Published in final edited form as:
Rev Environ Health. 2009 ; 24(2): 129–145.

Ecotoxicology of Hexavalent Chromium in Freshwater Fish: A Critical Review

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Abstract

Chromium (Cr) is a naturally occurring element found in rocks, animals, plants, and soil, predominantly in its insoluble trivalent form [Cr(III)]. Intense industrialization and other anthropogenic activities have led to the global occurrence of soluble Cr(VI), which is readily leached from soil to groundwater or surface water, in concentrations above permissible levels. The ecotoxicology of Cr(VI) is linked to its environmental persistence and the ability to induce a variety of adverse effects in biologic systems, including fish. In aquatic ecosystems, Cr(VI) exposure poses a significant threat to aquatic life. This paper reviews the fate and transport of Cr(VI) in the environment and its acute and chronic effects on fish. We also discuss Cr(VI) toxicity at the cellular, biochemical, and genetic levels. An attempt is made in this review to comprehend the staggered data on the toxic effects of Cr(VI) to various species of fish. Such data are extremely useful to the scientific community and public officials involved in health risk assessment and management of environmental contaminants as a guide to the best course of action to restore ecosystems and, in turn, to preserve human health.

Keywords

Cr(VI); acute and chronic effects; aquatic pollutants; risk assessment

INTRODUCTION

Pollution by heavy metals has become a serious environmental and public health hazard because the concentrations released into the environment from industrial processes often exceed permissible levels. Due to their bioaccumulative and non-biodegradable properties, heavy metals constitute a core group of aquatic pollutants /[1]/. Their high toxicity even in low concentrations can produce cumulative deleterious effects in a wide variety of fish and other aquatic organisms.

Chromium, one of the most common ubiquitous pollutants in the environment, does not occur naturally in the pure metallic form. The element is present in divalent [Cr(II)], trivalent [Cr(III)], and hexavalent [Cr(VI)] oxidation states, with Cr(VI) and Cr(III) being

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the most stable forms. Chromium enters into various environmental matrices (air, water, soil) from a wide variety of natural and anthropogenic sources in the Cr(III) or Cr(VI) form. The health hazards associated with exposure to Cr are dependent on its oxidation state, ranging from the low toxicity of the metal form to the high toxicity of the hexavalent form. Trivalent Cr plays an important role in glucose metabolism by serving as a cofactor for insulin action /[2-3]/.

Hexavalent chromium is a toxic industrial pollutant and classified carcinogen possessing mutagenic and teratogenic properties. All Cr(VI)-containing compounds were once thought to be man-made, with only Cr(III) naturally ubiquitous in air, water, soil, and biological materials. Recently, however, naturally occurring Cr(VI) has been found in ground and surface waters at values exceeding the World Health Organization limit for drinking water of 50 μg of Cr(VI) per liter //.

Generally, the aquatic environment is the ultimate sink for metal pollutants. Several industrial applications like leather tanning, electroplating, and corrosion protection contaminate ground water /[4]/, whereas surface waters are polluted by discharges from manufacturing processes and cooling towers /[5]/. On the other hand, the combustion of fossil fuels /[6]/ and manufacturing processes of iron and steel industries release Cr into the atmosphere /[7]/ in particulate form. Most of the Cr in air will eventually settle and end up in waters or soils. When released to land, Cr compounds bind to soil and are not likely to migrate to ground water. In water, however, these compounds are very persistent as sediments, with a high potential for accumulation of Cr in aquatic life. In its dissolved form, Cr is present as either the anionic trivalent $Cr(OH)_3$ or hexavalent CrO_4^{2-}.

Interest in the field of ecotoxicology—fundamental research on the impact of toxic chemicals on terrestrial, freshwater, and marine ecosystems at the population or community level—has undergone a particularly strong surge in aquatic systems. Several recently published reports have linked the occurrence of anomalies in various fish at physiological, histological, biochemical, enzymatic, and genetic levels to the toxic effects of Cr(VI). In many Cr(VI)-exposed fish, certain species have demonstrated extra sensitivity whereas others are tolerant. More in-depth studies in toxicodynamics and toxicokinetics are needed, however, to establish an exact cause-effect relation. The scientific data discussed in this review provide a basis for understanding the potential impact and for advancing our knowledge of the eco-toxicological effects and risk assessment of Cr.

FATE OF CHROMIUM IN THE ENVIRONMENT

Once in the environment, several physico-chemical reactions reduce Cr(VI) to the relatively less toxic trivalent form, catalyzed by different reducing agents like sulfide compounds /[8]/. In solution, Cr^{+6} ions can exist as monomers or dimers. The following equation shows the polymerization reaction of monomers to form dimers /[9]/.

$$HCrO_4^- + HCrO_4^- \iff Cr_2O_7^{2-} + O$$

The pH of water has a profound influence on the reduction of chromium in the environment. Dimers predominate at pH 6.5, whereas monomers are more abundant at extremely low pH values (0.1). A significant occurrence of dimers is observed in Cr^{+6} solutions of greater than 30 mM, whereas monomers are dominant in concentrations below 30 mM. In contrast, Cr(III) can be hydrolyzed to form hydroxides > pH 3.5, whereas no change occurs to this form in solutions below this pH value /[10]/. In addition, common electron donors like aqueous ferrous iron (Fe^{+2}) and reduced sulfur reduce Cr(IV) to Cr(III) /[11,12]/. In the environment, silicates, oxides, and sulfides and minerals that are rich in ferrous iron reduce

Cr(IV) very rapidly. Additionally, Cr(IV) could be reduced by organic matter in the soil, such as humic acid and fulvic acids, but pH plays a vital role on the rate of reduction by these acids /[13,14]/. The reduction reaction with organic matter is shown by the following equation:

$$2Cr_2O_7^{2-} + 3C^\circ + 16H^+ \rightarrow 4Cr^{3+} + 3CO_2 + 8H_2O$$

Microbial processes also transform Cr(IV) to the insoluble trivalent form /[15]/, which adsorbs o solid surfaces /[16]/. Noteworthy is that most microbial reduction reactions of Cr(IV) occur anaerobically as a part of a detoxification process or by extra-cellular reactions /[17]/. Under certain conditions, Cr(IV) can be reduced to Cr(III) that precipitates as a fairly insoluble hydroxide, thereby immobilizing it within the soil /[18]/.

