RESEARCH COMMUNICATION

# Evaluation of Genotoxic Potential of Chromium (VI) in *Channa punctata* Fish in Terms of Chromosomal Aberrations

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## Abstract

**Chromium, a widely recognized carcinogenic, mutagenic and redox active metal, is released into aquatic environments by electroplating, tannery and textile industries. Elevated concentrations in sediments and interstitial waters are well documented. Fishes dwelling in chromium waste infested waters are presumed to be affected by its deposits. To evaluate the genotoxic potential of chromium [Cr(VI)] on aquatic bio-system, bottom feeding fishes, *Channa punctata*, as model fish, were exposed to [Cr(VI)]. The chromosomal aberration test (CAT) was used as biomarker of [Cr(VI)] induced toxicity. The fish were divided into three groups:Group I non-treated controls; group II positive controls, treated with an intra-muscular injection of mitomycin-C at 1 mg/kg body wt; group III exposed to a sublethal concentration (7.689 mg/l) of [Cr(VI)], dissolved in the water. For CAT estimation, short term static bioassays were conducted and samples were collected from the kidneys of fish after 24, 48, 72, 96 and 168 hrs of exposure. The remarkable chromosomal aberrations recorded in the present investigation included chromatid breaks, chromosome breaks, chromatid deletions, fragments, acentric fragments, and ring and di-centric chromosomes, along with chromatid and chromosome gaps. A significant increase in chromosomal aberrations was observed after 72 hrs of [Cr(VI)] exposure. The present study, thus reveals that even for acute exposure, [Cr(VI)] is a genotoxic agent for *C. punctata*.**

**Key Words**: *Channa punctata* - chromosomal aberrations - Cr(VI) - fish - mitomycin-C

*Asian Pacific J Cancer Prev,* **7**, 472-476

## Introduction

Chromium is virtually omnipresent in the environment. It enters the environment through natural and several anthropogenic sources (Abbasi et al., 1991a). Chromium naturally occurs in air, rocks, soil, water and biological materials (Adriano, 1980; Abbasi et al., 1988; Soni, 1990). It is more abundant in the earth crust than other hazardous metals viz., cobalt, copper, zinc, lead, nickel and cadmium; ranking 21st in abundance among all elements, with an average concentration in the order of 100 ppm. Chromium is widely used in industrial process viz., production of ferrochromium, chromates, refractory materials, chromium steels, cement, fungicides, pigments, oxidants, catalysts and fertilizers etc. and is released into aquatic environments largely by electroplating, tannery and textile industries (Steinhagen et al., 2004). The other anthropogenic sources for introduction of chromium in environment include burning of oil and coal and oil well drilling (Soni, 1990; Abbasi et al., 1991a).

In freshwater, instances of anthropogenically introduced chromium contamination are occurring with increasing frequency. In polluted freshwater chromium levels up to 371 ppm, and 9.6 ppm have been recorded in rivers and lakes respectively. This indicates multiple build-up of chromium over levels found in unpolluted freshwater. Like other metallic pollutants, chromium not only alters the quality of water by changing the physico-chemical equilibrium but also affect the normal functioning of the vital activities of the body of aquatic organisms (Thaker et al., 1996; Steinhagen et al., 2004) The discharge of chromium containing wastewater on land surface may result in greater than natural or permissible levels of chromium in the tissues of receiving organisms resulting in toxicological insults of varying degree. The toxicity of chromium to aquatic life is strongly influenced by the chemical speciation of chromium and water quality (Abbasi and Soni, 1985; Soni, 1990) and considerably varies between and within groups of organisms. Growing awareness for the aquatic pollutants generated potential hazards has stimulated much interest in the use of fishes as indicators for the monitoring of environmental mutagens, carcinogens and teratogens (Krishnaja and Rege,1982). Ample experimental evidence is now available that fishes are extremely sensitive animal models to record genotoxic insults on account of environmental contamination in the form of induction of tumors by chemical carcinogens like dimethyl nitrosamine, 2-acetylaminofluorine and aflatoxin B1, and have indicated the advantage of using fish

