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SHORT REPORTS

THE EFFECT OF TRIPHENYLTIN HYDROXIDE TO CHROMOSOMES OF CATFISH (HYBRID OF *CLARIAS MACROCEPHALUS* AND *C. GARIEPINUS*)

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ABSTRACT

The effects of triphenyltin hydroxide (TPTH) pesticide on chromosomes of the F_1 hybrid catfish (Clarias macrocephalus x C. gariepinus) *were studied by using a recycling water exposure system. The fishes were divided into 4 groups, i.e., control, control solvent (dimethylsulfoxide, DMSO), and 2 treatment groups which were exposed to TPTH at concentrations of 1 μg/l and 3 μg/l. The results showed that the organotin compound, TPTH, had deleterious effects on the chromosomes of catfish. Fish exposed to TPTH at a concentration of 1 μg/l had chromosome deletions (5.3%) and non-specific chromosome aberrations (18%). The percentage of non-specific chromosome aberrations was higher (23.3%) when the fish were treated with TPTH at 3 μg/l. The percentages of chromosome deletion occurred at both TPTH treated groups were not significantly different. The effects of TPTH on chromosomes of the hybrid catfish were not dose dependent.*

INTRODUCTION

Triphenyltin hydroxide (TPTH) is an organotin compound having biocidal properties. Hence it has been widely used as a pesticide to control plant diseases such as rice blast, root rot of rice, potato leaf blight, etc. At the present time in Thailand this compound is not widely used in agriculture due to its high price, compared with other compounds which have similar properties. Malaysia, however, has just set up an industry for synthesizing organotin compounds. Thus, the prices of the compounds may soon decrease in Thailand, resulting in wider use in the future, and the compounds may contaminate the aquatic environment, either directly or indirectly. TPTH is toxic to fish, molluscs, algae and cladocerans (Duncan, 1980). It has also been reported that adverse effects caused by the organotin pesticides are more pronounced in the larvae and the younger stages of organisms than the adult stages. As a matter of fact, many countries in Europe have set the standard of this chemical in surface freshwater at **10 ng/l** above which water pollution is indicated (Smith, 1989).

Freshwater fish are commonly harvested food both commercially and artisanally. For example, hybrid catfish, resulting from artificial insemination between males of African catfish (*Clarias gariepinus*) and females of Thai catfish (*Clarias macrocephalus*), possesses better

characteristics than the parental species, including a fast growth rate, better taste, and greater tolerance to adverse environmental conditions and diseases. Hence, hybrid catfish has been promoted for farming in reservoirs and also in rice fields. Farmers have been taught to make troughs along the rice fields for rearing hybrid catfish. as part of an agricultural policy to increase the income of the farmers and also provide more protein for their families.

As TPTH is one of the pesticides applied to protect against rice diseases, the compound will contaminate water in the rice paddy and may cause adverse effects on the hybrid catfish. This could also pose a problem for humans who are the top consumer of the food chain. This study was thus conducted to see whether chromosomes of the hybrid catfish could be used as an indicator of the presence of TPTH in water even at very low concentrations. This study concentrated only on the effect of TPTH on chromosomes because chromosomes can be observed quite easily with a compound microscope. The hybrid catfish have a diploid chromosome number of 54 (Visoottiviseth *et al,* 1997).

MATERIALS AND METHODS

Chemicals

Triphenyltin hydroxide (TPTH), (90 % purity) was obtained from Aldrich Chemical Co.

Dimethyl sulfoxide (DMSO) (AR grade) was obtained from E. Merck, Darmstadt, Germany. DMSO was used as solvent of TPTH.

All other chemicals were obtained from Sigma Co., U.S.A.