CHROMIUM TOXICITY TO FISH

The aquatic toxicology of Cr depends on both biotic and abiotic factors. The biotic factors include the type of species, age and developmental stage. The temperature, concentration of Cr, oxidation state of Cr, pH, alkalinity, salinity, and hardness of water constitute the abiotic factors. Moreover, lethal and sub-lethal concentrations of the metal and its speciation also determine the sensitivity of the individual organism. This section reviews the acute and chronic toxic effects of Cr(VI) to various fish species.

Acute Effects

Knowledge of the acute toxicity of a xenobiotic helps in predicting and preventing acute damage to aquatic life in receiving waters. In addition, this information is useful to regulate toxic waste discharges /[19]/. Moreover, acute toxicity studies can rapidly and inexpensively provide environmentally relevant and useful data. Short-term studies on the acute effects of Cr revealed that the metal exerts its toxicity at various functional levels across fish species. Further, individual variations exist across species in terms of susceptibility. For instance, recent studies designed to test the sensitivity of five fish—rainbow trout, three-spined stickleback, roach, perch, and dace—exposed to acute concentrations of Cr(VI) revealed that rainbow trout is 1.16 to 2.52 times more sensitive than the other test species to the metal /[20]/.

Earlier data also indicate that both lethal and sublethal concentrations of the metal determine the sensitivity of individual organisms across species. A review of the acute toxicity of Cr to fish reveals that the differences in the 96h-LC_{50} values between fish species can be attributed to the complicated metal-induced changes in the physiology and survival of aquatic organisms under metallic stress. Such changes differ from metal to metal, from species to species, and from one experimental condition to another. The exact causes of death due to heavy metal poisoning are multiple and depend on time-concentration combinations. Tables 1-2 summarize the acute effects of Cr to freshwater fish.

Effect of pH—The degree of toxicity depends on the pH of the water in which the fish resides. Several studies cited in this section underscore the point that the pH of the water has a tremendous influence in determining the bioavailability of the metal to the fish and its associated toxic effects. For example, the toxicity of Cr(VI) to the teleost fish, *Nuria denricus*, was found to be influenced by changes in pH value /[21]/, which is in accordance with another laboratory study on the rainbow trout demonstrating variable susceptibility to Cr at pH values of 7.8 and 6.5 /[22]/. The results of the latter investigation indicated that at pH 7.8, a considerable amount of Cr accumulates in the internal organs rather than in the gills, whereas the gills retain a greater amount of Cr at pH 6.5 than do other organs. Although, further investigations by the same authors revealed histologic changes at both pH values, the

predominant changes occurred in the gills at pH 6.5 /[23]/. In another study in young rainbow trout, Cr toxicity was 50-200 times higher at pH 6.4 to 7.4 than at pH 7.8 to 8.0 /[24]/. In addition, comparative studies also clearly indicate that Cr levels in gills are higher than in other organs /[25]/.

Cytotoxicity—When bioconcentrating in the food chain, heavy metals, including Cr, are potentially cytotoxic to aquatic biota. Therefore, an early detection and ecotoxicologic evaluation of a sensitive biomonitoring system comprising both in vivo and in vitro test systems is essential. Recent research suggests, however, that in vitro systems using cell cultures derived from aquatic animal species are preferred over in vivo tests because of the inherent economic and ethical constraints associated with using live organisms. In vitro culturing of established fish cell lines is relatively rapid, cost-effective, readily reproducible, and can be easily adapted to automated high-throughput screening technologies /[26-28]/. In addition, in vitro cytotoxicity assessments readily examine multiple parameters, including measurements of cell death, viability, morphology, metabolism, cell attachment/detachment, cell membrane permeability, proliferation, and growth kinetics /[29-31]/.

The toxic effects of Cr and other heavy metals at the cellular level are being investigated by various researchers across the globe and thus form one of the major thrust areas of current toxicologic research. Recent studies have demonstrated the cytotoxic and genotoxic effects of sodium chromate, a soluble form of Cr(VI) in a medaka (Oryzias latipes) cell line /[32]/. In this investigation, the authors used a clonogenic cytotoxicity assay to measure sodium chromate (Na_2CrO_4) cytotoxicity, gamma-H2A.X immunofluoresence to measure DNA double-strand breaks, and chromosome damage to measure clastogenicity. According to this study, Na_2CrO_4 is highly cytotoxic to medaka fin cells in a dose-dependent manner. DNA double-strand breaks visualized by the formation of gamma-H2A.X foci could be detected even at very low concentrations (1 μM) of Cr. Further, Na_2CrO_4 induced chromosomal aberrations, causing chromatid lesions and exchanges that increased with concentration.