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bioassays for evaluating the potential of environmental carcinogens (Sato et al., 1973; Matsushima and Sugimura, 1976; Kraybill and Helmes, 1978). Mutagenic potential of heavy metals viz., Hg, Pb, Zn, Cd, Fe, Mn, Ni and Cr is well documented in aquatic animals. Their ecotoxicological manifestations, even in sub-lethal concentrations have been reported to cause undesirable alterations in genetic materials of the individuals. (Ansy Mathew and Jahageerdar, 1999; Tolga et al., 2005). Further, these mutagens may adversely affect the fertility and fecundity of living organisms.

Genotoxic studies using cytogenetic analysis in fishes have been demonstrated by number of workers (Das and Nanda, 1986; Al-Sabti, et al., 1994, Al-Sabti and Metcalfe, 1995; Rishi and Sunita, 1995; Ansy Mathew and Jahageerdar, 1999; Clarice, et al., 2001; Maples and Bain, 2004). Both, Chromosomal aberrations test (CAT) and Micronuclei tests (MNT) in fish have been reported to be useful biomarker of in vivo techniques for genotoxicity testing and show potential for in situ monitoring of water (Al-Sabti and Metcalfe, 1995; Kushwaha, et al. 2003). Fishes have also been used in several biochemical and toxicological studies (e.g. developmental, carcinogenic and teratogenic), both in vitro and in vivo (Nakatsuru, et al., 1987; De Flora, et al., 1991 and Bailey, et al., 1992). The toxicity of chromium on the haematology, biochemistry and histopathology of a variety of fishes have been well documented (Steinhagen et al., 2004; Krumschnabel and Nawaz, 2004), however, only scanty information is available regarding the chromium induced genotoxicological manifestations among fishes (De Lemos et al., 2001; Maples and Bain, 2004).

The aim of present study is to evaluate the genotoxic potential of chromium (VI) in aquatic environment using CAT in the kidney cells of C. punctata after exposure to sublethal concentration of chromium (VI).

## Materials and Methods

Channa punctata (Bloch, 1793, FishBase name - Spotted snakehead) (2n = 32), a common pond murrel (class - Actinopterygii (ray-finned fishes), Order - Perciformes (perch-likes), family - Channidae (Snakeheads)), widely occurring in both lentic and lotic habitats of India, Bangladesh, Pakistan and Sri Lanka was selected as the test species for present investigations on account of its abundance and ready availability throughout the year, economic, biological and ecological importance, suitability and susceptibility for bioassays, ease of maintenance and its sensitivity and endurance to the heavy metal Arsenic. Live and apparently healthy specimens of C. punctata (13-15 cm; 28-32 g), procured from local lentic habitats, subjected to repeated washing with tap water and various dip treatments viz., in formaline (0.4%) for 15 min., in Benzylkonium Chloride (1-4 ppm) for 1 hr. and in KMnO4 solution (1 mg/l) for 2hr., were acclimatized to laboratory conditions (pH 7.0 – 7.2, temperature 27 – 29˚C, D.O. 5.97, C.O.D. 65.1, Hardness 73.6, Alkalinity 75 and T.D.S. 228.3) in large well aerated glass aquaria of100l capacity for 15 days prior to exposure to test chemicals. Fishes were fed on minced goat liver daily. Every effort was made to provide optimal condition for fish; no mortality occurred during this period. 96 hr. $LC_{50}$ of chromium (VI) (taken as $CrO_3$) for the fish was determined by "Trimmed Spearman-Karber Method" (Hamilton et al.,1977). The fish were then divided in to three groups each containing 10 specimens. Group I (control) was maintained on normal diet of minced goat liver. A single dose, by intramuscular injection, of Mitomycin – C, dissolved in double distilled water, (@ of 1 mg/kg body wt. was administered to group II (positive control). Group III (experimental group) was exposed to sublethal concentration (7.689 mg/l; 1/10 of 96hr LC50) of chromium (VI) dissolved in test medium water. Fishes of all the groups were subjected for 24, 48, 72, 96, and 168 hrs. of exposure periods..

