Fishery Technology
1996, Vol. 33(1) pp : 6 - 9

Uptake and Depuration of Phenol by Rohu (*Labeo rohita*) during a Chronic Sublethal Bioassay

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Studies on the body residue levels in surivors of bioassay tests can provide valuable information on bioaccumulation and organism tolerance, while residue in survivors allowed to depurate in clear water could furnish data on detoxification and elimination of pollutants. Early juveniles of rohu (*Labeo rohita*) were exposed to five sublethal concentrations of phenol (0.1, 0.5, 2.0, 5.0 and 10.0 mg l^{-1}) and a control under a static-with 48 h replacement procedure with nominal concentrations for a period of 28 days. The phenol body residue concentration increased from 8.17 to 24.85 mg kg^{-1} whereas the bioaccumulation factor decreased from 81.7 to 2.5 as the phenol concentration in water increased from 0.1 to 10.0 mg l^{-1}. The 72 h percentage depuration of the accumulated body phenol residues ranged from 58.5 to 71.2% on exposure to clear water. The study reveals that phenol as a pollutant has a low bioaccumulation potency and a high rate of depuration in fishes.

Key words : Sub-lethal bioassay, phenol bioaccumulation, depuration, *Labeo rohita*

The need for hazard evaluation of accident prone chemicals for non-aquatic use has been emphasised by Brungs & Mount (1978). The June, 1993 phenol tanker accident and the resultant spillage of phenol into the Peechi Reservoir coupled with the absence of information on water quality criteria for phenol for tropical freshwaters underscored the need for sub-lethal bioassay. Studies on residue levels in surivors provide data on bioaccumulation factor, organism tolerance and resistance while residue in survivors allowed to depurate in clear water furnish additional data on rate of depuration and detoxification. The Indian major carp, rohu (*Labeo rohita* Hamilton) was selected as a test animal because of its presence in almost all the freshwater reservoirs in India and also for its suitability to toxicity monitoring (Ashraf *et al.*, 1992)

Materials and Methods

Early juveniles of rohu (*Labeo rohita*) obtained from the carp hatchery, College of Fisheries, Cochin were acclimatized to the experimental conditions for a period of ten days prior to the start of the bioassay. They were fed *ad libitum* on a pelleted carp feed. The sublethal treatment concentrations were computed from the preliminary 24 h LC_{50} experiment, based on Konar (1969) and Sprague (1973). The 28 days sublethal bioassay was carried out with a control and five exposure levels (0.1, 0.5, 2.0, 5.0 and 10.0 mg l^{-1}) in triplicate. The static bioassay method, with replacement of the treatment medium every 48 h was followed. Twelve numbers of randomly selected rohu juveniles (26.0 - 33.0 mm TL and 226.0 - 273.0 mg, wet weight) from the main stock were kept in 12 l of well-water containing the required phenol concentration for each treatment replicate. The ratio of animal weight to treatment volume ranged from 0.22 - 0.50 g l^{-1} during the 28 days exposure period. The fish were fed once a day on a pelleted carp feed at 6% of the wet body weight. The dissolved oxygen levels and pH values of the water are presented in Table 1.

Table 1. The range of dissolved oxygen and pH values in the six different treatments during the 28 days sublethal bioassay

No.	Nominal phenol concentrations mg l^{-1}	Dissolved oxygen mg l^{-1}	pH
T1	0.00	7.2-8.0	6.83-7.75
T2	0.10	6.6-7.8	6.82-7.50
T3	0.50	5.8-7.8	6.82-7.40
T4	2.00	5.4-7.4	6.75-7.40
T5	5.00	4.2-7.2	6.70-7.20
T6	10.00	3.8-7.0	6.60-7.10

At the end of the 28 days exposure period, 3 animals from each treatment replicate (9 animals from each treatment) were sacrificed and the carcass analysed for phenol as per the amino-antipyrene method of Ochynski (1960). Of the rest, 3 animals from each treatment were released into clear well-water for depuration (72 h) and then sacrificed for the analysis of phenol residue.

