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Uptake and Distribution of ^{203}Hg by Fish Fingerlings, *Cirrhina mrigala*, Exposed to Linear Alkyl Benzene Sulphonate

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Ecological changes caused by the continued pollution of the aquatic environment by chemicals through industrial effluents and domestic sewage and emanations settling into water pose grave concern. Synthetic detergents are one of the most important in this respect since they find their way into aquatic ecosystems thereby affecting the food chain. Our earlier studies with diverse aquatic fauna and flora suggested the potential ecotoxicological impact of synthetic detergents (Lal et al. 1983; Misra et al. 1985, 1987a; Chawla et al. 1987). Fish are nekton in an aquatic environment and are the primary, secondary or even the top consumer in an aquatic ecosystem. A large number of reports are available on the pollutants toxic to fish (Bromage and Fuchs 1987; Mc Kim et al. 1975). Evans (1987) selected fish gill, a site of action and model for toxic effect of pollutants and these pollutants also can interfere with the development of the aquatic organisms by disrupting metabolic processes. Fish are known to accumulate mercury by virtue of efficient uptake and slow rate of elimination (Rouhtula and Miettinen 1975). Mercury also causes morphological and physiological defects with consequent behavioral abnormalities in fish (Gill and Pant 1985). Passino and Kramer (1982) studied the subcellular distribution of mercury by measuring the labelled mercury in centrifuged fractions of disrupted tissue cells. Even though in actual situation, often the stress to the ecosystem is caused by a mixture of pollutants, the interactive effect of two or more pollutants present together at present poorly understood. Also, in the presence of one toxicant, the capacity of the ecosystem to deal with others can be impaired so that even biodegradable water pollutants may tend to accumulate (Misra and Viswanathan 1987b).

Therefore, an attempt has been made to study the uptake and distribution of mercury in presence and absence of detergent to test for any combined effects.

MATERIALS AND METHODS

Fish fingerlings *(Cirrhina mrigala)* of 5.2-5.5 cm size were obtained from the State Department of Fisheries, Gomti Nagar, Lucknow

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(Uttar Pradesh), India and acclimatized for 2 wk to the laboratory conditions in aged tap water (tap water left uncovered for 3 days). Chemicals used in the study were from BDH Analar or E. Merck extra pure. ^{203}Hg was procured from Bhabha Atomic Research Centre, Isotopic Division, Bombay, India. LAS (Linear alkyl benzene sulphonate) was obtained directly from the Indian Petrochemicals Ltd., Baroda, India. The aged tap water used in the study had the following characteristics: pH 7.2-7.4, DO 6.7-7.2 mg/L, alkalinity 95-100 mg/L, hardness 116-122 mg/L as $CaCo_3$ at 25 ± 1°C as measured by the method of APHA (1975) using a Century Digital Portable Kit, Century Instruments Ltd., Chandigarh (India).

For preparation of ^{203}Hg solution, 0.02 mL ^{203}Hg with counts 7.6 x 10^5/min was tagged with 3 mg/L, 7.6 mL $HgCl_2$. LAS solution of concentration 0.005 mg/L (25% LC_{50}) was prepared by the method of Lal et al. (1983). During acclimatization, fish were provided with Shalimar synthetic diet and tubifex worms. Fish were not fed during experiments because feeding could have increased the rate of metabolism and excretory substances which may influence the toxicity of the test solutions. After acclimatization, 6 jars of 5-L capacity were taken and filled with 2.5-L of aged tap water. Out of 54 fish, 9 fish were transferred into each jar. Three jars served as control and 3 as experimentals. One mL of ^{203}Hg solution was added to each experimental and control jar. LAS solution of 0.005 mg/L was introduced in experimental jars whereas control was devoid of detergent.

The fish fingerling from each jar were taken at intervals of 12, 24, 36 and 48 hr. Various tissues were excised, weighed and kept in tubes for γ-counting on LKB Wallac Ultrogramma II 1280, Pharmacia, Sweden. In case of whole fish fingerlings, the fingerlings were taken out washed and weighed and kept in tubes as above for procedure. The student's 't' test described by Fisher (1950) was employed to calculate the statistical significance between control and experimental values.

RESULTS AND DISCUSSION

The uptake and distribution of radioactive mercury by fish fingerlings is given in Table 1. The table shows that the presence of detergent enhanced the uptake of ^{203}Hg in the order gill > liver > brain > muscles. The increase in ^{203}Hg content in gill was found to be (120-140%) $p < 0.001$, in liver (51-88%) $p < 0.001$, in brain (33-42%) $p < 0.001$ and decrease in muscle (31-15%) $p < 0.01$ to $p < 0.02$ on exposure from 12 to 48 hr. The amount of mercury taken up by the individual fish in experimental group was 70% higher in 12 hr ($p < 0.001$), 74% in 24 hr ($p < 0.001$), 78% in 36 hr ($p < 0.001$) and 86% in 48 hr ($p < 0.001$). The increase in the content of ^{203}Hg from 12 to 48 hr in gill, brain and liver suggested its accumulation with the time of exposure whereas decrease in ^{203}Hg in case of muscles from 12 to 48 hr suggested its slow elimination.