Tan et al. /[33]/ evaluated the sensitivity of six fish cell lines to four heavy metals. The cell lines GCF (grass carp fins), CIK (Ctenopharyngodon idellus kidney), EPC (epithelioma papulosum, cyprini), CCO (channel catfish ovary), BB (brown bullhead caudal trunk), and FHM (fathead minnow muscle) were compared for their cytotoxic sensitivity to cadmium (Cd), chromium (Cr), zinc (Zn), and copper (Cu). The cells were differentiated by morphology, viability and proliferation after 24 h exposure to metal salts at selected concentrations. The test results indicated that all six fish cell lines showed sensitivity to all metals tested. Although variations in these effects were noticeable, the inhibitory concentration (IC_{50}) values indicate that both Cr and Cd exert a more pronounced cytotoxic effect than the other two metal salts. Of the six fish cell lines studied, the EPC cells demonstrated more sensitivity to Cr and Zn. This study concluded that CIK, EPC, and CCO cell lines could serve as valuable bio-indicators for monitoring and assessing the acute toxicity of metals at the cellular level in an aquatic environment /[33]/.

Prabakaran et al. /[34]/ studied the immune response and non-specific immunity in the tilapia (*Oreochromis mossambicus*) exposed to sublethal concentrations of tannery effluent containing Cr (88.2 mg L^{-1}) and significant amounts of calcium carbonate, and sodium sulphate. Both ELISA and bacterial agglutination assays were used to assess the specific immune response of fish to heat-killed *Aeromonas hydrophila.* Nonspecific immune mechanisms were evaluated in terms of serum lysozyme activity, the production of intracellular reactive oxygen species (ROS), and reactive nitrogen intermediates (RNI) by peripheral blood leucocytes (PBL). The chronic exposure of fish to 0.53% of TE significantly suppressed the antibody response, nonspecific serum lysozyme activity, and ROS and RNI production. Similar responses were observed in fish exposed to a low

concentration of 0.053% (1% LC_{50}) of TE, although to a lesser extent. The authors conclude that such findings could play an important role in monitoring fish health and risk assessment during periods of fluctuating levels of pollutants in both natural and farm environments.

In natural waters and/or aquaculture facilities, fish are often exposed to Cr waste and demonstrate cumulative deleterious effects as a function of time. Steinhagen et al. /[35]/ examined the effect of Cr(VI) on carp (*Cyprinus carpio*)-derived immune cells. The results demonstrated that at concentrations between 2 and 200 μmol Cr L^{-1}, the metal induced cytotoxicity and decreased the activation of mitogen-induced lymphocytes, as well as phagocyte functions. Neutrophils showed changes in cell shape together with reduced nitric oxide and reactive oxygen production at concentrations much lower than for the cytotoxic effects. The altered lymphocyte and neutrophil functions reflect the decreased resistance to pathogens observed in fishes under chronic Cr challenge.

The acute exposure of gold fish hepatocytes to 250 μM Cr(VI) significantly reduced cell viability and stimulated ROS production, but did not alter cellular calcium ion (Ca^{2+}) homeostasis /[36]/. In this study, the sources of ROS were identified as the lysosomal Fe^{2+} pool and the mitochondria.

In a study on the effects of several heavy metals and cyanide on fertilization in rainbow trout, the sperm of Cr-exposed fish showed high sensitivity even at a the minimum concentration of 5 μg L^{-1}, whereas the ova were only slightly sensitive, suggestive of a differential toxicity of the metal to gametes /[37]/.

Biochemical toxicity—Biochemical studies across various species have revealed that Cr induces cumulative deleterious effects at both biochemical and enzymatic levels. Bozcaarmutlu and Arinç /[38]/ studied the in vitro effect of mercury (Hg^{+2}), Cd^{+2}, nickel (Ni^{+2}), Cr^{+3}, and Zn^{+2} ions on the kinetic properties of NADPH-cytochrome P450 (CYP450) reductase purified from leaping mullet (*Liza saliens*). The results indicate that Cr, independent of its oxidation state, is a strong inhibitor of CYP450 reductase activity in fish /[38]/.

In another study in the Indian major carp, Cr had no significant effect on the activities of alanine amino transferase (ALT) or aspartate amino transferase (AAT, *Labeo rohita* /[1]/. This experiment was designed to investigate the hepatotoxicity of arsenic (As) and Cr(VI) to *L. rohita*, at LC_{50} concentrations for 24 and 96 hours exposure. Although elevated levels of ALT and AAT were observed at LC_{50} (61 mg L^{-1}) of Cr(VI) for 24 h and 96 h, these activities were not significant when compared with the controls. On the other hand, the significant increase in ALT activity ($p < 0.01$) in As- exposed fish mirrors serious hepatic damage and a distress condition to the fish.

Chromium can alter the glucose transport rate in epithelial cells of the intestine. One of the earliest studies on glucose uptake by epithelial cells in the intestine of rainbow trout showed a diminished rate of glucose absorption /[39]/. Subsequent studies /[40]/ on the impact of Cr on glucose intake in *Channa punctatus* at different concentrations (10 mM, 1 mM, 0.1 mM, 0.01 mM, 0.001 mM) showed increased absorption of glucose at all Cr concentrations examined, with the highest absorption rate occurring at 0.001 mM Cr. In contrast, *Colisa fasciatus* exposed to a sublethal concentration of Cr(VI) (60 mg L^{-1}) exhibited hyperglycemic conditions in blood and depleted glycogen levels in liver compared with control groups /[41]/. In another study, some biochemical profiles were investigated in various organs like gill, liver, and muscle of *L. rohita* at lethal concentrations of chromium (39.4 mg L^{-1}). The results of this study showed that glycogen, lipids, and protein levels were diminished significantly in all three organs, which could be due to metallic stress or to the

prevalence of hypoxic or anoxic conditions /[19]/. The results are in accordance with an earlier study by the same group who reported that *L. rohita* exposed to Cr exhibits hypoxia with lower oxygen consumption /[42]/.

