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THE EFFECT OF POLLUTION
BY HEXAVALENT CHROMIUM
ON THE SURVIVAL AND PHYSIOLOGY
OF CERTAIN FISH AND CRUSTACEA
"THE EFFECT OF POLLUTION BY HEXAVALENT CHROMIUM ON THE SURVIVAL AND PHYSIOLOGY OF CERTAIN FISH AND CRUSTACEA"

The toxicity of hexavalent chromium (as \( K_2CrO_7 \)) on three species of fish; rainbow trout \( (Salmo gairdneri) \), minnow \( (Phoxinus phoxinus) \) and Guppy \( (Poecilia reticulata) \) and on the crustacean \( Daphnia magna \) was investigated. The 24 hr LC\(_{50}\) values of chromium for these animals were determined. Among the fish, rainbow trout was found to be the most susceptible to chromium and minnows were the most resistant. \( Daphnia \) proved to be far more sensitive to chromium than fish. When \( D. magna \) were exposed to 0.35 mg Cr \( \text{l}^{-1} \) for up to 6 days and then returned to chromium-free water, they were able to recover as their survival was similar to that of control animals. But when exposed to this concentration of chromium for 8 days and then returned to chromium-free water, their recovery was greatly impaired.

All the species of fish were able to accumulate measurable levels of chromium in their tissues when exposed to chromium for 24 hours. In general, the chromium level in the various tissues increased with increasing chromium concentration in the external medium. Few exceptions to this were observed. Gill, kidney, liver and stomach tissues accumulated the highest levels of chromium followed by heart and gonad tissues, while brain and muscle tissues accumulated the lowest levels.

The rate of oxygen consumption was significantly reduced in male guppies exposed to 35.35 and 106.05 mg Cr \( \text{l}^{-1} \) for 24 hours. These fish were able to recover their normal rate of oxygen consumption in 24 hours when returned to chromium-free water. But exposure to 7.07 mg Cr \( \text{l}^{-1} \) for 24 hours did not cause a significant effect on the rate of oxygen consumption in this species. Individuals of both control and chromium exposed \( D. magna \) exhibited a significantly reduced rate of oxygen consumption when kept without food for 24 hours compared to the rate of corresponding fed groups. Exposure of 3-day old \( D. magna \) to 0.18 and 0.35 mg Cr \( \text{l}^{-1} \) for 24 hours (between 2nd and 3rd days) significantly reduced the rate of oxygen consumption. While exposure to 0.035 mg Cr \( \text{l}^{-1} \) for 24 hours did not have a clear effect on the rate of oxygen consumption in \( D. magna \).

Hexavalent chromium affected the rate of reproduction of \( D. magna \) in two different ways. Rearing \( Daphnia \) in chromium concentration of 0.035 and 0.07 mg \( \text{l}^{-1} \) caused an increase in the total number of young produced (reproductive stimulation) and an unexpectedly longer life span was observed in these groups when compared with control animals. On the other hand, rearing \( D. magna \) in chromium concentrations of 0.11, 0.18 and 0.35 mg \( \text{l}^{-1} \) caused a significant reduction in the total number of young produced (reproductive impairment) and these chromium concentrations also caused significant reductions in the life span of these animals. However, individuals of \( D. magna \) were able to recover from exposure to 0.35 mg Cr \( \text{l}^{-1} \) for up to 6 days when returned to chromium-free water. This recovery was evident as their rate of reproduction and mean life span did not significantly differ from those of control animals. But recovery in the rate of reproduction was greatly impaired in animals exposed to 0.35 mg Cr \( \text{l}^{-1} \) for 8 days before returning to chromium-free water.

Liver and kidney tissue sections taken from minnows and guppies exposed to 176.75 and 141.40 mg Cr \( \text{l}^{-1} \) for 24 hours respectively did not show pronounced histological alterations when compared with control tissues. Similar results were found in liver and kidney tissues taken from guppies exposed to 53.03 mg Cr \( \text{l}^{-1} \) for 96 hours. Using Chrome-azurol S, it was possible to stain chromium in liver and kidney tissue sections taken from rainbow trout and guppies exposed to 7.07 and 53.03 mg Cr \( \text{l}^{-1} \) for 96 hours respectively.

Finally, the modes of action of hexavalent chromium on the survival and physiology of fish and \( Daphnia \) are discussed.
THE EFFECT OF POLLUTION BY HEXAVALENT CHROMIUM ON THE SURVIVAL AND PHYSIOLOGY OF CERTAIN FISH AND CRUSTACEA

A Thesis submitted to the University of Dublin in fulfillment for the degree of
Doctor of Philosophy

BY

NOHA MAHMOOD AMEEN, B.Sc.

August, 1983
I hereby declare that the work recorded in this thesis has been carried out by Noha Mahmood Ameen, and that it is her own composition. No part of this work has been submitted for any other degree.

Prof. J. N. R. Grainger
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I hereby declare that the work recorded in this thesis is entirely my own, unless otherwise stated, and that it is of my own composition.

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Noha Mahmood Ameen
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INTRODUCTION

Heavy metals are well known pollutants which cause disorders in aquatic ecosystems. Of these heavy metals, chromium is the subject of the present work. Chromium in different forms reaches the aquatic environment through a number of avenues mainly from the effluents of certain industrial processes and products such as electroplating, leather tanning, paints, graphic arts, printing, fungicides, wood preservatives, textile industries and corrosion inhibitors (Dugan, 1972; National Research Council, 1974; Yongue et al., 1979; Riva et al., 1981)

Hexavalent chromium in water is known to be toxic to aquatic organisms. This work has been carried out to evaluate the toxic effect on survival, bioaccumulation, rate of oxygen consumption and histological alterations in three species of fish; rainbow trout, minnow and guppy. Three fish species were used because different species of fish may have widely different sensitivities to the same metal. In addition the fish come from widely different habitats. Rainbow trout live in fast flowing streams and lakes, minnows live in slow flowing streams and guppies are tropical fish. Also the toxic effect (lethal and sublethal) of hexavalent chromium was investigated using a cladoceran crustacean, Daphnia magna. This crustacean was chosen because of the following advantages: (a) It is easily handled and reproduces well in the laboratory. (b) It is available at all times of the year. (c) It is neither too sensitive nor too resistant to chemicals. (d) It is present in ponds and is of value to fish. Anderson (1948) suggested that Daphnia should be considered as representative of other abundant zooplankton species in sensitivity tests to toxic substances.
So the main aim of this work is to evaluate the toxic action of hexavalent chromium on survival and to investigate some of the physiological aspects of fish and Daphnia in order to try to find out the mechanism by which chromium exerts its toxicity on these organisms.
General

With increasing industrialisation, the pollution of aquatic environments by heavy metals has become a serious problem in many areas because of the harmful effects that high levels of such substances can have on aquatic organisms including fishes. McKee and Wolf (1963) have summarised the sources of individual heavy metals and their salts. Information on the toxicity of metals has been reported by many workers (Doudoroff and Katz, 1953; McKee and Wolf, 1963; Raymont and Shields, 1964; Pickering and Henderson, 1966; Smith and Heath, 1979; Kaviraj and Konar, 1982).

It has also been reported that heavy metals usually accumulate in different tissues of fish (Knoll and Fromm, 1960; Olson et al., 1973; Lock, 1979; Van Hoof and Van San, 1981; Van der Putte et al., 1981 a). Several studies have shown that exposure of fish to heavy metals leads to a number of disturbed physiological processes (Alabaster and Lloyd, 1980). Some work has been focused on the respiratory changes induced by these compounds. This approach was stimulated by the suggestion that the action of metals might be primarily on the gill surface and might consequently affect gaseous exchange (Jones, 1947; Skidmore, 1970; Sellers et al., 1975). Other studies have emphasized the effect of metals on water – and ion – balance in fish (McKim et al., 1970; Lewis and Lewis, 1971; Larsson et al., 1981; Lock et al., 1981).

It has frequently been observed that acute or subacute exposure to
metals causes histological alterations in organs involved in respiration (gills) and osmoregulation (gill, kidney and intestine). This has been reported for metals like copper (Baker, 1969), cadmium (Gardner and Yevich, 1970), zinc (Skidmore and Tovell, 1972), mercury (Wobeser, 1975) and hexavalent chromium (Van der Putte et al., 1981 b).

Some work has also been carried out on the effect of heavy metals on the reproduction of different aquatic organisms; Daphnia (Biesinger and Christensen, 1972); brook trout (Benoit, 1976); and annelids (Reish and Carr, 1978; Oshida et al., 1981).

Chromium with an atomic number of 24 and an atomic weight of 52 has oxidation states ranging from $\text{Cr}^{2-}$ to $\text{Cr}^{6+}$, but it most commonly occurs as $\text{Cr}^0$, $\text{Cr}^{2+}$ and $\text{Cr}^{6+}$. Divalent chromium, however, is relatively unstable being rapidly oxidized to the trivalent form, thus only two forms – trivalent and hexavalent – are found in nature (Strik et al., 1975). Chromium is an essential trace element and has been found in almost all living things. It is also found in the air, soil and water. In its hexavalent state (as chromic oxide, chromate or dichromate), chromium is a strong oxidizing agent and readily reacts with organic matter in acidic solution leading to reduction to the trivalent form. Chromium occurs in most biological material in the trivalent form, in which it is strongly associated with proteins, nucleic acids and a variety of low-molecular - weight ligands. The hexavalent form is more toxic than the trivalent because of its oxidizing potential and its easy permeation of biological membranes (National Research Council, 1974). According to Fales (1979) previous work with heavy metals has focused on toxic elements such as cadmium, lead, copper, zinc and mercury. More attention should be directed towards chromium which is often found in industrial effluents.
(Dugan, 1972) and sewage (Weaver et al., 1974).

Since this work is concerned wholly with the effects of hexavalent chromium on survival and some physiological processes in three fish species and in Daphnia, the literature review will be restricted to similar work dealing with chromium. The work on other heavy metals will be reviewed whenever chromium literature is scarce.

1. Acute Toxicity

According to Doudoroff and Katz (1953), dichromates and chromates (hexavalent chromium) belong to a class of compounds that are very different chemically and toxicologically from the typical heavy metals. In aqueous solution, hexavalent chromium almost exclusively exists in the form of oxo-anions (Cr$\text{O}_4^{2-}$, HCr$\text{O}_4^-$, Cr$_2\text{O}_7^{2-}$), which have been observed to pass readily through the gill membrane and to accumulate in various tissues and organs (Knoll and Fromm, 1960). Thus the hexavalent chromium could elicit its toxic effect at some internal site. Direct evidence of an internal site of hexavalent chromium toxicity has been reported by Fromm and Schiffman (1958), who observed severe pathological changes in the intestine immediately behind the pyloric caeca in largemouth bass (Micropterus salmoides) exposed to 96 mg Cr(VI) l$^{-1}$. Also Kuhnert et al. (1976) showed that the Na$^+$, K$^+$-ATPase activity of kidney and intestine, but not of gill, significantly decreased in rainbow trout (Salmo gairdneri) exposed to 2.5 mg Cr(VI) l$^{-1}$ for 48 hours.

Verriopoulos and Moraïtou - Apostolopoulou (1981) suggested that results on acute toxicity are of value in the toxicity tests because of
their minimum variability. But they may have serious limitations because they ignore differences in the sensitivity of a test species throughout consecutive generations or prolonged exposure (Saliba and Krzyz, 1976; Stockner and Antia, 1976; Winner and Farrell, 1976) and the impairment of various physiological processes at concentrations at which there is no clear effect on survival (Mount, 1968; MoraItou - Apostolopoulou and Verriopoulos, 1979; MoraItou - Apostolopoulou et al., 1979).