Water quality of all the sets of experiments was analyzed at 0 hr, and after 24, 48, 72, 96, and 168 hrs following standard methods (APHA, 1998). The notable parameters of water analyzed were hardness, alkalinity, dissolved oxygen (DO), chemical oxygen demand (COD), chloride, total dissolved solids (TDS), pH and temperature. Hardness, alkalinity and chloride were estimated by volumetric titration, EDTA method, HCl method and Silver nitrate method respectively. DO was determined by Azide-modification method. COD was estimated by dichromate reflux method. TDS was estimated by portable water analysis kit (JSGW) developed by ITRC, Lucknow. pH was measured by digital pH meter and temperature by celsius thermometer.

In the present study kidneys were used for chromosomal preparation. Being a haemopoietic organ, fish kidney is mitotically highly active and contains a sufficient number of rapidly dividing cells. The fishes of all the three groups were injected with 0.05 % colchicine, dissolved in double glass distilled water, intramuscularly (@ 1 ml/100 gm of body weight) one hour prior to dissection to arrest the metaphase stage. After one hour the kidneys were taken out by dissecting the fishes. The kidneys were cut into small pieces and homogenized in 8 ml hypotonic solution (0.56 % KCl) in glass tissue homogenizer to prepare cell suspension. The cell suspension was poured in a 15 ml centrifuge tube and incubated for about 25-35 minutes at room temperature for swelling of the cells. The hypotonic treatment was stopped by adding 1 ml of freshly prepared, chilled Carnoy's fixative (Methanol : Acetic Acid in 3 : 1 ratio). The fixative was mixed gently with Pasteur pipette. Now the cell suspension was centrifuged at 1200-1500 rpm for 10 min. The supernatant was discarded and the cell pellet was resuspended in 7 - 8 ml of chilled fixative and again centrifuged for 10 min at 1200-1500 rpm. The process of washing of the cell pellet with fixative was repeated thrice to get clear whitish pellet. The slides were prepared by the flame drying technique and stained with 4-5% Giemsa in phosphate buffer (pH 6.8). Well-spread metaphase chromosomes were selected and scoring of aberrations was done at a magnification of 100x under oil immersion microscope. Scoring was limited to only those metaphase

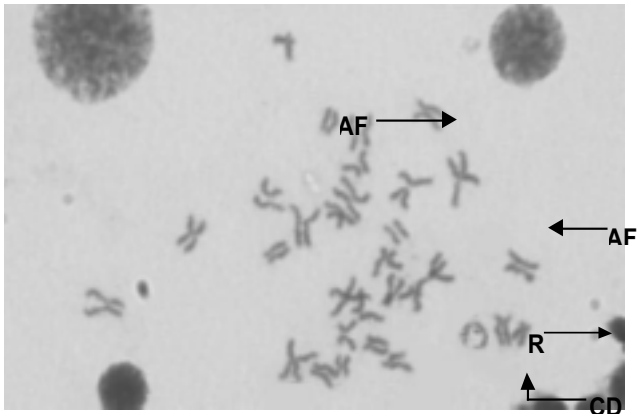


**Figure 1. Metaphase Spread Showing Acentric Fragments (AF), a Ring chromosome (R) and Chromatid Deletion (CD) induced by Mitomycin-C**