Trivalent chromium alters the osmoregulatory function of various fish. Experiments designed to study the levels of the plasma electrolytes sodium (Na^+), potassium (K^+), and choride (Cl^-), as well as Na^+K^+-dependent ATPase activity in the fish *C. carpio var. communis* exposed to sublethal concentrations of chromium sulphate revealed that the metal has the potential to cause fluctuations in the osmoregulatory functions of fish. This substance forms hydrogen ions and trivalent chromium when it reacts with tissue. During chromium sulphate treatment, the level of plasma Na^+ increased while the plasma Cl^- decreased throughout the experimental period. The plasma K^+ level increased up to the tenth and then declined for the rest of the study period. The Na^+,K^+-ATPase activity decreased up to the fifteenth day of treatment and then slowly recovered, showing significant increase up to the twenty-fifth day of treatment /[43]/.

Additionally, Cr(VI) has been reported to inhibit the ion-dependent ATPases in gills, kidney, and intestine of coastal teleost at different concentrations (5, 10, 15 mg L^{-1}) with a general dose- and duration-dependent trend /[44]/. The finding that chromium inhibits the activity of the ATPases in all three organs is crucial to understanding the toxic effects of the metal because such alterations have a great impact on osmoregulatory function and the transport system along the cell membrane. Similar results on the loss of osmoregulatory and respiratory abilities have been reported in rainbow trout /[45]/.

Hematology and immune system studies—Hematologic indices have different sensitivities to various environmental factors and chemicals. In fish, changes in these parameters and their peculiarities depend upon the concentrations of heavy metals and the duration of exposure. The alterations in the hematologic indices of freshwater fish exposed to Cr(VI) are well documented, and the metal is reported to induce a decrease in most blood parameters investigated. Studies on *L. rohita* exposed to Cr(VI) (39.4 mg L^{-1}) revealed significant decreases in the percent of hemoglobin (Hb) and the total erythrocyte count at the end of both 24 h and 96 h /[46]/. The study further reported that the results reflect the anemic state of the fish and could be due to iron deficiency and its consequent decreased utilization for Hb synthesis.

Blood coagulation studies on *Tilapia sparrmanii* exposed to potassium dichromate (0.098 mg L^{-1}) at different pH levels have shown an increase in the clotting time, with a concomitant rise in pH /[47]/. The results are typical of thrombocytopenia, a condition caused by a deficiency of platelets in the blood. The same authors further investigated hematologic studies in *T. sparrmanii* at the same concentration using acute and chronic exposures at pH 5.0, 7.4, and 9.0 /. At pH 7.4 to 9.0, the experimental organisms showed anemia and leukopenia at pH 5.0. The Hb concentration significantly decreased at high pH and slightly increased at pH 5.0.

In another important study on /[49]/, freshwater fish *Saccobranchus fossilis* were exposed for 28 days to 0.1, 1.0, and 3.2 mg L^{-1} concentrations of Cr(IV) in a static bioassay test to investigate humoral and cell-mediated immune responses, blood parameters, bacterial susceptibility to infection, and macrophage activity. The ratio of spleen to body weight increased significantly at the end of the experiment. Fish exposed to all Cr concentrations had lower antibody titer values, reduced numbers of splenic and kidney plaque-forming cells, and higher counts of splenic lymphocytes but reduced counts of kidney cells when compared with the control group. At all concentrations of Cr, a dose-dependent Cr accumulation in kidney, liver, and spleen was found. A dose-dependent decrease in

erythrocyte counts, Hb content, and packed cell volume indicated anemia. Interestingly, even the total number of leukocytes decreased to less than 12.9% compared with control values, with a significant decrease in large and small lymphocytes and increase in neutrophils and thrombocytes. Similarly, the oxidative function of mitochondria was affected even at mM concentrations of Cr(VI). The in vitro phagocytic activity of splenic and pronephros macrophages significantly decreased. Fish exposed to Cr for 28 days also exhibited higher susceptibility to *Aeromonas hydrophila* infection than control fish. Taken together, the results suggest that Cr exposure reduces the resistance of catfish to bacterial infections.

Trout liver mitochondria were incubated in the presence of a micromolar concentration of potassium dichromate under several experimental conditions /[50]/. Chromium(VI) strongly inhibited both state 3 and state 4 of respiration supplemented by NAD-linked substrates and slightly affected the respiration of FAD-linked substrates. A decrease in the acceptor control index (ratio of state 3:state 4 respiration rates (oxygen consumed in the presence and absence of ADP, respectively) and alterations in respiration rate were also observed /[50]/. The authors provided evidence that the respiratory inhibition induced by dichromate is partially coupled to the Cr(VI) reduction mechanism occurring in mitochondria.

Chronic Effects

Chromium exerts long-term effects in various fish species at different functional levels (Table 3). Such effects clearly demonstrate concentration-duration relations. The early life stage of Chinook salmon (*Oncorhynchus tshawytscha*) exposed to Cr(VI) (0 to 260 μ L^{-1}) from a contaminated groundwater source was evaluated for 98 days /[51]/. The study revealed no significant changes in the survival rate, development, and behavior. Chromium accumulation, however, was increased in tissues with respect to concentration and the time factor.