Although there is an extensive literature concerning the acute toxicity of hexavalent chromium it is difficult to compare these results with each other and to draw a general conclusion regarding the toxicity of this metal because of the variability in both chemical and physical characteristics of dilution water including the pH, hardness and temperature (Ellis, 1937; Trama and Benoit, 1960; Ruesink and Smith, 1975; Cairns et al., 1978; Chapman, 1978; Fales, 1978; Andros and Garton, 1980; Riva et al., 1981; Van der Putte et al., 1981 b).

The pH value of the test solution has been found to affect the toxicity of chromium to aquatic organisms. Ellis (1937) concluded that the toxicity of any solution below a pH value of 5 is due at least in part to its acidity, and in a solution with a pH value greater than 5 lethal factors other than pH play a major part. Grindley (1946) has found that hexavalent chromium in the form of potassium dichromate (acidic salt) was more toxic to rainbow trout than that in the form of potassium chromate (basic salt). While Trama and Benoit (1960) conducted studies to determine the effect of pH on the toxicity of hexavalent chromium on bluegills (*Lepomis macrochirus*). They found that 24-hr LC$_{50}$ (the chromium concentration causing 50% mortality in 24 hours) and 96-hr LC$_{50}$ values were 175 and 113 mg Cr $l^{-1}$ for K$_2$Cr$_2$O$_7$, while these values were 225
and 170 mg Cr 1⁻¹ for K₂CrO₄ respectively. Similar results have been reported by Pickering and Henderson (1966) who found that the 24-hr and 96-hr LC₅₀ values of hexavalent chromium for the fathead minnows (Pimephales promelas) in the form of K₂Cr₂O₇ was significantly less than those of K₂CrO₄. Hogendoorn - Roozemond et al. (1978) found in the rainbow trout that the 48-hr and 72-hr LC₅₀ values of hexavalent chromium at pH 6.8 (0.28 and 0.22 mg Cr 1⁻¹ respectively) were significantly lower than those at pH 7.9 (58.4 and 36.3 mg Cr 1⁻¹ respectively). More recently Van der Putte et al. (1981 b), working on rainbow trout, found that the acute toxicity of hexavalent chromium increased with decreasing the pH in the range from 7.8 - 6.5. To explain this, two hypotheses based on the assumption that acute hexavalent chromium toxicity is attributable to HCrO₄⁻ (hydrochromate) and CrO₄²⁻ (chromate) species have been put forward. The first suggests that only the availability of Cr(VI) to the fish increases with an increasing HCrO₄⁻/CrO₄²⁻ ratio at lower pH levels, possibly because monovalent ions tend to be more readily absorbed than divalent ions (Trama and Benoit, 1960; Becker and Thatcher, 1973). The second takes the enhanced oxidizing action of hexavalent chromium at a decreased pH, which is associated with an increased HCrO₄⁻/CrO₄²⁻ ratio, also into consideration (Van der Putte et al., 1981 a). Both hypotheses suggest that the apparent toxicity of HCrO₄⁻ is higher than that of the CrO₄²⁻. However, whether this is the case and to what extent has not been ascertained yet. Finally Kaviraj and Konar (1982) found that chromium was more toxic to the fish (Tilapia mossambica) at pH 7 (96-hr LC₅₀ = 170 mg Cr 1⁻¹) than at pH 8 (96-hr LC₅₀ = 217.5 mg Cr 1⁻¹).

Hardness has been found, as well, to alter the toxic effect of hexavalent chromium to a number of animals. Ellis (1937) reported that goldfish (Carassius auratus) survived for more than 96 hours in a solution
of 100 mg $l^{-1}$ chromium trioxide ($CrO_3$)($52$ mg Cr $l^{-1}$) in hard water, while the same concentration of chromium killed goldfish in 30 - 35 minutes in very soft water. This variation in the toxicity could be due to difference in pH. Cairns and Scheier (1959) demonstrated that the 96-hr LC$_{50}$ value of hexavalent chromium (as $K_2Cr_2O_7$) for the bluegill was highest in hard water and lowest in soft water. Trama and Benoit (1960) showed that the 24-hr LC$_{50}$ values of hexavalent chromium for bluegills in soft water (hardness 45 mg $l^{-1}$ as CaCO$_3$) were about 175 mg Cr $l^{-1}$ for $K_2Cr_2O_7$ and 225 mg Cr $l^{-1}$ for $K_2CrO_4$. On the other hand Abegg (1950) working on the same fish, found that 24-hr LC$_{50}$ values of hexavalent chromium in a synthetic dilution water having a hardness between 75-150 mg $l^{-1}$ as CaCO$_3$ were 290 mg Cr $l^{-1}$ for $Na_2Cr_2O_7$ and 300 mg Cr $l^{-1}$ for $Na_2CrO_4$. Pickering and Henderson (1966) working on toxicity of hexavalent chromium in four fish species, reported that the 24-hr, 48-hr and 96-hr LC$_{50}$ values of hexavalent chromium (as $K_2Cr_2O_7$) for the fathead minnows were higher in hard water (of 360 mg $l^{-1}$ hardness) than those in soft water (of 20 mg $l^{-1}$ hardness). But the same authors reported that the 96-hr LC$_{50}$ values of hexavalent chromium for bluegills in both hard and soft water were not significantly different. Olson and Harrel (1973) looked at the effect of salinity on the toxicity of $K_2Cr_2O_7$ for Rangia cuneata (an estuarine pelecypod) and found that the 24-hr, 48-hr and 96-hr LC$_{50}$ values increased as the salinity rose from 1 to 5.5 to 22 parts per thousand. Finally Bellavere and Gorbi (1981) found that the 24-hr LC$_{50}$ values of hexavalent chromium for Daphnia magna were 1.57 and 0.83 mg Cr $l^{-1}$ in water having a hardness of 200 and 100 mg $l^{-1}$ as CaCO$_3$ respectively.

Temperature is another environmental factor which has been found to influence the toxic action of hexavalent chromium on aquatic organisms.
Cairns and Scheier (1959) concluded that the toxicity of $\text{K}_2\text{Cr}_2\text{O}_7$ on the bluegills increased with increasing test temperature from 18-30°C. Ruesink and Smith (1975) found for the fathead minnow that the 48-hr and 96-hr LC$_{50}$ values of hexavalent chromium were 61 and 52 mg Cr l$^{-1}$ respectively at 15°C, while these values were 58 and 37 mg Cr l$^{-1}$ respectively at 25°C. Cairns et al. (1978) working on a number of invertebrates and species of fishes found an increase in the toxicity of hexavalent chromium with increasing the test temperature. Fales (1978) found that the 48-hr LC$_{50}$ of hexavalent chromium for the grass shrimp (Palaemonetes pugio) was 81, 39, 37 and 21 mg Cr l$^{-1}$ at 10°, 15°, 20° and 25°C respectively. Smith and Heath (1979) found that the 24-hr LC$_{50}$ value of hexavalent chromium (as $\text{K}_2\text{Cr}_2\text{O}_7$) for goldfish and golden shiner (Notemigonus crysoleucus) decreased when the test temperature was increased from 5° to 15° to 30°C. But they concluded that the toxicity of hexavalent chromium on bluegills and rainbow trout was unaffected by variations in the test temperatures. Finally Riva et al. (1981) reported that the toxicity of hexavalent chromium on goldfish increased with increasing test temperature.

The acute toxicity of hexavalent chromium on different species of fish has been well established in the literature. In general, it appears that the toxicity of chromium varies with the different species and among the same species as a result of variations in experimental procedures and conditions. Over the years, rainbow trout has been used by different workers in determining the toxicity of chromium. The results seem to be variable when compared with each other. Rainbow trout failed to recover from a 6-hr exposure to a solution of $\text{K}_2\text{Cr}_2\text{O}_7$ containing 35 mg Cr l$^{-1}$ even when returned to freshwater (Rushton, 1922). Grindley (1946) concluded that concentrations of hexavalent chromium below 20 mg l$^{-1}$ would be non toxic to yearling rainbow trout. He also demonstrated that
trout survived for about 60 and 70 hours in distilled water containing 20 mg Cr(VI) \(1^{-1}\), as \(K_2CrO_4\) and \(K_2Cr_2O_7\) respectively. While at 50 mg Cr \(1^{-1}\), the average survival time was about 30 hours for both chromium salts. But at higher concentrations \(K_2Cr_2O_7\) was more toxic than \(K_2CrO_4\). Olson and Foster (1956) reported a sublethal effect of hexavalent chromium on rainbow trout at concentrations as low as 13 \(\mu g\) \(1^{-1}\). While Schiffman and Fromm (1959) performed a carefully controlled experiments on rainbow trout in hard water (hardness, 334 mg \(1^{-1}\); pH, 8.5 - 8.8) using \(K_2CrO_4\). They found that the 24-hr LC\(_{50}\) was 100 mg Cr \(1^{-1}\), but exposure of the fish to only 20 mg Cr \(1^{-1}\) or even as low as 2 mg Cr \(1^{-1}\) caused significant pathological changes including a striking response in hematocrit value. Benoit (1976) reported that 96-hr LC\(_{50}\) values for brook trout (\(Salvelinus fontinalis\)) and rainbow trout were 59 and 69 mg Cr(VI) \(1^{-1}\) respectively in water with hardness of 45 mg \(1^{-1}\) as CaCO\(_3\) and a pH range of 7-8. He concluded that the higher LC\(_{50}\) established for rainbow trout was undoubtedly due to the difference in age (14-month old rainbow trout versus 5-month old brook trout). However, Cairns et al. (1978) showed that the 24-hr LC\(_{50}\) values of hexavalent chromium (as \(K_2Cr_2O_7\)) in soft water with a total hardness of 40 mg \(1^{-1}\) for 10 cm long rainbow trout were 58.9, 141 and 95.5 mg Cr \(1^{-1}\) at 5\(^\circ\), 12\(^\circ\) and 18\(^\circ\)C respectively. Smith and Heath (1979) found that the 24-hr LC\(_{50}\) of hexavalent chromium (as \(K_2Cr_2O_7\)) at pH mean value of 5.8 and hardness of 36 mg \(1^{-1}\) as CaCO\(_3\) for the rainbow trout at 12\(^\circ\)C was around 110 mg Cr \(1^{-1}\). Recently Van der Putte et al. (1981 b) found that the 24-hr LC\(_{50}\) value of hexavalent chromium (as \(Na_2CrO_4\)) for 6 g rainbow trout at pH 7.8, 7.0, and 6.5 were 180.2, 136.7 and 90.6 mg Cr \(1^{-1}\) respectively.

Some work has been published concerning the toxicity of hexavalent chromium on minnows. LeClere and Devlaminck (1950) reported the failure
of minnows to survive exposure to 40 mg l\(^{-1}\) of trivalent chromium in distilled water for as long as 6 hours. The 24-hr and 96-hr LC\(_{50}\) values of hexavalent chromium (as K\(_2\)Cr\(_2\)O\(_7\)) for fathead minnows in soft water (hardness 20 mg l\(^{-1}\)) were 39.6 and 17.6 mg Cr l\(^{-1}\) respectively. While these values in hard water (hardness 360 mg l\(^{-1}\)) were 63.5 and 27.3 mg Cr l\(^{-1}\) respectively (Pickering and Henderson, 1966). Whereas Ruesink and Smith (1975) reported 48-hr and 96-hr LC\(_{50}\) values of hexavalent chromium for fathead minnows in nonchlorinated well water of 61 and 52 mg Cr l\(^{-1}\) respectively at 15\(^\circ\)C, and 58 and 37 mg Cr l\(^{-1}\) respectively at 25\(^\circ\)C. More recently Broderius and Smith (1979) found that the 96-hr LC\(_{50}\) value of hexavalent chromium for the fathead minnow at pH 7.8 and at a temperature of 25\(^\circ\)C was 33.2 mg Cr l\(^{-1}\).