Treatment of the hybrid catfish

The experiment was performed at the breeding section, National Inland Fisheries Institute, Bangkhen, Bangkok. Well water was pumped into a storage tank and was stored for at least one week before use. An acute toxicity test of TPTH was performed on hybrid catfish (same size as the experimented fish) prior to the experiment to obtain the no-observed-effect concentration (NOEC). The TPTH concentrations which were used for the treatment groups must be lower than the NOEC dose, but higher than the standard TPTH dose for freshwater, *viz.* 1 mg/l and 3 mg/l, respectively. Two-hundred hybrid catfish, 2.5-3 cm in length, were reared in each 1-ton fiberglass tank, which contained about 800 litres of water per tank. There were 16 tanks divided into 4 groups, *i.e.*, control, control solvent (DMSO), and 2 TPTH-treated groups. Each group had 4 replicates. The test animals in the control solvent group were exposed to 0.0015% DMSO. The test animals in the two treatment groups were exposed to TPTH at a concentration of 1 mg/l and 3 mg/l, respectively, in addition to 0.0015% DMSO which was used as solvent of TPTH. Water from tanks within each group was recycled for 2 weeks. After that half of the water in each tank was replaced by fresh water. The water from the fish tank must be treated for TPTH removal before discharged to the natural reservoirs. Water sampling for TPTH determination was performed before and after the addition of TPTH to maintain its concentration at the desired level. The experiment was conducted for 8 months. Daily observations were made on mortality, behavior and appearance of the animals in each tank.

Three tested animals in each replicate of a group were sampled once a month during the 1^{st}-5^{th} month of the experimental period. At the 6^{th}, 7^{th}, and 8^{th} month, the number of fish collected from each replicate of a group was increased to 10. All the animals sampled within each group were combined together for chromosome studies. Tissues from the kidney were used for chromosome preparations. The technique for preparation and staining of chromosomes was modified from the technique of Kligerman and Bloom (1977).

Tissue preparation techniques

After samping, the fish were individually injected with 0.1% solution of colchicine at a dose of 0.1 ml per 20 g body weight of fish by the intraperitoneal (IP) route. The fish were then placed in well-aerated water for 6 hours. After that, kidney tissues were removed from the fish and placed in distilled water for 10 minutes. Then the tissues were transferred to Carnoy's fixative (4:1 solution of methanol: glacial acetic acid), and were ready for chromosome staining.

Chromosome staining procedure

The procedure for chromosome staining followed that of Kligerman and Bloom (1977) with some modification. The tissues were cut into very small pieces (1-2 mm), then placed into a well, after which one drop of 60% acetic acid was added. A glass rod was used to mince the tissues for 1 min. After that, the well was filled with 60% acetic acid and left for 15 min. The cells were drawn from the well with hematocrit tubes, then dropped onto clean glass slides from a height of 12-24 inches above the slide. The slides were air dried and stained with 10% Giemsa solution in phosphate buffer, pH 7, for 25 min. They were then rinsed twice with distilled water, air dried, immersed in xylene for 10 min, air- dried, and mounted with diaphane.

RESULTS

The effect of TPTH on chromosomes of catfishes was started to recognize after 4 month of TPTH exposure. Only cells with clearly revealed chromosomes were observed and recorded for percentage of chromosome abnormality. Altogether there were 1200 cells observed; 400 cells from the water control group, 200 cells from the DMSO control group, and 300 cells each from the TPTH treated groups. The results of the effect of TPTH on chromosomes of hybrid catfish are summarized in Table 1. In both the control water and control solvent (DMSO) groups, the chromosomes appeared to be normal (Figs. 1 & 2) while in the treatment groups chromosomal abnormalities were observed (Figs. 3 & 4). The predominant type of chromosomal abnormality was non-specific chromosomal aberrations which occurred in 18.0%

Table 1 The effect of TPTH on chromosomes of hybrid catfish.

Concentration of TPTH (μg/l)	No. of cell examined	Level of chromosome abnormality[1]		Total
		non-specific aberrations	deletions	
0 Control water	400	0	0	0
0 Control DMSO	200	0	0	0
1	300	54* (18.0%)	16* (5.3%)	70 (23.3%)
3	300	70* (23.3%)	14* (4.7%)	84 (28%)
Total	1200	124 (41.3%)	30 (10.0%)	154 (51.3%)

[1] measured by number and % of cell examined

* t-test ($P < 0.05$)

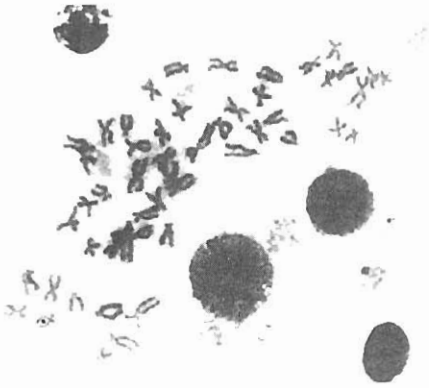

Fig.1 Metaphase plate of hybrid catfish (*Clarias macrocephalus* x *C. gariepinus*) from the control group (4 months of exposure).