Even the growth and survival of fish appears to depend on the dose and duration of exposure to Cr. Farag et al. /[52]/ reported that Chinook salmon exposed to Cr concentrations of 0-266 μg L^{-1} had no significant effect on growth. Increasing the concentration of chromium from 24-120 μg and 54-266 μg L^{-1} for 105 to 134 days exposure, however, significantly affected both survival and growth rate. Physiological alterations occurred after exposure to ≥ 120 μg L^{-1}, with DNA damage occurring after exposure to a concentration of 24 μg L^{-1}. At the end of 105 days exposure, lipid deposits were observed and attributed to altered lipid peroxidation.

A Cr concentration ≥ 36 mg L^{-1} was found to affect embryo survival and significantly reduced the larval growth even at concentrations ≥ 11 mg L^{-1} in African catfish (*Clarias gariepinus*) exposed for 5 days /[53]/. In another study, rainbow trout exposed to Cr at 0.02, 0.2, or 2 mg L^{-1} showed greater susceptibility to the metal at lower pH (6.5) than at higher pH (7.8). Further, the lowest concentrations of Cr at different pH values induced the mortality of embryos (0.2 mg L^{-1} at pH 6.5 and 2.0 mg L^{-1} at pH 7.8). At pH 6.5, a 2.0 mg L^{-1} Cr slightly affected the hatching of embryos, but no growth retardation occurred in this experiment /[54]/.

Hexavalent chromium suppresses in vivo immune responses more effectively than trivalent chromium. Weight reduction in the spleen of the African mouth breeder (*Oreochromis mossambicus*) exposed i.p. to either Cr(VI) or Cr(III) was greater in Cr(VI)-treated than in Cr(III)-treated fish. Lymphocyte and leukocyte counts decreased in both groups /[55]/.

Chronic exposure to Cr alters the activities of enzymes like pyruvate dehydrogenase (PDH), succinate dehydrogenase SDH, and lactate dehydrogenase (LDH) in kidney, brain, liver,

gills, intestine, and muscles of *Channa Punctatus* exposed for 60 or 120 days to 2.6 mg L^{-1}. In fish exposed for 60 days, no significant alterations in blood glucose or muscle glycogen content were noticed. Lactic acid content of blood and muscle was higher than in control fish, whereas the lactic acid content of liver decreased and liver glycogen was depleted. The activity of LDH significantly decreased in liver and kidney and that of PDH was inhibited in all organs (liver, kidney, intestine, brain, gills, and muscle). The activity of SDH was also inhibited in all organs except muscle, with highest percentage of decreased enzyme activity occurring in liver. In contrast, after 120 days exposure, fish showed hypoglycemia and hyperlactamia. The glycogen content increased in liver but decreased in muscle. The activity of LDH was inhibited in all organs, and the inhibition was greatest in liver. The activity of PDH significantly decreased in liver, intestine, gills, and muscles; SHD activity decreased in all organs except muscle /[56]/.

The freshwater fishes *C. carpio* and brown trout (*Salmo trutta L.*) were exposed for 38 weeks to 1,010 μg L^{-1} of potassium dichromate /[57]/. The primary and secondary humoral responses were diminished for MS2 bacteriophage. In trout, the primary antibody response was diminished by 10%, and the secondary antibody response by 50%. In carp, the serum proteins level was reduced by 25%. In this study, common carp appeared to be more sensitive to Cr than trout. On the other hand, prolonged exposure to CR(VI) has been shown to induce adaptability in fish. For example, hematologic studies in *T. sparrmanii* chronically exposed to 0.098 mg L^{-1} showed no significant changes in leukocytes or erythrocytes counts, whereas hemoglobin concentrations significantly decreased /[48]/.

BIOMARKERS OF CHROMIUM TOXICITY

Biomarker responses, although transient, are effective indicators when detected at the correct moment /[1]/. Certain biomarkers are mechanistically linked to the toxic modes of action and thus are classified as ‘biomarkers of effect’ at the level of individual organism /[58]/. Hexavalent chromium is a highly toxic metal that induces alterations at biochemical, histologic, genetic, and immunologic levels as a function of time (Table 4). Therefore, these changes in fish upon exposure to a pollutant may elicit a response that can be used as an early warning signal of putative stressors and can be often used to identify the type of stressor(s).

Biochemical Markers

Stress proteins are induced in an organism when its biological system is under stress. In Sea bream (*Sparus sarba*) blood cells, the 70 KDa heat shock proteins (HSP 70) were significantly induced in fish exposed to sublethal concentrations (0.1-150 μm) of Cd(II), Pb(II), or Cr(VI) /[59]/ The authors suggest that the HSP 70 response could be due to an adverse effect of metal on protein synthesis. On the other hand, as no overstimulation of metallothionein (MT) occurred in exposed animals, the authors reasoned that MT expression was delayed or might take a longer time to be expressed or not at all in sea bream blood cells. The authors further stated that no data are available on MT induction in fish as a result of chromium exposure. Nevertheless, the results indicate that blood cells could constitute an interesting biological model for experimental and applied toxicology to monitor environmental pollution.