For the guppy (Lebistes reticulatus), Pickering and Henderson (1966) reported that the 24-hr and 96-hr LC\(_{50}\) values of hexavalent chromium (as K\(_2\)Cr\(_2\)O\(_7\)) in soft water (20 mg l\(^{-1}\) hardness) were 113 and 30 mg Cr l\(^{-1}\) respectively at 25\(^\circ\)C. More work has been published which deals with the toxic effect of hexavalent chromium on goldfish (Martin and Rostenbach, 1953; Pickering and Henderson, 1966; Cairns et al., 1978; Smith and Heath, 1979; Riva et al., 1981); bluegills (Abegg, 1950; Doudoroff and Katz, 1953; Trama and Benoit, 1960; Pickering and Henderson, 1966; Cairns et al., 1978; Smith and Heath, 1979); the fish Brachydanio rerio (Bellavere and Gorbi, 1981); the fish Tilapia mossambica (Kaviraj and Konar, 1982) & many others.

The toxic effect of hexavalent chromium to a variety of invertebrate species has also been reported by numerous workers. One of the species which has been well studied is Daphnia. The reported results about the acute toxicity on Daphnia species are variable. Anderson (1944) reported threshold concentrations of both salts, chromic acid and potassium
dichromate, of less than 0.6 mg l\(^{-1}\) for 8-hr old *Daphnia magna* in Lake Erie water at 25°C. Freeman and Fowler (1953) found that the 100 hour toxicity threshold of Na\(_2\)CrO\(_4\) on *Daphnia magna* at pH 7.8 was 0.42 mg l\(^{-1}\).

Sherr and Armitage (1971) reported concentrations of dichromate (Na\(_2\)Cr\(_2\)O\(_7\)) of 50, 10, 1.0 and 0.1 mg l\(^{-1}\) at 21°C caused 100% mortality for *Daphnia pulex* in 2, 5, and 24 hours respectively. They also concluded that 12-day old *Daphnia pulex* were somewhat more resistant to dichromate than 6-day old animals. Winner (1976) noted that sensitivity to acute chromium stress was quite variable among *Daphnia*. Whereas Batac-Catalan and Cairns (1977) reported the 24-hr and 48-hr LC\(_{50}\) values for *Daphnia pulex* of 0.68 and 0.26 mg l\(^{-1}\) K\(_2\)CrO\(_4\) respectively in water having a hardness of 171 mg l\(^{-1}\) as CaCO\(_3\) at 21°C. Trabelka and Gehrs (1977) found an apparent effect of chromium on *Daphnia magna* at a concentration of 10 µg l\(^{-1}\).

Cairns et al. (1978) reported 24-hr LC\(_{50}\) values of hexavalent chromium for *Daphnia magna* and *Daphnia pulex* of 0.76 and 0.56 mg Cr l\(^{-1}\) at 25°C, and these values were 1.0 and 0.8 mg Cr l\(^{-1}\) at 20°C for the two species respectively in soft water (40 mg l\(^{-1}\) total hardness) at pH 7.5. They also found that 24-hr and 48-hr LC\(_{50}\) values of hexavalent chromium decreased with increasing experimental temperature, and in all experiments *Daphnia magna* was more resistant to chromium than *Daphnia pulex*.

Recently, Bellavere and Gorbi (1981) found that 24-hr LC\(_{50}\) values of hexavalent chromium for *Daphnia magna* in water with hardness of 100 and 200 mg l\(^{-1}\) as CaCO\(_3\) were 0.83 and 1.57 mg Cr l\(^{-1}\) respectively.

Other invertebrate species have been found to be affected by the presence of hexavalent chromium in their environment. The acute toxicity of this metal on a number of aquatic invertebrates has been reported by many workers. Working with hexavalent chromium, Yongue et al. (1979) found that *Euglena gracilis* tolerated chromium as high as 1 mg l\(^{-1}\) for 3
hours or longer at room temperature. Rotifers have been found to tolerate high concentrations of hexavalent chromium. This has been shown by Schaefer and Pipes (1973) who found that 24-hr $LC_{50}$ was 28 mg Cr(VI) $L^{-1}$ at 25°C for *Philodina roseola*, and they also gave values of 48-hr, 72-hr and 96-hr $LC_{50}$ of hexavalent chromium at different temperatures for this species. Earlier work conducted by Allee and Rosenthal (1949) showed that this species has a rather wide tolerance range for a variety of environmental factors and thus it would have a higher tolerance for toxic materials such as hexavalent chromium than many other aquatic organisms.

Different workers have reported contradicting results for the toxicity of hexavalent chromium on the polychaete *Neanthes arenaceodentata*. The 96-hr $LC_{50}$ for this species was reported to be 3.1 mg Cr(VI) $L^{-1}$ as potassium dichromate at 20°C (Mearns et al., 1976) and more than 1.0 mg Cr (VI) $L^{-1}$ as chromic acid (Reish et al., 1976). While the 7-day $LC_{50}$ of hexavalent chromium for this species ranged from 1.44 – 1.89 mg Cr(VI) $L^{-1}$ in the form of $K_2Cr_2O_7$ (Oshida et al., 1981). Another polychaete *Nereis virens* has been found to have 96-hr $LC_{50}$ value of hexavalent chromium (as $K_2CrO_4$) of 2 mg Cr $L^{-1}$ at 20°C (Eisler and Hennekey, 1977). Toxicity of hexavalent chromium on different species of crustacea has been determined. The 24-hr $LC_{50}$ values of hexavalent chromium for the brine shrimp (*Artemia salina*) and the zoea larva of the crab (*Sesarma hematocheir*) were 40-70 mg Cr $L^{-1}$ and 56-200 mg Cr $L^{-1}$ respectively (Okubo and Okubo, 1965); the 48-hr $LC_{50}$ values of hexavalent chromium for the grass shrimp (*Palaemonetes pugio*) at 25°C were 21 and 77 mg Cr $L^{-1}$ in water containing 10 and 20 parts per thousand salinity respectively (Fales, 1978); the 96-hr $LC_{50}$ value of hexavalent chromium (as $K_2Cr_2O_7$) for the amphipod *Allorchestes compressa* at 20°C was 5.56 mg Cr $L^{-1}$ (Ahsanullah, 1982); the 96-hr $LC_{50}$ values of hexavalent chromium for the copepod *Cyclops viridis* at 27°C were 92.5, 109.0 and 97.0 mg Cr $L^{-1}$ at pH values of 6, 7 and 8 respectively (Kaviraj
and Konar, 1982); and the 48-hr LC$_{50}$ value of hexavalent chromium for the marine copepod *Tisbe holothuriae* at 24°C was 8.14 mg Cr l$^{-1}$ (Moraïtou - Apostolopoulou and Verriopoulos, 1982). In general, it could be said that invertebrate animals are more sensitive to hexavalent chromium than fish.

2. **Chronic Toxicity**

Up to now we have been concerned with the literature for the acute toxicity of hexavalent chromium on the aquatic organisms. Tests on the toxicity of pollutants for longer periods of exposure time are important to gain some idea about the harmful effects of low concentrations of metals which might be found in natural bodies of water and they could serve in setting up standard values for the maximum allowable concentration of a pollutant in the environment. Olson and Foster (1956) exposed young chinook salmon (*Oncorhynchus tshawytscha*) and rainbow trout continuously to various concentrations of Na$_2$Cr$_2$O$_7$ in running water for periods up to 7 months and found that survival was impaired at 0.08 mg Cr l$^{-1}$ and growth was inhibited at only 0.013 - 0.022 mg Cr l$^{-1}$. Raymont and Shields (1964) reported in *Nereis* that no deaths occurred in control worms in sea water and in toxic concentrations of hexavalent chromium up to 0.5 mg l$^{-1}$ over a period of 5 weeks, but at a concentration of 1.0 mg Cr l$^{-1}$ 50% mortality took place after 3 week exposure. The 3-week LC$_{50}$ value of trivalent chromium for *Daphnia magna* in Lake Superior water was 2.0 mg Cr l$^{-1}$ (Biesinger and Christensen, 1972). The long-term toxic effect of hexavalent chromium on different organisms has also been studied by Benoit (1976) on brook trout over 22 months period and on rainbow trout for 8 months; Reish et al. (1976) on the polychaetes *Neanthes arenaceodentata* and *Capitella capitata*; and Oshida et al. (1981) on the polychaete *N. arenaceodentata*. 

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3. **Tissue Accumulation**

It has been well established that heavy metals tend to accumulate in tissues of aquatic organisms including fish (Knoll and Fromm, 1960; Olson et al., 1973; Lock, 1979; Van Hoof and Van San, 1981; Van der Putte et al., 1981 a and 1982; and many others). In fish, three avenues of entry are available to heavy metals including hexavalent chromium; (1) absorption through the skin, (2) ingestion into the stomach, or (3) entry across the gill membrane. The possibility of chromium gaining entrance via the skin would tend to be discredited due to a consistently low concentration of chromium found in muscle samples (Knoll and Fromm, 1960; Van Hoof and Van San, 1981; Van der Putte et al., 1981 a). This avenue of entry cannot be ruled out entirely, however, since muscle may not bind chromium even if it is present. The assumption that chromium from the environmental media reaches the gut as a result of drinking is somewhat doubtful, since fresh water fish supposedly drink very little water. Fromm and Schiffman (1958) suggested that chromium accumulated in the gut as a result of excretion by liver via the bile. Knoll and Fromm (1960) showed that radioactive chromate placed directly into the stomach of trout failed to be absorbed in significant amounts within 24 hours, thus tending to minimize the importance of uptake via the digestive system. They also showed that tissue distribution of chromium in the oesophageal occluded fish compared favorably with those of normal fish exposed to hexavalent chromium. With these observations in mind it is postulated that the gill is the most likely path for the entrance of hexavalent chromium.

Although there is a considerable amount of literature available concerning the presence of metals in fish, it deals almost exclusively with levels in muscle tissue or in whole fish after chronic exposure. Informa-
tion about the distribution of metals in different tissues is scarce and its value for the study of acute exposures resulting in kills has hardly been explored (Van Hoof and Van San, 1981).