Fish like other aquatic organisms can accumulate certain pollutants from, and release them to their environment thus playing an important

Table 1. Uptake and distribution of ^{203}Hg by fish fingerlings (*Cirrhina mrigala*) in presence and absence of linear alkyl benzene sulphonate

Organs	Counts/g tissue			
	12 hr	24 hr	36 hr	48 hr
Gill	5454±53.10 (2083±26.40) p< 0.001	4875±51.28 (2000±30.23) p< 0.001	5604±66.60 (2312±33.91) p< 0.001	6014±71.83 (2500±31.79) p< 0.001
Liver	4116±49.30 (2722±38.70) p < 0.001	6025±57.38 (3200±40.32) p < 0.001	6117±62.34 (3300±42.89) p< 0.001	6527±72.79 (3465±41.80) p < 0.001
Brain	2265±43.13 (1699±25.43) p < 0.001	2100±22.19 (1466±29.92) p<0.001	2243±13.26 (1600±31.12) p < 0.001	2645±22.50 (1862±23.36) p < 0.001
Muscles	1052±35.80 (800±44.70) p < 0.01	1149±25.80 (906±46.41) p < 0.001	1225±41.80 (998±39.56) p < 0.01	1145±38.90 (995±38.35) p < 0.02
Remaining tissue	339±12.45 (270±8.96) p < 0.001	389±11.79 (310±19.37) p < 0.01	350±12.72 (272±13.40) p < 0.01	372±15.12 (292±11.91) p < 0.01
Whole fish	14200±325.60 (8327±157.00) p < 0.001	14979±422.58 (8563±175.30) p < 0.001	15700±485.39 (8820±187.50) p < 0.001	17012±505.27 (9125±180.30) p < 0.001

Data are expressed ± S.D. of six control and six experimental fish fingerlings. Control values (absence of LAS) are given in brackets. Radioactivity is measured in counts/g tissue.
p < 0.001, Highly significant; p < 0.01, More significant; p < 0.02, Significant.

role in the environmental fate of the substance. The path through which the pollutants enter fish body may be directly through uptake of water, or may be indirectly through the food chain. Surfactants decrease the surface tension and alter the permeability of biological membranes to water and ions and could therefore modify the uptake of heavy metals in fish. Liver is the organ involved in the biotransformation of mercury (Olson et al. 1978) but the site of biotransformation is unknown. Baatrup et al. (1986) found mercury within the lysosomes of the hepatocytes in the fish. The excretion of mercury is much slower in fish than in mammals but the excretory route of the mercury compounds has not been thoroughly elucidated. It is well known that mercury has high affinity for -SH groups and practically all mercury in tissues is bound to proteins (Routhula and Mietinen 1975). Most of the mercury is absorbed in the form of Hg^{2+}, but it is likely that some may be converted by the organisms or mucosal reactions and absorbed by fish as organic mercury. The ecotoxicological properties of mercury depends principally on its capacity to complex with free radicals or with macromolecules (Boudou et al. 1980).

The enhanced uptake of ^{203}Hg in presence of detergent by different tissues in fish as evident from Table 1 depends upon the concentration of mercury in water, time of exposure, rate of elimination, valence state of mercury and solubility. An additive toxicity was noticed in rainbow trout for mixture of anionic detergents ABS and LAS and copper and mercury and it was also found that the divalent ions Ca^{2+} and Mg^{2+} promoted the uptake and tissue retention of anionic detergent in rainbow trout (Calamari and Marchetti 1973). Earlier studies from this laboratory showed that under scanning electron microscopy, gills were found to be covered with mucus in case of fish exposed to detergent (Misra et al. 1985). An increase in the fluidity of mucus secreted by the epidermis of the fish after contamination with $HgCl_2$ showed that mercuric chloride has higher capacity for accumulation in the bronchial mucus (Lock and Vanoverbreeke 1981). As detergent and mercury both enhance the secretion of mucus, the higher concentrations of Hg in the gill in the experimental cases may be due to its capacity to complex with glycoprotein of the mucus or due to change of redox potential at the surface of the gill during the process of osmoregulation.

The concentration of Hg in liver, brain and muscles may be due to its interference with the enzymatic functions and chemical speciation of mercury leading to some favourable conformational changes. For mixture of metals (Cu and Hg) and surfactants (LAS), Calamari and Marchetti (1973) suggested that anionic surfactants bind the divalent heavy metal ion in a non-polar complex containing two surfactant molecules and one metal ion. The non-polar complex would be expected to be more freely available to fish than the components individually. Thus, the sunergistic effect by detergent and mercury in the present suty may be indicative of some specific additional responses.

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