0.911 | 7.649 | 239 | 2.982 | 7.143 | 1.092 | 11.216 | 633.5 |
| 0.01 | 1.325 | 2.747 | 0.558 | 4.650 | 465 | 1.782 | 3.699 | 0.804 | 6.285 | 628.5 |
| Control | 0.011 | 0.006 | ND | 0.016 | | 0.026 | 0.008 | 0.065 | 0.099 | |
| | | | | | LIVER | | | | | |
| 0.32 | 3.303 | 5.614 | 2.101 | 11.018 | 34.4 | 4.416 | 5.640 | 1.811 | 11.867 | 34 |
| 0.1 | 0.311 | 2.110 | 0.303 | 2.724 | 27.2 | 0.408 | 2.814 | 0.481 | 3.703 | 37 |
| 0.032 | 0.214 | 1.582 | 0.104 | 1.900 | 59.4 | 0.313 | 1.921 | 0.158 | 2.392 | 74.8 |
| 0.01 | 0.060 | 0.161 | 0.022 | 0.243 | 24.3 | 0.143 | 0.212 | 0.118 | 0.473 | 47.3 |
| Control | 0.002 | ND | 0.009 | 0.011 | | 0.009 | 0.008 | 0.044 | 0.061 | |
| | | | | | BRAIN | | | | | |
| 0.32 | 1.921 | 2.216 | 0.902 | 5.039 | 15.7 | 2.207 | 3.801 | 0.145 | 7.153 | 22.6 |
| 0.1 | 0.249 | 0.910 | 0.040 | 1.119 | 11.2 | 0.304 | 1.940 | 0.114 | 2.358 | 23.6 |
| 0.032 | 0.113 | 0.514 | 0.201 | 0.828 | 25.9 | 0.166 | 0.937 | 0.092 | 1.195 | 37.3 |
| 0.01 | 0.090 | 0.043 | 0.008 | 0.141 | 14.1 | 0.147 | 0.056 | 0.049 | 0.252 | 25.2 |
| Control | ND | ND | ND | - | | ND | ND | ND | - | - |

*Mean of 4-5 samples.

bioassay test conditions. Bioconcentration of HCH was rapid in gill, reaching 29.828 ug/g in 7 day of exposure at 0.32 mg/L. Thus, the pattern of bioconcentration of HCH was different in fish tissues, with maximum bioconcentration occur in gill in first few days of HCH exposure. In general, the bioconcentration rates decreased considerably after 7 days of exposure. The content of the gill HCH residue averaged 30.623 ug/g and this value indicates a bioconcentration factor (BCF) of 95.7% based upon the nominal concentration of 0.32 mg/L of HCH in water. From the results it can be derived that for the dose levels from 0.01 to 0.32 mg/L, the average BCF ratio in gill is about 235.2 (93-465) at 7 days.

The results of our investigations of sublethal toxicity of HCH of air-breathing catfishes, reinforces our opinion that the S. fossilis provides the best opportunity for evaluating toxicity of chemicals to freshwater fishes of Asian continent. This species is widely distributed in freshwater reserviors of many Asian countries and is easy to handle and maintained in the laboratory with minimum death for periods of several months. Several toxicological studies have been reported on S. fossilis (Singh and Singh 1980; Verma et al 1978; Khangarot et al 1988).

Our results indicate that various tissues of S. fossilis showed marked capacity to accumulate HCH from the medium. The highest amounts of total HCH were accumulated by gill. After 14 days of exposure, gill and brain accumulated the HCH an average of 628X and 24X respectively at 0.01 mg/L. These finding are similar to those observed by other authors. Schimmel et al (1977) reported that after 28 days of exposure of HCH the finfish bioaccumulated an average of 130X in their edible tissues and 617X in offal. Veith et al (1979) observed a bioconcentration factor of 180 for fathead minnows (Pimephales promelas) from water in 32 day and Macek et al (1976) reported a concentration factor of about 7079 in tissues of the fathead minnows after 10 days of exposure to lindane ( $\gamma$-HCH).

The results of present study have shown that S. fossilis bioconcentrated the significant amount of HCH from water. Additional bioaccumulation studies of HCH from water in controlled experiments in seminatural environment and with the sampling of S. fossilis and other aquatic organisms in natural environment are needed in order to verify the laboratory results.

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