In spite of the amount of data published on the metal contents in fish, comparison of data is quite difficult. The literature considers a wide range of species, in field and in laboratory, with samples analysed by different methods and using different digestion procedures on various tissues or whole body (Calamari et al., 1982). Of all studies concerning chromium accumulation in different tissues of fish, the rainbow trout seems to have attracted more attention by different workers. Rainbow trout exposed to 2.5 mg l\(^{-1}\) hexavalent chromium as K\(_2\)CrO\(_4\) for 12 days showed chromium concentrations in blood in amounts which never exceeded the concentration in the surrounding water. All other tissues studied, except muscle, accumulated chromium in concentrations exceeding that of the environment. The rate of accumulation by the spleen and stomach were of a similar magnitude but less than that of liver, gut, kidney and pyloric caeca. The pyloric caeca accumulated chromium more rapidly than any other tissue studied. The muscle tissue did not accumulate chromium during the exposure period (Knoll and Fromm, 1960). Fromm and Stokes (1962), after exposing rainbow trout to 0.0013 and 0.01 mg l\(^{-1}\) hexavalent chromium as K\(_2\)CrO\(_4\) for 10 days, found that the mean maximum concentration of chromium in fish was 16.6 and 133.6 \(\mu\)g Cr per gram respectively. Kuhnert et al. (1976) found that 2-year old control rainbow trout showed chromium levels of 0.114, 0.156, 0.181 and 0.218 \(\mu\)g Cr per gram wet weight in liver, gill, intestine and kidney respectively. While in trout exposed to 2.5 mg l\(^{-1}\) hexavalent chromium (as chromate) for 48 hours, the chromium content of the same tissues was 0.544, 2.141, 0.579 and 2.164 \(\mu\)g Cr per gram wet weight respectively. Buhler et al. (1977) found small amounts of chromium
in different tissues of 2-year old control rainbow trout. In Hanford
rainbow trout exposed to 2.5 mg Cr 1\(^{-1}\) (as Na\(_2\)Cr\(_2\)O\(_7\)) for 24 hours, the
chromium content in some of the tissues studied was 0.54, 0.91, 2.04,
2.52, 4.04, 5.86, 7.09 and 14.1 μg Cr per gram wet weight for white muscle,
stomach, liver, heart, anterior kidney, gill, posterior kidney and brain
respectively. They also showed that after a 22-day exposure to 2.5 mg Cr
1\(^{-1}\) the highest concentrations of chromium were found in opercular bone,
kidney and gall bladder, and the lowest in white muscle and skin while
other tissues showed an intermediate concentrations of chromium. Singh
and Ferns (1978) determined the chromium concentrations in rainbow trout
fed for 10 weeks with a diet containing 30% by weight of activated sewage
sludge found that the fish accumulated significantly elevated levels of
chromium. Ten Holder et al. (1978) working on rainbow trout exposed to
sodium chromate solutions containing \(^{51}\)Cr, found that the uptake increased
with increasing exposure concentration from 1 to 10 mg Cr 1\(^{-1}\) as well as
with increasing exposure time. They also reported that independent of the
concentration of chromium and duration of exposure, the kidney, liver and
gills accounted for 3, 4 and 13% of the chromium absorbed respectively.
More recently, Van der Putte et al. (1981 a) exposed yearling rainbow
tROUT to \(^{51}\)CrO\(_4\)\(^{2-}\) containing Na\(_2\)CrO\(_4\) solution having a chromium concen-
tration of 40 mg 1\(^{-1}\) for 2 and 4 days at pH values of 7.8 and 6.5. They
found for the 2-day and 4-day exposure experiments at pH 6.5 that the
order of chromium concentrations from lowest to highest were in white
muscle, brain, gonad, heart, stomach, liver, kidney and gill. But with
2-day exposure experiment at pH 7.8 this order was white muscle, brain,
gonad, heart, stomach, liver, gill and kidney. Finally Calamari et al.
(1982) reported that tissue chromium of rainbow trout exposed to 0.2 mg
Cr 1\(^{-1}\) as K\(_2\)Cr\(_2\)O\(_7\) for 30, 90 and 180 days were higher than control.
They found that kidney accumulated the highest concentration of chromium
followed by liver, while muscle accumulated the lowest concentration. The concentration of chromium in these tissues increased with increasing the exposure period.

Accumulation of hexavalent chromium in tissues of other species of fish has also been reported by many workers. Lucas et al. (1970) reported whole body concentrations of chromium in alewife (Alosa pseudoharengus), spottail shiner (Notropis hudsonius) and trout-perch (Percopsis omiscomaycus) collected from Lake Michigan were 1.1, 0.9 and 1.6 mg Cr per gram tissue respectively which showed variation in the metal concentration in the different species of fish. After 14 days of exposure, Hoss and Baptist (1973) found low concentrations of $^{51}$Cr in the muscle of Atlantic croaker (Micropogon undulatus) relative to $^{51}$Cr levels in other organs and tissues such as gills and liver. Elwood et al. (1980) found that muscle and whole body (excluding gastro-intestinal tract) chromium concentrations were not significantly different from each other in bluegills or largemouth bass collected from a lake chronically contaminated with chromates from cooling towers. Riva et al. (1981) showed that gills significantly accumulated chromium in goldfish exposed to different concentrations of hexavalent chromium (as $K_2Cr_2O_7$) ranging from 110-200 mg Cr l$^{-1}$ at 22°C when compared with controls. But there was no relation between the chromium dose and the levels of gill accumulation. Pfeiffer et al. (1982) found in the guppy (Poecilia reticulata) collected from a river containing 0.3 mg Cr l$^{-1}$ that chromium levels in whole fish and soft tissues were 39.97 and 23.31 µg per gram dry weight respectively. On the other hand, Starý et al. (1982) reported that when guppies (Poecilia reticulata) were kept in labelled Cr(III) solutions ($5\times10^{-8}$ mol. l$^{-1}$ and $2\times10^{-7}$ mol. l$^{-1}$), they showed after 1 - 2 hours an appreciable radioactivity which was caused mostly by the absorption of Cr(III) on the
surface of the fish. While fish kept in labelled Cr(VI) solutions (10^{-7} mol. l^{-1} and 2 \times 10^{-7} mol. l^{-1}) showed after a short exposure time only low radioactivity which explain that the absorption of Cr(VI) on the surface of the fish is small. Recently Van Hoof and Van San (1981) exposed the fish rudd (*Scardinius erythrophthalmus*) to acute lethal and subacute non-lethal concentrations of hexavalent chromium (as K_{2}Cr_{2}O_{7}) for 24 hours in water with a hardness of 200 mg l^{-1} as CaCO_{3}. They found in all acute concentrations of chromium that killed fish accumulated a measurable chromium levels in muscle, gill, opercle, liver and kidney, and most elevated values were detected in gill tissue. In all tissues except kidney, chromium levels found in the highest chromium concentration tested (145 mg Cr l^{-1}) were lower than those found after exposure to 80 mg Cr l^{-1} probably because of shorter exposure time before death. Chromium levels in all tissues of killed fish differ significantly from surviving fish exposed to 20 mg Cr l^{-1} which have higher chromium concentrations in opercle, kidney and liver than in gill tissue. On the other hand, they reported that exposure to subacute concentration (16 mg Cr l^{-1}) resulted in tissue levels below detection limit.

pH values have been proved to affect the rate of accumulation of chromium in fish. Riva et al. (1981) found that the chromium levels in gills of goldfish treated with 110, 150 and 170 mg Cr(VI) l^{-1} were significantly higher when pH was not controlled (acidic conditions) than in those in which the pH was maintained close to neutral. Similarly Van der Putte et al. (1981 a) exposed fingerlings of rainbow trout to 2.5 and 16.5 mg Cr(VI) l^{-1} for 4 days at pH 7.8 and 6.5. They found that at the lower pH the whole body and the gill tissue accumulated significantly more chromium than at the higher pH. In addition, the chromium contents in the gill of the fish exposed at the lower pH far
exceeded those in kidney, liver and digestive tract. They also reported higher concentrations of chromium in gill of yearling trout exposed to 40 mg Cr(VI) l\(^{-1}\) at the lower pH than at the higher pH.

4. **Oxygen Consumption**

Several studies have shown that exposure of fish to heavy metals leads to a number of disturbed physiological processes (Alabaster and Lloyd, 1980). Respiration is one of these physiological processes which has been studied to show the effect of poisoning by heavy metals on aquatic organisms (Jones, 1947; Skidmore, 1970; O'Hara, 1971; Sherr and Armitage, 1971; Chaisemartin and Chaisemartin, 1976; Van der Putte et al., 1982).

From the information available in the literature, it could be said that different species of fish may show different patterns in their oxygen consumption upon exposure to heavy metals. These variations depend on the species and on the nature and concentration of the heavy metal. In some, there could be no effect but in others the rate of oxygen consumption is either increased or decreased. For example Fromm and Stokes (1962) demonstrated that in vitro respiration of pyloric caeca, liver and kidney tissues from trout exposed to 1.0 mg Cr(VI) l\(^{-1}\) for as long as 39 days was not different from that of control fish. Skidmore (1970) reported that the rate of oxygen consumption in slightly sedated rainbow trout, exposed to 40 mg Zn l\(^{-1}\), remained steady at the resting level until 80% of the survival time has elapsed, but afterwards there was a rapid collapse in the oxygen uptake. Van der Putte et al. (1982) also found that there was no significant effect of hexavalent chromium on the rate of oxygen consumption of rainbow trout exposed to different concentrations.
of chromium for 4 days. They suggested, however, that alterations in the rate of oxygen consumption in chromium treated fish were possibly masked by variations in spontaneous activity of the fish.

Since the work on the effect of chromium on the rate of oxygen consumption is somewhat scarce, similar studies with other heavy metals will be discussed. Jones (1947) exposed stickleback (Gasterosteus aculeatus) to low concentrations of mercuric chloride, copper sulphate and lead nitrate, found that the rate of respiration increased at first (10 - 20 minutes). Then it declined, but the rate of opercular movement continued to increase reaching a rate of 180 - 240 per minute. It continued at this rate for some time and then fell rapidly when the rate of oxygen uptake was reduced to 38% normal. In largemouth bass exposed to 94 mg Cr(VI) l⁻¹ (half the concentration for 48-hr LC₅₀), Fromm and Schiffman (1958) reported that after up to 23.5 hours of exposure the rate of oxygen consumption was higher than control fish. At 49 hour exposure, the rate was lower than that of control and after 68 hour exposure, it gradually decline to 27% below normal. O'Hara (1971) exposing juvenile bluegills to different concentrations of copper ranging from 0.5 - 5.0 mg l⁻¹, found that there was at first an increase in the rate of oxygen consumption related to the concentration of the metal in the solution. The maximum increase in oxygen utilization occured between 3 and 6 hours after the copper was added. Then the fish entered a phase of apparent recovery with the oxygen consumption rate declining during the next 18 hours to base level. After 24 hours, the oxygen consumption in all fish was below that of the control. Calabrese et al. (1975) reported that the rate of oxygen consumption in gill tissue of the winter flounder (Pseudopleuronectes americanus) was significantly higher than control after exposure to 0.01 mg l⁻¹ mercury for 60 days. But flounder exposed
to 0.005 and 0.01 mg l\(^{-1}\) cadmium, respired at a significantly lower rate than control fish, while fish exposed to 0.005 mg l\(^{-1}\) mercury respired at the same rate as control fish. Hingorani and Diwan (1979) showed that industrial effluents containing heavy metals caused a decrease in the rate of oxygen consumption of the fresh water fish *Labeo rohita*. This decrease was more pronounced as the concentration of the effluents increased and they concluded that this decrease in the rate of oxygen consumption may be due to hypoxia.

It has been suggested that the increase in the rate of oxygen consumption in fish exposed to heavy metals may be due to increase in the activity of the exposed fish (Jones, 1947; Skidmore, 1970). This has been contradicted by O'Hara (1971) who stated that bluegills, which have an increase rate of oxygen consumption after exposure to copper, did not show any increase in activity other than in the rate of opercular movement that would normally be associated with a greater respiratory demand.