The bioaccumulation factor (BAF) was taken as the ratio of the phenol body residue concentration (PBRC, mg kg^{-1}) to the phenol concentration in water (mg l^{-1}) The percentage depuration (PD) was calculated as

$$\frac{\text{28 days PBRC - 72 h depurated PBRC x 100}}{\text{28 days PBRC}}$$

Results and Discussion

Phenols in both free and conjugated forms are normally found in mammalian tissues, but few observations have been made on fish (Alabaster & Lloyd, 1982). Phenol residue found in trace amounts (4.06±0.20 mg kg^{-1}) in control fish was subtracted from the residue levels of treated fish to arrive at the body residue levels for further analysis. The phenol body residue concentrations (PBRC) at the end of the 28 days exposure period to various sublethal concentration of phenol and after 72 h depuration in clear water are presented in Fig. 1 The PBRC ranged from 8.17 - 24.85 mg kg^{-1} in the fish exposed to 0.1 to 10.0 mg l^{-1} phenol concentrations. Schulz (1961) reported that carps exposed to 10.0 ppm phenol for 5 days showed the maximum accumulation of 19 mg kg^{-1} in the liver, followed by the gills (17 mg kg^{-1}) and intestine muscles (10 mg kg^{-1}), testes (9 mg kg^{-1}) and intestine (7 mg kg^{-1}). In the present study the maximum carcass accumulation was 24.85 mg kg^{-1} of phenol in fish exposed to 10.0 mg l^{-1} for 28 days. Mortality was noticed at this concentration after 23 days exposure and the percentage survival at the end of the experiment was 72.20±23.90.

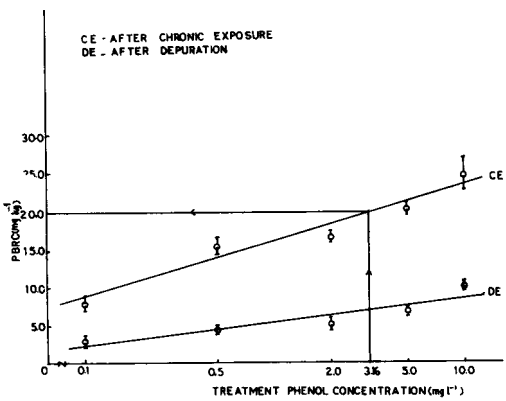

Fig. 1. Relationship between 28 days accumulation phenol body residue concentration (PBRC) and log phenol concentration (Y = 16.226 + 7.5534 log x; r = 0.9773**) and between 72 h depuration PBRC and log phenol concentration (Y = 5.5962 + 3.2031 log x; r = 0.9173**)

The relation between bioaccumulation factor and the treatment phenol concentrations are depicted in Fig. 2. The bioaccumulation factor decreased from 81.7 to 2.5 as the sublethal concentration increased from 0.1 to 10.0 mg l^{-1}. Comotto *et al.* (1979) working on a surfactant LAS (Linear alkylbenzene sulphonate) exposing fathead minnows up to 50 days found that

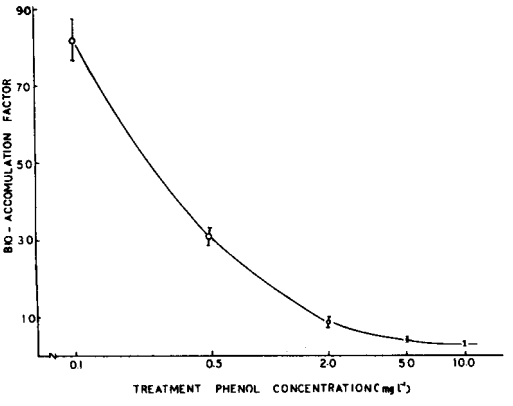

Fig. 2. Relationship between the bioaccumulation factor and log phenol concentration

the whole organism wet weight bioaccumulation factor ranged from 75 to 300. The highest value in the present study coincides with the lowest value for LAS showing the low potency of phenol to accumulate in fish when compared to LAS. About 85% depuration of LAS was noticed in all tissues of fathead minnows within 3-4 days, with nearly 100% clearance within 10 days (Comotto *et al.*, 1979). In the present study the 3 days phenol percentage depuration ranged from 58.5 - 71.2%.