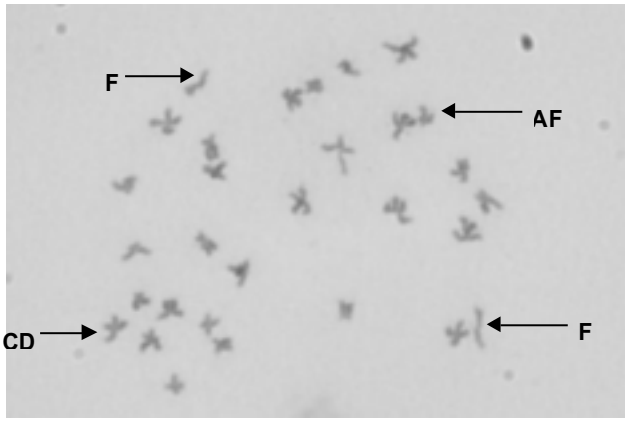


**Figure 2. Metaphase Spread Showing Chromatid Deletion (CD), an Acentric Fragment (AF) and Fragments induced by Chromium (72 hours exposure)**

plates that contained complete set of chromosomes. The common types of aberrations recorded during scoring were chromatid break, chromosome break, chromatid deletion, fragment, acentric fragment, ring and di-centric chromosomes. Chromatid and chromosome gaps were recorded separately. The mitotic index was established by estimating the number of metaphases in 2000 cells counted at a magnification of 40x. The mean values and S.E. were calculated using Sigma Stat Software.

## Results

In group III for both the setups hardness (2042 % after 24 hrs and 10.40 % after 168 hrs from group I) and alkalinity (10.06 % after 24 hrs and 21.08 % after 168 hrs from group I) were found variously decreased. DO (5.97 ± 0.0) and pH (7.2 ± 0.0) were recorded almost constant in group I and II whereas, DO was found increased (24.95 % after 24 hrs and 8.20 % after 168 hrs from group I) while pH registered negligible decrease (1.38 after 24 hrs and 5.50 after 168 hrs from group I) in group III. TDS increased sharply in group III. They were recorded 39.28 % over and above the group I. Chloride contents were recorded almost unchanged both in group I and II while, they were found decreased by 8.18 % after 24 hrs and 5.45 % after 168 hrs. Temperature (27˚C) almost remains unchanged throughout the experiment. In all the groups COD and TDS were found increased throughout the experiment. Chloride content was recorded constant in both the group I and II but a slight increase was recorded in group III. Temperature almost remained unchanged throughout the experiment.

The mitotic index evaluated as percentage of dividing cells was found to be 4.13 ± 0.010 in group I. The cytotoxicity of Mitomycin - C (group II) and chromium (group III) was evident as the mitotic index was found to be decreased in comparison to group I (Table 1). Treatment in vivo with mitomycin - C at a single dose at 1 mg/kg body weight resulted in a significant increase in the frequency of chromosomal aberrations (see Figures 1 and 2) per metaphase compared with the control. The highest number of chromosomal aberrations was recorded after 72 hrs of exposure period and then it was found slightly decreased after 96 and 168 hrs of exposure periods. Similar trend of aberrations were recorded in fish exposed to sublethal concentration of chromium but the frequency of aberrations was less in comparison to positive control. Maximum frequency of chromatid breaks was recorded after 72 hrs of exposure in both group II and III. In the present study it was also found that as the exposure time increased, the percentage of metaphase spreads with chromosomal abnormalities and percent incidence of aberrant cells also increased up to 72 hr period and then decreased in both groups II and III.

**Table 1. Frequencies of Chromosomal Aberrations Induced by Chromium and Mitomycin-C in Kidney Cells of *Channa punctatus***

| Exposure period | Mitotic index | Total no. metaphases | Metaphases with abnormalities | A | B | C | D | E | F | G | H | I | Total number | % of metaphases | % aberrant cells |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Control | 4.13 ± 0.01 | 136 | 11 | 2 | 1 | 1 | 3 | 4 | 3 | 0 | 0 | 0 | 11 | 8.1 | 1.3 |
| Mit-C 24 | 3.65 ± 0.01 | 159 | 70 | 15 | 14 | 28 | 8 | 12 | 9 | 5 | 3 | 0 | 65** | 40.9 | 9.0 |
| Mit-C 168 | 2.97 ± 0.01 | 160 | 75 | 27 | 15 | 22 | 10 | 28 | 20 | 5 | 5 | 1 | 93* | 57.8 | 13.2 |
| Ch 24 | 1.63 ± 0.14 | 144 | 45 | 10 | 5 | 7 | 2 | 12 | 7 | 3 | 1 | 1 | 33** | 22.9 | 4.1 |
| Ch 168 | 2.44 ± 0.01 | 151 | 57 | 23 | 12 | 16 | 3 | 25 | 18 | 2 | 1 | 1 | 66** | 43.7 | 9.6 |