However, the decline in the rate of oxygen consumption by fish exposed to heavy metals has been explained in different ways. Some authors suggested that this decrease in the rate of oxygen consumption is due to the accumulation of mucus which cover the gills of fish, thus impairing the function of gills as the site for gaseous exchange (Fromm and Schiffman, 1958; Hingorani and Diwan, 1979; Kaviraj and Konar, 1982). While Van der Putte and Pärt (1982) suggested that oxygen deficiency in rainbow trout exposed to hexavalent chromium may have been caused by gill epithelia damage. On the other hand, it has been shown by Skidmore (1970) that rainbow trout with a reduced arterial oxygen tension caused by exposure to zinc, exhibited a normal rate of oxygen uptake.
Very little work has been done on the recovery in the rate of oxygen consumption of fish exposed to heavy metals. Jones (1947) found that in sticklebacks exposed to 0.0001N mercuric chloride, there was at first an increase in the rate of oxygen uptake and after 20 minutes exposure the rate began to decline. But when the heavy metal solution was replaced at 50 minutes with well-aerated tap water, the rate of oxygen consumption began a slow upward climb, and after 24 hours in the metal-free water the fish appeared to have recovered completely. O'Hara (1971) reported that exposure of juvenile bluegills to different concentrations of copper ranging from 0.5 to 3 mg l\(^{-1}\) for 7 days resulted in an initial increase in the rate of oxygen consumption during the first few hours in copper solutions. Then the rate of oxygen consumption began to decrease and after 7 days in copper solutions this rate was lower than that of control fish. But when these copper treated fish were transferred to copper-free water and kept for 7 days, all the fish had either recovered their base oxygen consumption rate or showed strong evidence of recovery.

The rates of oxygen consumption in a number of invertebrate species have been determined as indicators of physiological stress following exposure to heavy metals. More or less, the results of work carried out in this direction, showed that the rate of oxygen consumption in invertebrates responded in a similar way as mentioned for fish (p.20) after exposure to heavy metals. For example Reeve et al. (1976) showed that the respiration and excretion in *Calanus plumchrus* and *Metridia pacifica* were not significantly changed after exposure to copper in concentrations ranging from 5 to 10 \(\mu g\) l\(^{-1}\). Similarly Engel (unpublished data; in Engel and Fowler, 1979) reported that excised gill tissues of clam exposed to acute concentrations of cadmium (0.1 to 1.0 mg l\(^{-1}\)) showed little effect on their rate of oxygen consumption. However other invertebrates exhibited
an increase in their rate of oxygen consumption following exposure to heavy metals. During 40 hours of exposure of *Daphnia pulex* to 0.01 mg l\(^{-1}\) sodium dichromate solution, Sherr and Armitage (1971) found that the rate of oxygen consumption per animal in the dichromate exposed group was approximately double that in the control group. Because some females produced young during the determination of oxygen consumption, they concluded that it is not possible to state with certainty that the oxygen consumption rate was increased as a result of exposure to dichromate. Also, exposure of *Mytilus edulis* and *Mya arenaria* to silver resulted in increased oxygen consumption (Thurberg et al., 1974). Chaisemartin and Chaisemartin (1976) demonstrated that exposure of two species of crabs *Macropodia rostrata* and *Pachygrapsus marmoratus* to 0.05 mg l\(^{-1}\) lead for 10 days caused an increase in the respiration rate at the end of exposure period in both species. While exposure to 0.1 mg l\(^{-1}\) lead for 6 days using *M. rostrata* and for 9 days using *P. marmoratus* caused an increase in the respiration rate at the end of the first day of exposure but the rate was greatly reduced in both species at the end of the exposure period. Engel and Fowler (1979) found in gill tissue from the oyster (*Crassostrea virginica*) exposed to either 0.05 or 1.0 mg l\(^{-1}\) copper or to 0.1 or 0.6 mg l\(^{-1}\) cadmium continuously for 14 days, that there was a significant increase in the rate of oxygen consumption in 0.1 mg l\(^{-1}\) copper exposed group and in 0.6 mg l\(^{-1}\) cadmium exposed group, but the data were more variable. On the other hand, the rate of oxygen consumption has been found to decrease following exposure to heavy metals in a number of invertebrate species. Exposure of mussel (*Mytilus edulis*) to copper in the form of copper sodium citrate at a concentration of 500 mg l\(^{-1}\) caused 50% depression in the respiratory rate of the whole animals as compared to unexposed controls and sodium citrate-exposed controls (Brown and Newell, 1972). Similarly Scott and Major (1972) reported a
reduction in the rate of oxygen consumption of *M. edulis* exposed to copper. Chaisemartin and Chaisemartin (1976) exposed two species of crabs *M. rostrata* and *P. marmoratus* to 1.0 and 0.5 mg L\(^{-1}\) hexavalent chromium for different periods. They found that the respiration rate in both species decreased after 10 day exposure to 0.5 mg Cr L\(^{-1}\) and after 5 to 7 day exposure to 1.0 mg Cr L\(^{-1}\) at 24°C. Reduction in the rate of oxygen consumption of excised gill tissue of *Mytilus edulis* was observed after exposure to 10 mg Cr L\(^{-1}\) and 1.0 mg Cr g clay indicating an inhibition of ciliary activity (Capuzzo and Sasner, 1977).

5. **Reproduction**

Sublethal effects of toxicants have been defined as a long-term biological effect on an organism as a result of some man-made change in the environment which may not necessarily cause death of the organism, but the effect may cause some alteration of a biological process(es) which would lead to the inability of the organism or its offspring to function normally (Reish, 1974). Such changes could be the prevention of feeding, a change in behaviour which would block some physiological processes, inhibition of reproduction or alter the ecosystem in such a way that the organism could no longer exist there (Reish, 1974; Waldichuk, 1974). The effect of toxicants on reproduction has been stressed (Waldichuk, 1974). But some work on the effect of heavy metals on aquatic organisms including some species of annelids, crustacea and fish could be found in the literature.

Heavy metals have been found to affect the reproduction in a number of annelid worms. Reish (1974) reported that abnormal larvae were induced in
the polychaete *Capitella capitata* when specimens were grown in concentrations as low as 0.01 mg Cu 1\(^{-1}\) and 0.05 mg Zn 1\(^{-1}\). While Reish and Carr (1978) studying the effect of six heavy metals on the reproduction of two species of polychaetous annelids *Ctenodrilus serratus* and *Ophryotrocha diadema*, concluded that there are three generalities which can be stated with regard to the effect of these metals on reproduction in these two species: (1) there was a low concentration of metal at which there was no statistical difference as compared to the control, (2) there was an intermediate concentration at which many of the initial specimens survived and reproduced but at a statistically significant reduction and (3) there was a high concentration of metal in which at least some of the adults lived but were unable to reproduce. Oshida et al. (1981) exposed the polychaete *Neanthes arenaceodentata* to different concentrations of hexavalent and trivalent chromium for 440 days (3 generations) and 293 days (2 generations) respectively. In the hexavalent chromium exposed group, they observed that the reproduction ceased at 100 μg 1\(^{-1}\) and the number of young produced was reduced at the 12.5 to 50 μg 1\(^{-1}\) levels and above. However, polychaetes that lived in trivalent chromium during the test showed no adverse effect in 50400 μg 1\(^{-1}\). Soni and Abbasi (1981) investigated the reproduction of the earthworm *Pheretima posthuma* after exposure to hexavalent chromium in concentrations ranging from 0 to 100 mg 1\(^{-1}\), found that the incidence of sexual reproduction (juvenile formation) was higher in case of Cr treated worms compared with controls. More recently Oshida and Word (1982) exposed the polychaete *Neanthes arenaceodentata* to different concentrations of hexavalent chromium for 2 generations (309 days) found that there were no significant changes in time to spawning in any Cr(VI) concentrations. There were no significant reductions in brood size among the parental (P) generation in polychaetes exposed to Cr(VI); however, the number of young per brood produced in
16.6 µg Cr l⁻¹ was significantly higher than that produced by control polychaetes. First filial generation (F₁) polychaetes that had spawned in 38.2 µg Cr l⁻¹ showed significantly reduced brood sizes when compared with the controls, while polychaetes in the lower Cr(VI) concentrations did not show significant changes in brood size. In addition they could not establish a direct relationship between tissue chromium concentration and reduced numbers of offspring.

The reproduction of some crustacean species exposed to heavy metals has also been studied. Arthur and Leonard (1970) reported that *Gammarus pseudolimnaeus* reproduced in copper concentrations of 8 µg l⁻¹ or less during both 6-week trials. After 9 week's additional exposure the newly hatched amphipods reached adult size in copper concentration of 4.6 µg l⁻¹. Sherr and Armitage (1971) showed that eggs in groups of *Daphnia pulex* exposed to potassium dichromate, were released from the ovaries and developed into young in a shorter time than in the control groups. While Biesinger and Christensen (1972) found that the concentrations of trivalent chromium causing 16% and 50% reproductive impairment in *Daphnia magna* were 330 and 600 µg l⁻¹ respectively. They also gave 16% and 50% reproductive impairment in this animal for other metals. Paffenhöfer and Knowles (1978) found that females of the copepod *Pseudodiaptomus coronatus* which grew and matured in 5 µg l⁻¹ cadmium produced only 50% as many nauplii as did non-cadmium treated females. Recently, Verriopoulos and Moraítou - Apostolopoulos (1981) investigated the impact of different concentrations of hexavalent chromium (0, 0.5, 1.0 and 2.0 mg l⁻¹) on the population dynamics of the copepod *Tisbe holothuriae*. They found that there was no inhibition of the ability to form egg sacs. The development in egg sacs was strongly influenced by chromium and an increase percentage of abortion was observed in direct relationship with chromium concentra-
tions. The number of F₃ offspring was decreased with increasing the chromium concentrations.

In fish, reduction in the number of eggs produced by females of zebra fish (Brachydanio rerio) exposed to phenylmercuric acetate (Kihlström et al., 1971); and by females of the brook trout exposed to 0.35 mg Cr(VI) l⁻¹ (Benoit, 1976) has been reported.

On the other hand, it has been reported that some heavy metals when present in low concentrations enhanced the reproduction in some organisms. Biesinger and Christensen (1972) reported that reproduction in Daphnia magna was stimulated by small metal addition. The percentage of hatching of eggs of zebra fish actually increased at 10 µg l⁻¹ phenylmercuric acetate (Kihlström and Hulth, 1972). This improved hatching at the low phenylmercuric acetate concentration was attributed to its bacteriocidal and fungicidal activity. Similarly Reish and Carr (1978) observed an enhancement of reproduction at lower concentrations of cadmium, copper and mercury in the polychaete Ctenodrilus serratus and at lower concentrations of cadmium, copper, mercury, lead and zinc in the polychaete Ophryotrocha diadema. But they offer no explanation to account for this reproductive enhancement. Also Soni and Abbasi (1981) found that the incidence of sexual reproduction (juvenile formation) in the earthworm Pheretima posthuma was higher in the case of worms treated with hexavalent chromium compared with control worms.

6. Histological Alterations

There have been several studies on the histological changes that take
place in different tissues of fish in the presence of environmental pollutants including salts of heavy metals. A number of studies showed histopathological alterations in gill tissue of fish exposed to zinc (Lloyd, 1960); cadmium (Voyer et al., 1975); hexavalent chromium (strik et al., 1975; Van der Putte et al., 1981 b; Van der Putte and Pärt, 1982). While other studies did not show any alterations in gill tissue of fish (Fromm and Schiffman, 1958; Lloyd, 1960)

However Fromm and Schiffman (1958) reported that exposure to hexavalent chromium in the largemouth bass caused significant changes in the histology of the intestine immediately below the level of pyloric caeca. There was widespread destruction of the intestinal epithelium and the intestinal folds were greatly reduced in size. Apparently exposure to chromium caused a sloughing off of practically all of the epithelium of the gut. This suggests that the digestive function is seriously impaired or lost entirely after exposure to chromium.