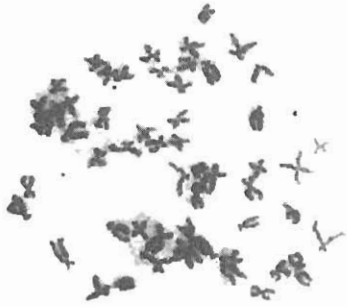

Fig.2 Metaphase plate of hybrid catfish (*Clarias macrocephalus* x *C. gariepinus*) from the control solvent (DMSO) group (4 months of exposure).

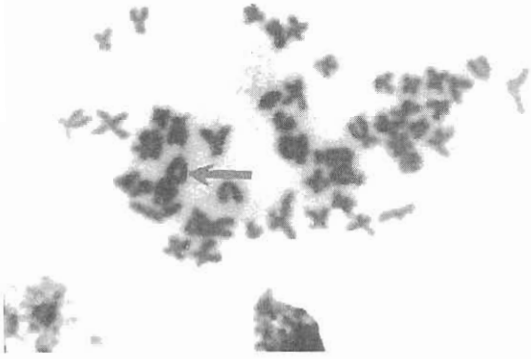

Fig.3 Metaphase plate of hybrid catfish (*Clarias macrocephalus* x *C. gariepinus*) from the 1 mg/l TPTH treated group (4 months of exposure) showing chromosome abnormalities (arrow).

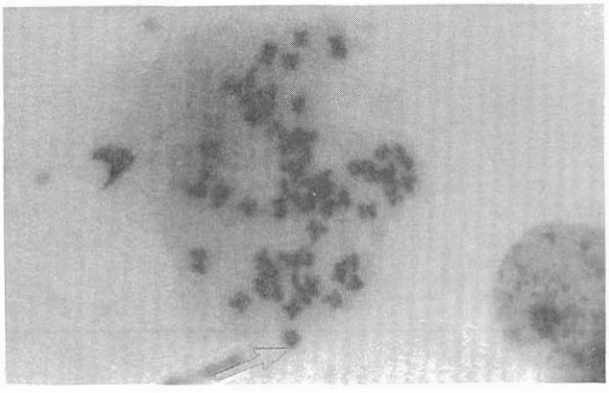

Fig.4 Metaphase plate of hybrid catfish (*Clarias macrocephalus* x *C. gariepinus*) from the 3 mg/l TPTH treated group (4 months of exposure) showing chromosome abnormalities (arrow).

and 23.3% of the total number of cells examined for the treatment groups which were exposed to 1 μg/l and 3 μg/l of TPTH, respectively. None were found in the control group. Chromosome deletions were also recognized, but at a lower percentage than the non-specific chromosome aberrations, i.e., 5.3% and 4.7% for the fish which were exposed to TPTH at concentrations of 1 μg/l and 3 μg/l, respectively. However, there was no significant difference in the percentage of chromosomal abnormalities, either the non-specific type of aberration or the chromosome deletion, between the two treatment doses ($p < 0.05$). In other words, the effect of TPTH on chromosomes of hybrid catfish was not dose dependent.

Daily observation on behavior of the experimental fishes was performed. Start from the 4th month of experiment, the TPTH treated fishes showed signed of abnormality, including reduction of food intake, and swimming style. At the end of the experiment the TPTH treated fishes were smaller sizes than those of the control fishes. Also the TPTH treated fishes swam perpendicular to the water surface. The fishes which had this behavior would eventually die.

DISCUSSION

Fish provide an excellent source of material for the study of the mutagenic and/or carcinogenic potential of water samples since they are aquatic vertebrate, which can metabolize, concentrate and store water pollutants.

There are many cytogenetic end points that can be used as an indication of exposure to genotoxic substances in aquatic organisms. Fish chromosomes can serve as an important indicator of the presence of mutagens in the aquatic environment. However, the detection of chromosome damage in fish is rather difficult because the chromosomes are small in size and large in number. The other cytogenetic end points for genotoxicity are sister chromatid exchanges (SCE) and micronucleus formation. Chromosome studies in fish have become increasingly important nowadays, not only in fish farming operations but also for detection of genotoxic pollutants on fish (Al-Sabti, 1991).