Recent research indicates that various types of rearing systems have an impact on fish health /[60]/. In this unique investigation, three different systems viz., flow-through (FTS), recirculating (RAS), and recirculating with a high-rate algae pond (RAS+ HRAP) were employed to rear European sea bass. Multiple biomarker responses to metal bioaccumulation in fish was used to demonstrate exposure and effects of the rearing water in the three systems, much like environmental risk assessment. Major biomarkers, including

ethoxyresorufin-O-deethylase (EROD) and superoxide dismutase (SOD), were measured in the liver. Moreover, 12 metals, including Cr that have regulations regarding human consumption were measured in the liver and muscle. Compared with FTS, a significant increase of EROD and SOD activities were evident in RAS, as well as a significant accumulation of seven and four metals in muscle and liver, respectively. Although eight metal concentrations were significantly higher in the liver than in muscle, these concentrations were below the FAO/WHO recommended values for human consumption. Such studies are extremely helpful in unraveling the underlying mechanisms of metal toxicity.

Alterations in the biochemical constituents of the fish *Cirrhinus mrigala* following exposure to chromium and withdrawal were also reported by Virk and Sharma /[61]/. They observed that the muscle carbohydrate content was significantly reduced while lipid levels in muscle significantly increased. In another such study on *L. rohita* a 96h-LC_{50} exposure to a concentration of Cr(VI) (39.4 mg/L) significantly decreased the glycogen content, total protein, and total lipids in liver, muscle, and gill tissues of the fish /[19]/. The enhanced utilization of glycogen and its subsequent depletion in tissues is attributed to hypoxia. The author reasoned that the consistent decrease in tissue glycogen reserves observed in this study was due to impaired glycogenesis and might be due in part to its utilization in the formation of glycoproteins and glycolipids, which are essential constituents of various cells and other membranes. This study showed that Cr is highly toxic to fish, and that biochemical changes can be used as biomarkers of exposure. Similar results were also evidenced in *Catla catla* exposed to Cr at 20, 25, 30, and 35 mg L^{-1} over a period of 30 days /[3]/.

Transition metal-mediated oxygen radical attack generated putative intra-strand cross-links and strand breaks in salmon sperm DNA /[62]/. In this investigation, 32P-Postlabeling analysis of DNA treated with hydrogen peroxide and either Cu(II), Cr(VI), Co(II), Fe(II), Ni(II), or vanadium(III) [V(III)] resulted in the detection of between four and eight radioactive TLC spots that could be hydroxyl radical-mediated oxidative DNA lesions. Copper generated the highest total yield of lesions (75.6 per 108 nucleotides), followed by Co (47.5), Ni (26.2), Cr (25.1), Fe (21.7), and V (17.1). Two spots, common to all these Fenton systems, were the major oxidation products in each case, which were postulated to be due to free radical-mediated intra-strand cross-linking reactions. Generation of the putative intra-strand cross-links increased in a concentration-dependent manner up to 1 mM Co, Ni, or Cr(VI) ions. Agarose gel electrophoresis demonstrating extensive DNA strand breakage with Cu, Fe, Cr(III), or V, but not with Ni, Cr(VI), Co, Cd, or Zn Fenton systems indicate that DNA intra-strand cross-links and strand breaks occur independently. Interestingly, ingested CR(III) is reported to be more genotoxic than Cr(VI) in mice and in yeast exposed to the ions in the medium //.

The acute effects of Cr(VI) on certain ion-dependent ATPases in gills, kidney, and intestine of a coastal teleost fish, *Periophthalmus apes* (mudskipper) were delineated /[44]/. This study examined the concentration and time-dependent effects of Cr(VI) on ATPase systems and found that exposure duration was more important than dose in the inhibition of enzyme activity. The authors speculated that this heavy metal ion alters the membrane permeability of the intestinal epithelial cells and other layer of cells by altering the activity of ATPases, resulting in a breakdown of the active transport mechanism needed for the absorption of nutrients, ions and metabolites.

Histologic Biomarkers

Hexavalent chromium, one of the core toxicants in the aquatic ecosystem, has been shown to induce alterations in the morphology of gills and liver in fish in a dose- and time-dependent manner. For example, in rainbow trout exposed to Cr (10 mg L^{-1}), the epithelial cells were

not much affected initially but an increase in exposure times for 28 days had a great impact on the epithelial cells, resulting in hyperplasia (thickness of the stratified epithelial layer of the primary lamellae), and epithelial lifting in secondary lamellae. Liver cells showed reduced nucleus to cytoplasm ratio /[63]/. In another study on the Indian major carp (*L. rohita*) exposed to 96 h LC_{50} concentration of hexavalent chromium (39.40 mg L^{-1}), the fish demonstrated degeneration of secondary gill lamellae, hyperplasia of lamellar cells, and atrophy of central axis at the end of the exposure. The data suggest that Cr is highly toxic to fish cutting across species /[64]/.

Genotoxic Biomarkers

Hexavalent chromium, a known carcinogen, exerts genotoxic effects in addition to endocrine disruption in freshwater fish. For example, the physiologic and genetic responses of the European eel (*Anguilla anguilla* L.) upon short-term exposure to chromium revealed genotoxic effects /[70]/. Biomarkers for endocrine function like plasma cortisol, thyroid-stimulating hormone (TSH), free triiodothyronine (T3), and free thyroxin (T4) were determined. The genotoxicity was scored by the frequency of erythrocytic nuclear abnormalities (ENA). The impact of the chromium was evident on the plasma T4 levels in eels that decreased only when exposed to the metal.

The genotoxic effect of CR(III) was studied using fish MN analysis in peripheral blood erythrocytes from *Pimephales promelas*, the fathead minnow /[68]/. The authors used 45 to 60 day-old fish to assess the spontaneity of genetic damage. The genotoxic effect of Cr(VI) in experiments performed for 7, 14, and 21-d exposure periods was estimated. Significant micro-nucleated erythrocytes (MNE) induction was detected in fish exposed for 7 d to 2.5 mg L^{-1} Cr(VI), and this trend decreased after 21 d of exposure. The study reported basal levels of MNE providing laboratory values for future assay quality control.