The teleost kidney is characterized by tubules surrounded by hemopoetic tissue. Normally the tubular cells are well formed with little cellular debris within the tubule lumen (Baker, 1969). The same author studied the effect of different concentrations of copper on the histology of kidney and liver of the winter flounder (Pseudopleuronectes americanus) for 700 hours exposure period. For both tissues of fish exposed to 560 μg l⁻¹ there was no appreciable change in their histology when compared with control tissues. But in liver of fish exposed to 1000 and 3200 μg l⁻¹ there was fat in the cells around the central vein. In the kidney of fish exposed to 1000 and 3200 μg l⁻¹ there was a considerable change. The hemopoetic tissue was necrotic and very much reduced in volume. The tubule cells themselves were vacuolated and reduced in size. The apical
portion of the tubule cells seemed to disintegrate and the lumen of the tubules showed considerable dilation and contained much dense material. Strik et al. (1975) reported, after exposure of rainbow trout to 10 mg l\(^{-1}\) hexavalent chromium for 15 - 22 days, that there was a severe necrosis of the kidney tubules. In the proximal parts of the nephron the tubular epithelium was completely disrupted and there was loss of nuclei. The cytoplasm of these necrotic cells was fractionated into hyalin globules. In less severely affected cells a hydropic degeneration was noticed. They added that 23 days after the end of the treatment, the kidney was completely restituted in 4 fish kept in clean water. More recently, Van der Putte et al. (1981 b) exposing rainbow trout to 44.8 mg l\(^{-1}\) hexavalent chromium for 96 hours at pH 7.8 found no alteration in the liver of chromium treated fish compared with that of the control. But in kidney there was dilation of the lumen of tubules and there was an increase in the nucleus-to-cytoplasm ratio of the tubule epithelium.

7. Histochemical Staining of Chromium

Before the work published by Suzuki et al. (1978), there have been neither effective reagents for histochemical staining of chromium nor masking reagents for the interfering substances. Because of this, it was not previously possible to be stained. However, an effective dye-stuff for chromium, namely Chrome azurol S (CAS) and a useful method for acceleration of the reaction between Cr and CAS by adding methanol to the staining solution have been recently discovered (Suzuki et al., 1975; Suzuki et al., 1976). The mechanism of the accelerative effect of methanol is yet unknown. The only difficulty remained unsolved was masking the interference due to presence of other heavy metals. This
difficulty was overcome by using a solution containing succinic acid, sodium borate and acetyl-acetone (Suzuki et al., 1978). The masking effect was remarkably influenced by the concentration of acetylacetone. Upon trials Suzuki et al. (1978) suggested a concentration of 15% solution of acetylacetone is ideal for masking other heavy metals excluding chromium within 30 minutes at room temperature. Basically chromium reacts with CAS to produce a chromium-Chrome azurol S chelate complex which usually colored blue in the sections of livers and kidneys of chromium injected rats. They reported that the blue coloration of the stained sections was identified as chromium with an electron probe micro-analyser. Decreasing the pH results in a uniform red staining of the background, which is the original color of the CAS, and no counter staining is necessary if the medium is prepared at a low pH.

Using this technique, Suzuki et al. (1978) were able to stain sections of liver and kidney of rat injected with 5ml of 0.05 M aqueous solution of CrCl₃ from the portal vein. In the liver sections of a chromium injected rat the Cr-CAS complexes filled the sinusoids. Cr-blue infiltrated the hepatic cell cytoplasm except for the nuclear area. The central vein was seen as a red spot. But in kidney sections of a chromium injected rat the Cr-blue appears in the capillary wall of the glomerulus and the interstitials. The proximal tubules and the erythrocytes were stained red which is the original color of CAS. To my knowledge this technique for staining chromium has not yet been used on tissues of fish exposed to chromium.
MATERIALS AND METHODS

General

This work has been carried out to evaluate some effects of hexavalent chromium on three species of fish; namely, rainbow trout (Salmo gairdneri Richardson), minnow (Phoxinus phoxinus Linnaeus), and guppy (Poecilia reticulata [Peters]), and a cladoceran crustacean (Daphnia magna Straus).

Rainbow trout were obtained from two fish farms; Mullingar fish farm in Co. Westmeath and Shankill fish farm in Brittas, Co. Dublin, in the Republic of Ireland. The standard size of fish used throughout this work ranged between 12 - 14 cm. On arrival to the department, the fish were immediately segregated into groups of 6 - 8 individuals. Each group was kept in a plastic fish tank (measuring 64.8 x 39.4 x 16.5 cm) filled to about three quarter of its volume with dechlorinated tap water (some of its properties are presented in Table 1) which had been previously passed through a column of glass wool and activated charcoal. The water was vigorously aerated using compressed air. These tanks were kept in a cold room at a temperature of 9 ±1°C. The fish were fed trout pellets daily and the water in the tanks was changed once every two days. The fish were kept under these conditions for about one week for acclimation before being used in any experiment.

Minnows, on the other hand, were caught using a trap from the Dodder river near Orwell bridge in the city of Dublin. After arrival at the department, the fish were sorted out and those individuals with a
standard size ranging between 5.5 - 6.5 cm were kept in plastic fish tanks (measuring 64.8 x 39.4 x 16.5 cm), each tank containing about 30 fish and was filled to about three quarter of its volume with canal water collected from the Grand canal in Dublin. Some of its properties are listed in Table 1. These tanks were also kept in a cold room at a temperature of 9 ±1°C. The water was continuously aerated with compressed air and the fish were fed daily with live Daphnia obtained from a stock culture maintained in the laboratory. The water in the tanks was changed twice a week. The fish were kept under these conditions for about a week for acclimation prior to any experimentation.

The guppies were reared in the laboratory in two plastic fish aquaria, each of about 30 liters capacity containing dechlorinated tap water. The temperature of the water was maintained at 23 ±0.2°C using an aquarium heater and the water was aerated continuously with compressed air passing through a filter of cotton wool and charcoal. The fish were fed with fish food flakes (TetraMin - a staple food for tropical fish). The water in the tanks was changed once every two weeks. The newly hatched individuals were constantly taken out from the large aquaria and kept in plastic standard aquaria (measuring 32.5 x 22.5 x 20.5 cm) and fed more frequently until fully grown. Individual guppies used in this work had a standard size that ranged between 1.7 - 2.2 cm unless otherwise stated.

Individuals of Daphnia magna used in the present work were obtained from a stock culture maintained in the laboratory as follows: a stock culture of Daphnia magna was maintained in a large glass beaker (of 3 liters capacity) filled with canal water filtered through Watman filter paper (No. 1) and kept at room temperature under natural day-night conditions. A suspension of mixed algae, mostly belonging to the genus
Chlorella, taken from cultures continuously maintained in the laboratory, was used as food. At intervals, the stock cultures of Daphnia were subcultured to maintain the availability of the large number of daphnias required for the experiments.

In almost all the experiments carried out in this work, the animals were starved for 24 hours prior to and during the test. The exceptions to this were the experiments concerning the recovery of the oxygen consumption of guppies and also of daphnias after exposure to hexavalent chromium. This also applies to those experiments investigating the effect of hexavalent chromium on the reproduction of Daphnia magna.

The hexavalent chromium used in the present work was in the form of the potassium dichromate salt $K_2Cr_2O_7$ (Merck). In all fish experiments the different concentrations of chromium solutions used were freshly prepared by dissolving the appropriate weight of the salt in either dechlorinated tap water in the case of rainbow trout and guppy or in canal water in case of minnow. But in the case of the Daphnia experiments a stock solution with a concentration of 1000 mg $K_2Cr_2O_7$ per liter was prepared with glass distilled water and kept in a cold room at a temperature of 9 ± 1°C. An appropriate amount was drawn from this stock solution and diluted with filtered canal water to get the desired concentrations of chromium used in the different experiments. Usually a fresh stock solution of potassium dichromate was prepared every week. The effect of the different concentrations of chromium on the initial and subsequent pH of the dilution waters are shown in Table 2.

In all the toxicity tests, fish were considered dead when they lay on their side on the bottom of the test chamber or when they floated.

*Analysis of the standard dichromate solutions gave a return of 99% Cr.
sideways on the surface of the test solution and showed no signs of opercular movement. *Daphnia* were considered dead upon cessation of body and antennal movements. None recovered from this state. At the end of the test period, the number of dead individuals in each toxicity test was observed and the percent survival was then calculated. The 24-hr LC50 (i.e. the concentration causing 50% mortality in 24 hours) was determined from the survival curve, and also by the log probit method. These gave similar results (see Tables 3, 4 and 5).

The results of all experiments carried out in this work were analysed using a student's t-test.

1. **Acute Toxicity**

1.1 *Rainbow Trout* (*Salmo gairdneri*)

The survival of rainbow trout was determined in a set of six chromium test concentrations; 20, 50, 100, 150, 200 and 250 mg K2Cr2O7 l⁻¹ equivalent to 7.07, 17.68, 35.35, 53.03, 70.70 and 88.38 mg Cr(VI) l⁻¹ respectively, along with a control, for a period of 24 hours. 10 individuals were used in each test concentration and 10 in the control. Each was divided into five groups of 2 specimens and placed separately in a 10 liter test solution in a plastic standard aquarium (measuring 32.5 x 22.5 x 20.5 cm), which served as test chambers, for a period of 24 hours in a cold room at a temperature of 9 ± 0.5°C. The test solutions were continuously aerated with compressed air. The test chambers were covered during the exposure period with plastic mesh to prevent the fish from jumping out. Each test was replicated four times and the number of dead specimens at the end of the experiment was counted.
out of 40 fish. Percent survival in each test solution and 24-hr LC$_{50}$ were determined.

1.2 **Minnow (Phoxinus phoxinus)**

The 24 hours survival of minnows was determined in different concentrations of chromium; 300, 400, 500, 600, 700 and 800 mg K$_2$Cr$_2$O$_7$ 1$^{-1}$ equivalent to 106.05, 141.40, 176.75, 212.10, 247.45 and 282.80 mg Cr(VI) 1$^{-1}$ respectively along with a control in a similar way as described for rainbow trout with the exception that 5 individuals were used in each test chamber with 5 liters of canal water containing the desired concentration of chromium. In all, 20 individuals were used in each test concentration plus a control. The percent survival in each test solution and 24-hr LC$_{50}$ were determined.

1.3 **Guppy (Poecilia reticulata)**

The 24 hours survival of adult guppies in solutions containing 100, 200, 250, 300, 400, 500 and 600 mg K$_2$Cr$_2$O$_7$ 1$^{-1}$ equivalent to 35.35, 70.70, 88.38, 106.05, 141.40, 176.75 and 212.10 mg Cr(VI) 1$^{-1}$ respectively plus a control was determined as follows: for each test concentration 10 individuals were used, which divided into two groups of 5 fish, each placed with 2 liters of test solution in a 4 liters capacity glass beaker (serving as test chamber). The test chambers were immersed in a constant temperature water tank set at 23 ± 0.1°C. Aeration was applied continuously during the test period. The test was repeated twice for each chromium concentration along with a control. The percent survival in each test solution and 24-hr LC$_{50}$ were determined.
Additional experiments were conducted to determine the survival of juvenile guppies (1.1 - 1.2 cm long) in 50, 100, 200, 300, 400 and 500 mg K$_2$Cr$_2$O$_7$ 1$^{-1}$ equivalent to 17.68, 35.35, 70.70, 106.05, 141.40 and 176.75 mg Cr(VI) 1$^{-1}$ respectively plus a control for a period of 24 hours in the same way described for adult guppies.