Al-Sabti (1985, 1986) reported that carcinogenic-mutagenic chemicals could induce chromosomal aberrations (CA) in the cells of common carp, *Cyprinus carpio* L., and rainbow trout, *Salmo gairdneri*. He proposed that the *in vivo* CA method in the fish system would be an excellent way to detect or investigate waterborne or internally administered pollutants (Al-Sabti, 1985; 1985; 1986). The results of this study show that TPTH is genotoxic to the catfish because it causes chromosomal abnormalities in the TPTH -treated groups. The chromosomal abnormalities found were chromosome deletions and non-specific chromosome aberrations. The percentages of non-specific chromosome aberrations were higher than the percentages of chromosome deletions. However, the percentages of chromosomal abnormalities occurring in the treatment groups were not dose-dependent, i.e., an increase in dosage of TPTH did not cause a significant increase in percentage of chromosomal abnormality ($P > 0.05$). These results are contrary to the work of Al-Sabti (1985; 1986) who studied the induction of CA in fishes by some pollutants, e.g., phenol, decamethrine, Neguvon, Malathion, Aroclor etc. Al-Sabti concluded that the percentages of induced CA by those pollutants were dose dependent. In another study with mussels by Al-Sabti and Kurelec (1985) who investigated the feasibility of using induced CA as an indicator of genotoxins under actual field conditions they concluded that the frequency of CA in the gills of mussels could serve as a relevant parameter in the assessment of genotoxic chemicals present. They even proposed that this method had advantages over the SCE (sister chromatid exchange) method because it could be applied under field conditions, in addition to the inability of SCE to detect the genotoxicity of some chemicals. Concerning TPTH, the chemical tested in this study, it can be shown that the induction of CA in the kidney cells of hybrid catfish can serve as an indication of the presence of TPTH, a pollutant, in water. Also this study suggests that TPTH is a mutagenic agent to catfish. When Tennant (1989) tested the mutagenicity of TPTH by other methods, he found that the

Salmonella mutagenesis assay was negative, the mouse lymphoma mutagenesis assay was positive and cytogenetic effects (aberrations and SCE) in Chinese hamster ovary cells were negative. Thus, from the results of Tennant's study and this study, it can be concluded that TPTH is a mutagenic agent in both mouse and catfish. This technique, the induced CA, has an advantage in that there is no need to concentrate the chemicals in water before analysis. Also, chromosomal aberrations can be detected at very low concentrations of TPTH (1 μg/l). Thus, this study suggests the finding of chromosomal aberrations in kidney cells of hybrid catfish is an indication of the presence of TPTH, a pollutant, in the water.

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บทคัดย่อ

การศึกษาผลกระทบของสารประกอบดีบุกอินทรีย์ Triphenyltin hydroxide (TPTH) ที่มีต่อโครโมโซมของปลาดุกบิ๊กอุย ซึ่งเป็นปลาดุกพันธุ์ผสมระหว่างปลาดุกรัสเซียเพศผู้ (*Clarias gariepinus*) กับปลาดุกอุยเพศเมีย (*Clarias macrocephalus*) โดยใช้ระบบ recycle water การทดลองแบ่งปลาออกเป็น 4 กลุ่ม คือ กลุ่ม control, กลุ่ม control solvent (Dimethylsulfoxide หรือ DMSO) และกลุ่มทดลองซึ่งได้รับสาร TPTH ขนาดความเข้มข้น 1 μg/l และ 3 μg/l ตามลำดับ จากการทดลองพบว่าสาร TPTH ก่อให้เกิดความผิดปกติต่อโครโมโซมของปลาดุกบิ๊กอุย ในกลุ่มทดลองที่ได้รับสาร TPTH ที่ความเข้มข้น 1 μg/l พบว่าเกิด chromosome deletion และ non-specific chromosome aberration 5.3% และ 18% ตามลำดับ ส่วนปลาในกลุ่มทดลองที่ได้รับสาร TPTH ที่ความเข้มข้นสูงขึ้นคือ 3 μg/l พบว่ามี chromosome deletion และ non-specific chromosome aberration เกิดขึ้น 4.7% และ 23.3% ตามลำดับ ซึ่งเมื่อทดสอบทางสถิติแล้วพบว่าความผิดปกติของโครโมโซมที่เกิดขึ้นในกลุ่มปลาทดลองที่ได้รับสาร TPTH ความเข้มข้น 1 μg/l และกลุ่มที่ได้รับสาร TPTH ความเข้มข้น 3 μg/l ไม่มีความแตกต่างกันอย่างมีนัยสำคัญทางสถิติ ($P < 0.05$)