Trivalent chromium can alter gene expression in the mummichog, *Fundulus heteroclitus* /[65]/. The authors examined altered gene expression both in laboratory-exposed fish and in those collected from a Cr-polluted estuarine site and found differential expression of 20 genes from either group of fish. Several genes were highly homologous to known sequences, including a fatty acid-binding protein (FABP), cytochrome P4502N2 (CYP2N2), and a precursor to the translation initiation factor eIF2B. Verification of the differentially expressed genes by real-time polymerase chain reaction (PCR) revealed that FABP was repressed to a 3.6-times greater extent (3.6-fold) in the field-site animals than in a reference site, eIF2B was repressed 2-fold, and an expressed sequence tag (EST) termed A31 was induced 2.6-fold. In laboratory-exposed animals, A31 was also induced between 2- and 4-fold. In contrast to the field-site fish, FABP was upregulated in Cr-exposed animals. The authors envisage using A31 as a biomarker for ascertaining the genotoxic impact of chromium to fish.

Nuclear abnormalities were reported in *Oreochromis niloticus* exposed to petroleum refinery or Cr-processing plant effluents. This study revealed that although both effluents had genotoxic potential on the fish, the frequencies of lobed nuclei (LN), blebbed nuclei (BL), and notched nuclei (NT) were not significant /[66]/. Similar results were also reported in *C. carpio* /[67]/. On the other hand, *Pimephales promelas* exposed to different concentrations of chromium viz., 1.0, 1.5, 2.0, 2.5 mg L^{-1} for different exposure times (7, 14, 21days) demonstrated significant induction of micronuclei (MN) at the 2.5 mg L^{-1} concentration after 7 days exposure /[68]/. Nuclear abnormalities other than MN were evaluated on erythrocytes to assess the genetic damage. This study demonstrated that both effluents are genotoxic, and the level of genetic damage induced by petroleum refinery effluent was relatively higher than the Cr-processing plant effluent. The results further indicate that

nuclear abnormalities other than MN, such as BN and LN, could also be used as biomarkers of genotoxic damage.

The differential expression of genes can be used as a biomarker to identify the type of toxicant and the health of fish. For instance, the Cr(VI) treatment of winter flounder (*Pseudopleuronectes americanus*) revealed alterations in ion-selenium glutathione peroxidases /[69]/. In this study, 29 differentially expressed genes products were sequenced and identified, substantiating that Cr(VI) significantly changes gene expression, including two potential glutathione peroxidases in the fish.

Multiple biomarker responses play a crucial role in establishing the exact cause-effect relations of toxicants in fish /[63]/. Recent research on Cr(VI) toxicity in rainbow trout showed that multiple biomarkers can be used at different levels of biological organization. Appreciable differences in MT induction, SOD activity, lipid peroxidation, cellular morphology, and growth have been reported. The results indicate that gill tissues exhibit more sensitivity than hepatic tissues to Cr toxicity. The liver, however, appears to play a larger role than gill tissues in an organism's adaptive response to Cr. This study underscores the importance of using a set of integrated biomarkers to assess heavy metal exposure and effects.

CONCLUSION

Although chromium is ubiquitous in the environment, Cr(VI) is an industrial pollutant and classified carcinogen possessing both mutagenic and teratogenic properties. Research indicates that Cr exposure can induce a variety of adverse effects in fish at physiologic, histologic, bio-chemical, enzymatic, and genetic levels. Certain fish species, however, appear to show more sensitivity to Cr toxicity than others. Hence, Cr-induced toxicological pathology in fish is influenced by such factors as species, age, environmental conditions, exposure time, and exposure concentration. The exact causes of fish death are multiple and depend mainly on time-concentration combinations. In-depth toxicodynamics and toxicokinetics studies are necessary to establish an exact cause-effect relation. The scientific data discussed in this review provide a basis for understanding the potential impact, as well as for advancing our knowledge of the ecotoxicology and risk assessment of chromium.

Acknowledgments

This research was supported in by the RCMI Center for Environmental Health (Grant No. 2G12RR13459-11) from the National Institutes of Health, and in part by the Environmental Cooperative Science Center (Grant No. NA17AE1626 & Subcontract No. 27-0629-017) from the National Oceanic and Atmospheric Administration.

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Table 1

Summary of fish sensitivity to Cr during an exposure period of 96 h-LC50 as reported by /[71]/.

Fish Species	Results	Exposure Type	Temperature	Test Conditions	
	(mg L^{-1})		°C	pH	(mg L^{-1})
Labeo rohita	39.40	Renewal	27.5 ±1	8.0±5	CO_3=61; HCO_3=301; Alk=501
Pimephales promelas	48	Flow through	25	7.5 to 7.9	Alk=200-230
Channa punctatus	50	Static	29.8±1	7.5 to 8.5	
Pimephales promelas	52	Flow through	15	7.6 to 8.2	Alk=230-232
Salvelinus fontinalis	59	Flow through	12	7.0 to 8.0	Hardness=44-46
Pimephales promelas	61	Flow through	15	7.6 to 8.2	Alk=230-232
Salmp gardnerii	69	Flow through	12	7.0 to 8.0	Hardness=44-46
Carassius auratus *	85.7	Renewal	26-28	7.0 to 8.0	Hardness=85-90
Catla catla	100	Static	28±1	7.1	CO3=604
Carassius auratus	120	Flow through	25	7.5 to 7.9	Alk=200-230