1.4 *Daphnia magna*

The toxicity of different concentrations of chromium 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0 mg K$_2$Cr$_2$O$_7$ 1$^{-1}$ equivalent to 0.18, 0.35, 0.53, 0.71, 0.88, 1.06, 1.24 and 1.41 mg Cr(VI) 1$^{-1}$ plus a control was determined using 6-12 hour old *Daphnia* for a period of 24 hours. Test individuals were obtained as follows: a number of mature female daphnias carrying eggs* were taken from the stock culture and subcultured in a freshly prepared medium (filtered canal water) which contained an excess amount of mixed algae as food. Usually 6-8 females were kept in each subculture in about 2 liters of freshly prepared culture medium to stimulate the development of the eggs.

For each chromium test concentration 4 pyrex test tubes (15 cm in length and 2 cm in diameter) were used as test chambers. Each test chamber contained 5 individuals and 10 ml of the test solution. This was prepared by diluting an appropriate amount of chromium stock solution in filtered canal water which has been aerated for at least 24 hours to ensure saturation with oxygen. The test chambers were placed in a rack and immersed in a constant temperature water tank set at 23 ± 0.1°C. The test was carried out in darkness. The tests were replicated 3 times for

*The eggs are laid into the brood pouch where they develop as embryos. For convenience all these stages are referred to as eggs throughout this work.*
each chromium concentration along with the control. The percent survival and 24-hr LC$_{50}$ were determined.

Another similar experiment was carried out in the same way with different chromium concentrations ranging from 1.6 - 4.0 mg $K_2Cr_2O_7$ l$^{-1}$ equivalent to 0.57 - 1.41 mg Cr(VI) l$^{-1}$ plus a control for 24 hours.

2. **Chronic Toxicity on *Daphnia magna***

The survival of *Daphnia magna* was measured following exposure to these sublethal concentrations of chromium: 0.1, 0.2, 0.3, 0.5 and 1.0 mg $K_2Cr_2O_7$ l$^{-1}$ equivalent to 0.035, 0.07, 0.11, 0.18 and 0.35 mg Cr(VI) l$^{-1}$ respectively. In addition a control experiment was run in chromium-free water. For each chromium concentration, a group of 30 individuals, 6-12 hour old, was used. The animals were obtained by subculturing mature females taken from a stock culture reared in the laboratory. They were kept individually in pyrex test tubes (19 cm in length and 2.6 cm in diameter) with 40 ml of the desired concentration of chromium solution and 0.5 ml of mixed algae was given as food. The test tubes were placed in a rack and suspended in a constant temperature water tank set at 23 ± 0.1°C with continuous light derived from a fluorescent lamp.

The test solutions in the tubes were changed once every 2 days. Keeping the test solutions for 2 days does not appear to cause a pronounced decrease in chromium concentration through absorption by the algae. This was demonstrated in a preliminary experiment in which the chromium concentration in the algae was analysed. The algae in each test tube accumulated 0.025 µg Cr (S.E.=0.001) in a 2 day period. This was
0.34% of the actual concentration of chromium in the test solution. From these results it appeared that the amount of chromium accumulated by algae during the 2 day period is negligible. The algal concentration was the same as in the experiments.

The animals were inspected daily during the experiment and whenever young Daphnia were observed they were removed. The number of dead animals was recorded until 100% mortality was reached. The percent survival was calculated. The survival curve for each chromium concentration was drawn and the duration required for 50% mortality (LD50) was read from the curves.

Another experiment was conducted in which the survival of groups of Daphnia magna was determined after exposure to 1.0 mg K2Cr2O7 1⁻¹ (0.35 mg Cr(VI) 1⁻¹) for 2, 4, 6 and 8 days and then subsequently transferred to the chromium-free water until 100% mortality was reached, using the same procedure as described above (p.38).

3. Tissue Accumulation

3.1 Rainbow Trout

Fish were exposed to 20, 50, 100, 150 and 200 mg K2Cr2O7 1⁻¹ (7.07, 17.68, 35.35, 53.03 and 70.70 mg Cr(VI) 1⁻¹ respectively) for 24 hours plus a control in the same way described for the acute toxicity (p.35). At the end of the exposure period, the fish which were alive were sacrificed, rinsed once in distilled water and kept in a deep freeze for chromium analyses of the tissues.
Before carrying out analysis all the glassware used was soaked for 24 hours in 25% solution of a concentrated cleaning agent, RBS (Chemical products R. Borghgraef s.p.r.l. Belgium) then washed thoroughly in tap water. In each container an amount of 50% nitric acid (Analar HNO₃ low in chromium) was placed and shaken vigorously for about 2 minutes to eliminate any ions present. Finally the glassware was rinsed in deionized distilled water at least 10 times and then left in an oven for drying.

For chromium analyses of tissues, fish previously exposed to chromium plus a control were taken out from the deep freeze, thawed and 8 tissues; brain, white muscle, gonad, heart, stomach (any food present was removed), liver, gill and kidney were dissected out. For each fish one sample of each tissue was taken and kept in a small glass Petri dish and dried to constant weight at 105°C (around 24 hours). After drying, these samples were cooled and weighed using an Oertling balance. A suitable weight was taken from each tissue in order to get a limited range of AAS absorbance (see p.41) for the different tissues (this was previously determined by a preliminary analysis). Each sample was placed in a large borosilicate-glass digestion tube (25 cm in length and 2.4 cm in diameter) with 3 ml concentrated nitric acid (Analar HNO₃ low in chromium), plus 2 digestion tubes containing 3 ml concentrated HNO₃ serving as blanks. The digestion tubes were placed in a digestion block (Tecator 1006) with an automatic control unit (Model 1008) set at 160°C for 4 hours to complete digestion and then left at 200°C for nearly 2 hours to evaporate most of the liquid. After that each sample was poured into its own volumetric flask which was then filled to the mark with deionized distilled water. For brain and gonad samples 10 ml volumetric flasks were used while for the remaining tissue samples 25 ml volumetric flasks were used. One of the blank samples was diluted to 10 ml and the other to 25 ml.
A series of chromium standard solutions containing 0.00, 0.025, 0.05, 0.075, 0.10 and 0.15 mg Cr(VI) l⁻¹ were prepared from chromic nitrate standard solution.

Chromium analyses were made by electrothermal atomic absorption spectrophotometry using a Perkin-Elmer 372 Flameless Atomic Absorption Spectrophotometer (AAS) equipped with HGA 500 programmer and an auto sampling-system AS-1.

Tissue samples, deionized distilled water, chromium standard solutions and the blank samples were each placed in an appropriately labelled AAS polyethylene sample cup (about 1 ml capacity). The sample cups were arranged on the AAS automatic sampling tray in the following order: deionized distilled water, chromium standard solutions, blanks and tissue samples. After the instrument had warmed up, 20 µl of aliquot from each sample cup was drawn automatically and injected into a graphite tube inside HGA graphite furnace, the sample was dried at 110°C for 20 seconds, charred at 1200°C for 10 seconds and atomized at 2700°C for 6 seconds. Then the absorbance was read directly from the dial of the AAS. Two absorbance readings were taken for each sample. Any absorbance noted in the blank was subtracted from the absorbance readings of tissue samples. Using a computer calculator (Hewlett-Packard HP-97) programmed for a linear regression analysis of the absorbances recorded, the chromium concentration corresponding to the average absorbance were computed in mg Cr(VI) l⁻¹. All results of tissue accumulation were expressed in µg Cr(VI) per gram dry weight. For each tissue the chromium analysis was repeated 5 times using tissue samples from 5 different fish, and the final results are presented as mean of 5 samples ± 1 standard error.

The method could detect 1 µg.l⁻¹ Cr. To determine the loss of Cr during the analytical procedures a known volume of a Cr solution containing 1.76 µg Cr was added to a number of tissue samples. The mean recovery (3 estimations) was 96.6% in the case of gill and 92% in the case of liver.
3.2 Minnow

Accumulation of chromium in brain, white muscle, gonad, heart, stomach (without food), liver, gill and kidney was determined after fish have been exposed to 300, 400, 500, 600 and 700 mg K$_2$Cr$_2$O$_7$ 1$^{-1}$ (106.05, 141.40, 176.75, 212.10 and 247.45 mg Cr(VI) 1$^{-1}$ respectively) for 24 hours plus a control. For exposing the fish to chromium the same procedure described earlier (p.36) for acute toxicity tests was followed.

Analysis of chromium in the different tissues of both chromium treated and control fishes has been carried out following the same procedure described for chromium analysis in rainbow trout with the following exceptions: small borosilicate-glass digestion tubes (15 cm in length and 1.6 cm in diameter) were used, each with a tissue sample and 1 ml of concentrated nitric acid. The tissue samples and the blanks were digested using Tecam dri block (Model DB-4) set at 100°C for 2 hours. Then the temperature of the block was raised to 160°C to evaporate most of the solution. The content of each tube was then poured into its own 10 ml volumetric flask and made up to the volume with deionized distilled water.

The chromium concentration in tissues is presented as a mean of five samples, each taken from different fish ± 1 standard error and expressed in µg Cr(VI) per gram dry weight.

3.3 Guppy

The accumulation of chromium in brain, white muscle, gonad, heart, stomach (without food), liver and gill was determined in adult guppies which had been exposed to 100, 200, 250 and 300 mg K$_2$Cr$_2$O$_7$ 1$^{-1}$...
(35.35, 70.70, 88.38, and 106.05 mg Cr(VI) \text{ l}^{-1} \text{ respectively}) \text{ for 24 hours plus a control. Exposure to chromium was carried out in a similar way as in the acute toxicity test described earlier (p.36). Because of the small size of the fish, one tissue sample (except for brain and muscle) for each chromium determination was dissected out from each of 3 fish in order to obtain sufficient material for chromium analysis. Material from brain and muscle was taken from one fish for each chromium determination. It was difficult to dissect out the brain because of its tiny size so it was removed from the head along with the surrounding part of the skull.}

The samples, after drying and weighing, were digested following the same procedure used for chromium analysis in minnow's tissues. On completion of the digestion process each tissue sample was diluted to 10 ml in a volumetric flask, except heart samples which were diluted to 5 ml with deionized distilled water.

The chromium concentrations in the samples were determined similarly as described for rainbow trout (p.39). The results are presented as a mean of 5 samples taken from different fish ± 1 standard error and expressed in \( \mu \text{g} \text{ Cr(VI)} \text{ per gram dry weight.} \)

An additional experiment was conducted to determine the chromium concentration in juvenile guppies exposed to 50, 100, 200, 300 and 400 mg \( \text{K}_2\text{Cr}_2\text{O}_7 \text{ l}^{-1} \) (17.68, 35.35, 70.70, 106.05 and 141.40 mg Cr(VI) \text{ l}^{-1} \text{ respectively}) for 24 hours plus a control following the same procedure described above. The body of each fish was divided into 3 parts; anterior portion including the head and the gills, middle portion containing the viscera, and a posterior portion representing the part from the anal opening to the beginning of the caudal fin.
The results are presented as a mean of 5 samples, each taken from different fish ± 1 standard error and expressed in μg Cr(VI) per gram dry weight.

