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Induction of Micronuclei in Peripheral Erythrocytes of *Misgurnus anguillicaudatus* by Polychlorinated Biphenyls

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Polychlorinated biphenyls (PCBs) are ubiquitous environmental contaminants (WHO 1993). They accumulate in the fatty tissues of humans and other animals and have caused toxic effects in both (Safe 1987). Surface water may become contaminated with PCBs from atmospheric fall-out or from direct point sources. Fish have been shown to take up PCBs readily from water, while their bioconcentration factors are rather high. There have been many suggestions in the literature that PCBs might affect populations of fish in the wild. Studies attempting to demonstrate such an effect are few.

In the present study, the incidence of micronuclei (MN) in the peripheral erythrocytes of *Misgurnus anguillicaudatus* treated with contaminated water by commercial polychlorinated biphenyls (PCBs) was analyzed. PCB_3, a commercial PCB product made in China, was used for the experiments. China produced about 10000 ton PCBs, the trade names are PCB_3 and PCB_5. The composition of PCB_3 and PCB_s are similar to that of Aroclor 1242 and Aroclor 1254, respectively. The aim of the experiment is to use a model test system for rapid assessment of the genotoxicity of PCBs in aquatic environment.

MATERIALS AND METHODS

The *Misgurnus anguillicaudatus* was chosen for this study because it is very hardy and available all the year round. The fish were purchased from fishing ground and about 12 mon old. They were kept in water in laboratory aquaria for 1 wk for acclimatization before use. The strong and active individuals with a body weight of 10-20 g were selected and allocated at random to various groups.

Cyclophosphamidum *pro* injection (Shanghai Twelfth Pharmaceutical Factory,

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China) and polychlorinated biphenyls PCB_3 (Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences) were used in the tests. PCB_3 was dissolved in acetone and then added to water. The final concentration of acetone in water for each test set was 0.1 mL/L

As a first step, we have investigated the suitability of *Misgurnus anguillicaudatus* for the micronucleus test (MNT). A batch of four fish were injected i.p. once with doses (200 mg/kg) of cyclophosphamid dissolved in sterile, distilled water and another four fish were used as control group. After 2 d the fish were examined.

In the second set, 12 fish were released into different aquaria containing PCB_3 of different concentrations (2 mg/L, 1 mg/L, 0.5 mg/L) for 7 d. For each concentration level four fish were sampled.

In the third set, nine fish were released into aquarium containing PCB_3 of 1 mg/L concentration. Three fish were removed at each sampling period (2 d, 4 d, 7 d).

The experimental groups and control groups were reared under the same conditions of temperature range from 25 °C to 28 °C in 5-L aquaria filled with 3 L well-aerated tap water. The hardness of the water was 112 mg/L $CaCO_3$ and pH range 7 to 8.

At the end of the treatment period the fish were sacrificed, cut in the caudal region and smears were carried out in the usual way on heparinized microscope slides and then dried. After fixation for 3 min in methanol, the slides were stained for 15 min in 10% Giemsa, washed with water and then dried. Microscopic examination was carried out using an immersion lens (x 100)

RESULTS AND DISCUSSION

Several testing protocols have been developed for the mutagenic properties of various chemical substances that enter the environment. Among the tests for the detection *in vivo* of genotoxic pollutants in fresh water, various authors here suggested the use of fish (Hooftman 1982; Jaylet 1986; Das 1986). It is also essential to know, therefore, what effects, if any, water-borne pollutants have on the genetic material of fishes because numerous fish species provide important sources of protein and other nutrients in the diet of man and certain animals raised for human consumption.

Cyclophosphamide is a well known mutagen-clastogen. In the present study, two

days after the start of treatment, the incidence of the particles noted as MN in the cytoplasm of the blood cells in the cyclophosphamide injected fish was 1.39 ‰, which shows significant difference in comparison with control group (0.25 ‰) by ' t ' test. This consideration suggests that this species seems to be suitable for *in vivo* detection of chemicals that can cause chromosomal abnormalities.

Table 1 summarizes the incidence of MN induced in the peripheral erythrocytes of fish exposed to different concentrations of PCB_3. The result demonstrates a gradual increase in the incidence of MN with the increase of the concentration of PCB_3. Examination of the data in Table 2, a set of time-response analysis, shows the increase in the incidence of MN also with an increase in the exposure period.

Table 1. Incidence of MN in peripheral erythrocytes of *Misgurnus anguillicaucaudatus* exposed to water of different PCB-concentrations

Groups	Number of animals	Number of cells scored	Micro-nucleus	Frequency of micronucleus ‰
control	4	8155	2	0.25
0.5 mg/L	4	8141	10	1.23[a]
1.0 mg/L	4	8131	13	1.60[a]
2.0 mg/L	4	8083	13	1.61[b]

[a,b] significantly higher than the respective control value ('t' test) [a]P<0.05, [b]P<0.01

Table 2. Incidence of MN in peripheral erythrocytes of *Misgurnus anguillicaudatus* exposed for different periods

Groups days	Number of animals	Number of cells scored	Micro-nucleus	Frequency of micronucleus ‰
control	4	8155	2	0.25
2 days	3	8114	7	0.86[c]
4 days	3	8116	10	1.23[c]
7 days	3	8131	13	1.60[c]

[c] significantly higher than the respective control value ('t' test), [c]P<0.05

PCBs have been shown to interact with proteins, RNA and DNA after metabolic activation (Robbiano 1981; Mendoza 1985). The potential of readily metabolizable PCB congeners to cause primary DNA damage has been indicated (Stadnicki 1979). Dose-related chromosome breakage was found in human lymphocytes exposed to the planar PCB congener (Sargent 1989). Many

mutagenicity tests have been carried out over the years with different commercial PCB mixtures. Our results show that water polluted by PCBs have a possible mutagenicity for aquatic organisms. These results also support the suitability of assessing peripheral blood of fish for the induction of MN as a short-term test for monitoring environmental genotoxicants.

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