*Source: Velma et al. /[72]/

Table 2

Acute effects of chromium to freshwater fish

Fish Species	[Cr]	Acute Effects	Ref
Salmo gairdneri	0.005 mg L^{-1}	Effect on fertilization.	37
Tilapia sparrmanii	0.098 mg L^{-1}	Decrease in blood clotting time	47
Tilapia sparrmanii	0.098 mg L^{-1} at pH 7.4-9.0	Decrease in WBC, RBC counts, and Hb concentration. Increase in ALA-D activity.	48
Sccobranchus fossilis	0.1-3.2 mg L^{-1}	Increases in spleen to body ratio, WBC, RBC, Hb, MCV, PVC, and splenocytes. Decreased antibody production and increased susceptibility to bacteria.	49
Periophthalmus dipes	5-15 mg L^{-1}	Decrease in ion-dependent ATPase activity.	44
Labeo rohita	39.40 mg L^{-1} 96 h-LC50	Decrease in glycogen content, total lipid content and total protein content of liver, muscle and gill.	19
Colisa fasciatus	60 mg L^{-1}	Reduction in liver glycogen content. Hyperglycemic response.	41
Carassium auratus	250 μM	Decrease in cell viability. Increase in ROS.	36

All data are derived from in vitro experiments. ALA-D – δ-amino levulinic dehydratase; WBC – white blood cells; RBC – red blood cells; Hb – hemoglobin; MCV – mean red cell volume; PVC – packed cell volume; ROS – reactive oxygen species

Table 3

Chronic effects of chromium to freshwater fish

Exposure type	Fish Species	Cromium concentration	Chronic Toxicity of Chromium	Ref
In vivo*	*Oreochromis mossambicus*	7.5 μg/fish Cr(VI) 100 μg/fish Cr(III)	Decrease in antibody production. Reduction in splenic weight. Decrease in lymphocyte count.	55
In vitro	*Oncorhynchus tshawytscha*	24-120 μg L^{-1}l 54-266 μg L^{-1} 24 μg L^{-1}	Decrease in survival rate. Decrease in growth rate. DNA damage.	52
In vitro	*Cyprinus carpio*	1010 μgL^{-1} 38 wk	Diminished humoral responses. Reduced serum proteins level by 25%.	57
In vitro	*Salmo gairdneri*	0.2 mg L^{-1} pH 6.5; 2 mg L^{-1} pH 7.2 and pH 6.5	Induction of mortality rate. Effect on embryo hatching.	54
In vitro	*Channa punctatus*	2.6 mg L^{-1} 60 days exposure	Increased muscle and blood lactic acid. Decreased liver lactic acid and glycogen. LDH activity inhibited in liver and kidney. PDH and SDH activities nhibited in all the tissues except muscle	56
		120 days exposure	Glycogen increased in liver but decreased in muscle. LDH inhibited in all six tissues. Decreased activity of PDH in liver, intestine, gill, muscles; SDH elevated in muscle but inhibited in other tissues	56
In vitro	*Clarias gariepinus*	>/ = 36 mg L^{-1} >/ = 11 mg L^{-1}	Decreased embryo survival rate. Decreased larval growth.	53
In vitro	*Nuria denricus*	0-100 mg L^{-1}	Erosion of fin and fin rays	21

* i.p. injection

LDH = lactate dehydrogenase; SDH = succinate dehydrogenase; PDH = pyruvate dehydrogenase

Table 4

Biomarkers of chromium in freshwater fish

Fish Species	Chromium form and concentration	Effect[1]	Ref
Sparus sarba	0-150 μM	Significant induction of 70 KDa heat shock proteins (HSP 70) in blood cells	/59/
Cirrhinus mrigala	31-35 mg L^{-1}	Significant reduction of muscle carbohydrate content. Significant induction of lipid levels in muscle	/61/
Labeo rohita	39.40mg L^{-1}	Decreased glycogen, total lipid and total protein contents of liver, muscle and gill	/19/
Catla catla	20, 25, 30 and 35 mg L^{-1}	Impaired glycogenesis. Decreased glycogen, total lipid and total protein contents of liver, muscle and gill	/3/
Salmon fish	1 mM	Intra-strand cross-links and strand breaks in salmon sperm DNA, extensive DNA strand breakage	/62/
Periophthalmus apes	5, 10 and 15 mg L^{-1}	Decrease of calcium and magnesium ATPases	/44/
Oreochromis niloticus	Effluent	Significant increase in the frequency of micro nucleated erythrocytes and gills cells	/66/
Rainbow trout	10 mg L^{-1}	Hyperplasia of epithelial cells and epithelial lifting of secondary lamellae. Reduction of nucleus to cytoplasm ratio in liver cells.	/63/
Pimephales promelas	2.5mg L^{-1}	Significant induction of micronuclei.	/68/
Labeo rohita	39.40 mg L^{-1}	Degeneration of secondary gill lamellae, hyperplasia of lamellar cells, atrophy of central axis.	/64/
Saccobranchus fossilis	0.1, 1.0, & 3.2 mg L^{-1}	Significant change in spleen to body weight ratio. Lower antibody titer values. Decrease in the numbers of splenic and kidney plaque-forming cells. High counts of splenic lymphocytes with reduced counts of kidney cells. Dose-dependent decrease in red blood cell counts, hemoglobin content, and packed cell volume.	/49/

[1] All data are derived from in vitro experiments