A, Chromatid gap; B, Chromosome gap; C, Chromatid break; D, Chromosome break; E, Chromatid deletion; F, Fragment; G, Acentric fragment; H, Ring; and I, Di-centric chromosomes *P < 0.05, **P< 0.01 compared to the controls

## Discussion

Although chromium is a trace element for biological systems, necessary for normal glucose tolerance in mammals (Schwarz and Mertz, 1959), suppressing serum cholesterol level and having a tendency to decrease cholesterol level with age (Schroeder, 1968), but above than permissible limit it causes abnormal body physiology at cellular and molecular levels. In the present study we found that when the fishes were exposed to sublethal concentration of chromium (VI), the total number of metaphase spreads with chromosomal aberrations, total number of aberrations, percentage of aberrations per metaphase and percent incidence of aberrant cells were significantly higher than the group I but lower than the group II.

Mathew and Jahageerdar (1999) have shown that when the fish *C. punctata*, exposed to lead at a very low concentration of 0.012mg/l and for just 96 hours of exposure induced chromosomal aberrations in fish. In other study De Lemos et al (2001) showed significant induction of micronucleated erythrocytes in fishes exposed to chromium (VI) for 7, 14 and 21 days and the induction was found to be decreased after 21 days of exposure. Studies on chemical speciation of chromium reveals that chromium (III) is less toxic, while chromium (VI) is extremely toxic as it is more mobile than trivalent form. Chromium (VI) is a strong oxidizing agent, diffuses readily in the tissues and can easily penetrate cell membranes (Piscator, 1986; De Flora and Wetterhahn, 1989). The toxic action results from its strong oxidative effect on membrane phospholipid proteins and nucleic acids (Chorvatovicova, et al., 1992). However, genotoxic activity occurs through intracellular reduction of chromium (VI) to chromium (III), the most stable form of chromium ion and reactive intermediates such as chromium (IV) and chromium (V). During reduction process, oxygen generated as highly reactive free radical species that can react with DNA (Tsalev and Zaprianov, 1983; Mirsalis, et al., 1996). The chromium associated with DNA is usually found in the trivalent form (Hughes, 1995), may form single strand breaks and DNA cross-links (Manning, 1994).

In the present study, chromosomal aberrations increased initially and then decreased gradually. This is probably due to the fact that, in the initial stage chromium may damage the cell and the genetic material may induce more chromosomal aberrations but later on with increase in exposure time animals developed a mechanism by which they accumulate chromium in their body and the cells in which the chromosomal aberrations formed, fail to divide and multiply. The results are quite suggestive that chromium is a potent genotoxic agent and it brings about chromosomal aberrations in exposed fish.

Fish is a rich source of protein (15 – 25%) and also healthy fats, vitamins and minerals. Therefore, it is important that the release of effluent from electroplating, tanneries and textile industries should be minimized and fishes should be reared in chromium free water.

## Acknowledgments

The first author is thankful to CSIR, New Delhi for providing JRF. The authors are also grateful to Dr. Nirupama Agrawal, Prof. and Head, Department of Zoology, University of Lucknow, Lucknow for providing necessary facilities and Dr. N.S. Nagpure, NBFGR, Lucknow for providing training programme on genotoxic assays. Thanks are also due to Dr. Y. Shukla, ITRC, Lucknow for his valuable suggestions.

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