The linear relationship between the $\log_{10}$ phenol test concentration in water and the PBRC after exposure and after 72 h depuration is clearly seen in Fig.1.

The bioaccumulation curve (Fig. 2) almost plateus when the phenol test concentration goes above 2 mg l^{-1}. The accumulation of a toxicant is a function of both uptake and elimination which occur simultaneously and a steady state will depend on the balance among factors determining the two processes (Mason, 1981) viz., toxicant concentration, duration of exposure, physiological condition of fish and their detoxifying mechanism. Hickman & Trump (1969) have stated that the process of phenol detoxification in the liver and gills occurs by the formation of conjugation products with glucuronides and sulphates in fish.

Simultaneous to the present study, biological and growth parameters viz., Specific Growth Rate (SGR), Food Conversion Ratio (FCR), Food Conversion Efficiency (FCE) and apparent digestibility coefficient for dry matter and protein were also analysed (Nair & Sherief, manuscript under publication) to arrive at a Maximum Allowable Toxicant Concentration (MATC) of 3.16 mg l^{-1} during the 28 d exposure period.

$$\text{MATC} = (\text{NOEC} \times \text{LOEC})^{1/2}$$

where NOEC = No observble effect concentration; and

LOEC = Least observable effect concentration

The maximum allowable body residue concentration (MABRC) was found to be 20.0 mg kg^{-1} corresponding to the maximum allowable toxicant concentration.

Because of the lower levels of dissolved oxygen in water (Table 1) at the higher concentration (5.0 and 10.0 mg l^{-1}), the uptake of phenol from the medium through the gills may be high as suggested by Jones (1964). But the bioaccumulation factors were low (4.11±0.22 and 2.49±0.21) in these test concentrations indicating enhanced detoxification and excretion. Although uptake through gills may be low in the lower concentrations (0.1 and 0.5 mg l^{-1}), the bioaccumulation factors were comparatively high (81.7±10.9 and 31.3±2.29) suggesting a low level of detoxification and excretion at these concentrations. Based on studies in rainbow trout, Alabaster and Lloyd (1982) state that a phenol test concentration of less than 1.0 mg l^{-1} is unlikely to lead to an increased phenol excretion rate.

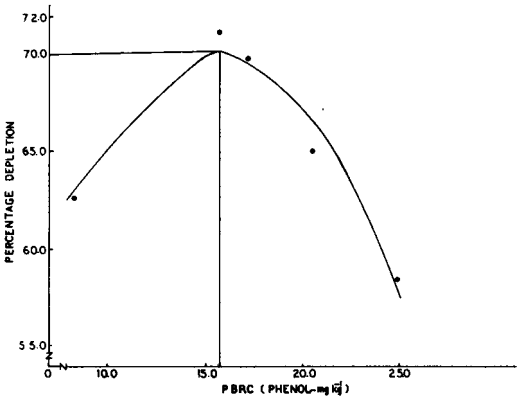

Fig. 3. Relationship between percentage depuration and accumulation phenol body residue concentration ($Y = 37.58 + 4.2174x - 0.1366x^2$)

A curve (Fig. 3) was fitted between percentage depuration and accumulation PBRC. An optimum percentage depuration of 70.2% was obtained at 15.7 mg kg^{-1} PBRC. This value was below the maximum allowable body residue concentration computed (20.0 mg kg^{-1}), which suggests that the depuration efficiency reaches a maximum before the MABRC is reached.

The study thus revealed that phenol as a pollutant had a low bioaccumulation potency and a high rate of depuration in rohu juveniles.

The authors thank Dr. M.J. Sebastian, Dean, College of Fisheries for the facilities provided and for all encouragement.

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