4. Oxygen Consumption

4.1 Guppy

Adult male guppies were used to determine the effect of chromium exposure on their oxygen consumption and the subsequent recovery of the oxygen consumption after transferring the chromium treated fish to chromium-free water. Three sets of experiments were carried out using 3 different concentrations of chromium which were 20, 100 and 300 mg K₂Cr₂O₇ l⁻¹ (7.07, 35.35 and 106.05 mg Cr(VI) l⁻¹ respectively).

In each set, before treatment with chromium, individual fish serving as control were taken and their oxygen consumption was determined. Then each individual fish was exposed to the chromium solution separately in a 800 ml glass beaker containing 400 ml of the appropriate chromium concentration. The treatment beakers were immersed in a constant temperature water tank set at 23 ± 0.1°C and aerated continuously throughout the course of the experiment. At the end of 24 hour exposure period to chromium, individuals were taken and their oxygen consumption was determined in dechlorinated tap water. Then the fishes were kept individually in 800 ml glass beakers containing 400 ml chromium-free water immersed in the water tank to allow recovery from chromium treatment. The oxygen consumption of fish was determined daily during the recovery period which was 2 days for fishes exposed to 20 mg K₂Cr₂O₇ l⁻¹ (7.07 mg Cr(VI) l⁻¹) and 7 days
for those fish exposed to 100 and 300 mg $K_2Cr_2O_7 \cdot 1^{-1}$ (35.35 and 106.05 mg Cr(VI) $1^{-1}$). During the recovery period the water was continuously aerated, the fish were fed daily and the chromium-free water was changed once every 2 days. The oxygen consumption of the same individual fish was followed from the beginning to the end of the experiment.

The oxygen consumption was determined using a Gilson differential respirometer. Fish were kept individually in a 65 ml reaction flask with 50 ml chromium-free water. 0.4 ml of 10% KOH was placed in the side well with a piece of filter paper for carbon dioxide absorption. Oxygen consumption was determined at a temperature of $23 \pm 0.01^\circ C$ and at a shaking rate of 72 S.P.M. In all determinations, flasks containing the fish were left for about half an hour at the beginning to allow equilibrium to take place and to overcome any disturbance of the fish due to handling. Readings for each flask were recorded at 15 minute intervals for 2 hours. The results were converted to standard conditions by multiplying the micrometer reading of the instrument by a correction factor. Then the results are expressed in $\mu l \text{O}_2$ per gram wet weight per hour.

4.2 Daphnia magna

A number of sets of experiments were conducted to evaluate the effect of chromium on the oxygen consumption of Daphnia magna. The oxygen uptake of both control and chromium treated daphnias was determined by the Cartesian diver microrespirometer as described by Klekowski (1971) for 2-day and 3-day old animals reared individually in pyrex test tubes (19 cm in length and 2.6 cm in diameter) immersed in a constant temperature water tank set at $23 \pm 0.1^\circ C$. 

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The Cartesian divers were made as follows: Thin-walled capillary tubes (of about 2 mm in diameter) were made by pulling apart rapidly the opposite ends of melted pyrex test tubes (2 cm in diameter). From these capillary tubes 2 pieces which fit tightly inside one another were selected for the making of one diver. Using a micro-burner, one end of the larger tube was sealed. The other end was melted and then sealed by pulling with a fine forceps, this end was expanded by heating uniformly on all sides to form a small regular bulb which constituted the diver head-bulb. After cooling, the excess length of the diver was cut off. A suitable length of the other piece of capillary tube was taken to be used as the stopper of the diver. The stopper end which was to be inserted inside the diver was sealed with the micro-burner. At a suitable length, the other end of the stopper was sealed and thickened to some extent to form the tail of the stopper. The approximate length of the divers used in these experiments ranged between 3 - 3.5 cm from the head-bulb to the end of the tail.

Braking pipettes were made to be used in the calibration of the divers. A thin-walled capillary tube (about 15 cm long) was heated evenly at its mid point and then drawn out quickly on either side of the heated point to a very fine bore, and broken in two. The capillary tube was mounted in an open-ended glass tube (about 5 mm in diameter) with the fine bore inside using sealing wax. A drop of mercury was drawn up into the capillary and its length was measured in different parts of the capillary. It must have the same length of mercury throughout (i.e. the capillary must have a constant inner diameter).

Some distilled water was drawn up into the capillary of the braking pipette, its length was measured and blown out into a small tin-foil
weighing dish. The water was weighed and the volume of the water was calculated per 1 mm length, taking the density of the water to be 1. This was used to give the gas volume per 1 mm length of the braking pipette capillary.

The divers were calibrated in order to determine the gas volume (diver constant), at which the diver floats at a definite level, i.e., equilibrium level. The diver was submerged and filled with water. A measured length of air bubble (with mm graph paper) was inserted into the neck of the diver using the braking pipette. The water behind the air bubble was replaced by 0.1 N NaOH solution then the diver was closed by turning the stopper gently until firm. Closing the diver should be done while it is immersed in the sodium hydroxide solution. The floating of the diver in the flotation fluid (sodium hydroxide solution) was checked. If the diver sinks, a small piece of glass from the stopper tail was removed and if it rises, a small piece of glass was added to the tail until the diver floats. Finally each calibrated diver was marked and its gas volume was recorded.

For the determination of oxygen consumption, a single Daphnia was dropped inside the diver head-bulb, after the diver was filled with filtered canal water and immersed in a crystalline dish full of canal water. The appropriate length of air bubble was introduced into the neck of the diver behind the water with organism. The water behind the air bubble was replaced with 0.1 N NaOH solution and the diver was stoppered. Each diver was placed vertically into the flotation vessel filled with 0.1 N NaOH solution (the flotation vessels containing the sodium hydroxide solution were previously boiled for at least 45 minutes to remove all the air bubbles). The flotation vessels then were attached
to a series of stoppers connected at one end to a manometer and at the other end with rubber tubing to a syringe. This was used to apply pressure in order to raise or lower the divers. The flotation vessels containing the divers were immersed in a constant temperature water bath set at 23 ± 0.01°C and left for 15 minutes for equilibrium. Readings for each diver were recorded at 15 minute intervals for 90 minutes. At the end of each determination the animals were taken out. The divers were cleaned by rinsing twice in 0.1 N H$_2$SO$_4$ solution then rinsed several times in distilled water and dried in the oven.

In the first set of experiments, 20 individuals of 2-day old Daphnia were obtained as usual by subculturing adult females from the stock culture. Immediately the oxygen consumption of those individuals was determined. They were then divided into 2 groups of 10 individuals each. Animals of the first group were kept separately for 24 hours in pyrex test tubes, each containing 40 ml of filtered canal water with no food. But in the second group, animals were exposed to 1.0 mg K$_2$Cr$_2$O$_7$ l$^{-1}$ (0.35 mg Cr(VI) l$^{-1}$) for 24 hours by placing individuals separately in pyrex test tubes with 40 ml of chromium test solution with no food as well. At the end of the 24 hours, the oxygen consumption of 3-day old individuals in group 1 (control) and group 2 (chromium treated animals) was determined. This experiment was repeated following the same procedure with the exception that 2-day old individuals of both groups (i.e. control and chromium treated animals) were fed by adding 0.5 ml of mixed algae to each test tube in order to investigate any possible effect of food on the rate of oxygen consumption. In the second set, the effect of exposure of fed Daphnia to 0.1 and 0.5 mg K$_2$Cr$_2$O$_7$ l$^{-1}$ (0.035 and 0.18 mg Cr(VI) l$^{-1}$) was determined following the same procedure described above.
The oxygen consumption of the same individual *Daphnia* was followed from the beginning to the end of the experiment. Each result is presented as a mean of the rate of oxygen consumption determined for at least 8 individuals ± 1 standard error and expressed in μl O₂ per animal per hour.

5. **Reproduction of *Daphnia magna***

A series of experiments were conducted to determine the effect of 0.1, 0.2, 0.3, 0.5, and 1.0 mg K₂Cr₂O₇ l⁻¹ (0.035, 0.07, 0.11, 0.18 and 0.35 mg Cr(VI) l⁻¹ respectively) on the rate of reproduction in *Daphnia*. For each chromium concentration a group of 30 individuals was used. The experiment was started with 6 - 12 hour old animals kept individually in pyrex test tubes (19 cm in length and 2.6 cm in diameter), each containing 40 ml of the proper chromium test solution to which 0.5 ml of mixed algae was added as food. The test tubes were placed in a rack and suspended in a constant temperature water tank set at 23 ± 0.1°C under a fluorescent light switched on continuously during the course of the experiment. The test solution was changed every 2 days with a freshly prepared solution. Keeping the test solution for 2 days does not appear to cause a pronounced decrease in chromium concentration by loss to algae, as was shown by a preliminary experiment. From the results obtained it appeared that the amount of chromium accumulated in algae during 2 days is negligible (see p.38).

Animals were inspected daily to observe the formation of eggs and emergence of young. The number of young were counted and discharged. The experiment was terminated when the last *Daphnia* died. Along with these
experiments, the reproduction rate of a group of 30 individuals reared in chromium-free canal water was used as control. From the observations made, the mean of the peak number of young produced per female, the mean of the total number of young produced per female, the mean life span and the mean period of egg incubation with the standard error were computed.

Following the same procedure, an additional experiment was carried out to determine the rate of reproduction in 4 groups of Daphnia magna (each of 30 individuals) which were exposed to 1.0 mg K$_2$Cr$_2$O$_7$ 1$^{-1}$ (0.35 mg Cr(VI) 1$^{-1}$) for 2, 4, 6 and 8 days, and then transferred to and reared in chromium-free water. This experiment was carried out to investigate the ability of Daphnia to recover from exposure to hexavalent chromium.

6. Histological Alterations

Individual minnows were exposed to 500 mg K$_2$Cr$_2$O$_7$ 1$^{-1}$ (176.75 mg Cr (VI) 1$^{-1}$) for 24 hours as described for the acute toxicity test (p.36). The guppies were exposed to 400 mg K$_2$Cr$_2$O$_7$ 1$^{-1}$ (141.40 mg Cr(VI) 1$^{-1}$) for 24 hours and 150 mg K$_2$Cr$_2$O$_7$ 1$^{-1}$ (53.03 mg Cr(VI) 1$^{-1}$) for 96 hours. The same procedure as with acute toxicity test (p.36) was followed except that the chromium test solution was changed every day for the 96-hour test. At the end of the test living individuals from both species (chromium exposed and control fishes) were anaesthetized in a solution of 150 mg MS-222 1$^{-1}$. For minnow liver and kidney were dissected out. For guppies a small portion from the middle region of the body (containing liver and kidney) was chopped off. The tissues were then fixed in Bouin's fluid for 24 hours. 6 μm thick paraffin sections were made from the
tissues and stained with Hematoxylin and Eosin.

7. Histochemical Staining of Chromium

7.1 Rainbow Trout

Two groups of rainbow trout were used. The first group was exposed to 50 mg $K_2Cr_2O_7$ l$^{-1}$ (17.68 mg Cr(VI) l$^{-1}$) for 24 hours and the second group was exposed to 20 mg $K_2Cr_2O_7$ l$^{-1}$ (7.07 mg Cr(VI) l$^{-1}$) for 96 hours similarly as described for the acute toxicity test (p.35) except that the chromium test solution was changed every day in the 96 hours experiment. At the end of the exposure time, live fish from each group and from control were anaesthetized in a solution of 150 mg MS-222 l$^{-1}$. Immediately after that, liver and kidney were dissected out from these fish, frozen right away in liquid nitrogen and kept in a cryostat chamber set at $-25^\circ$C for about half an hour. 10 μm thick sections were prepared by cryostat, fixed to microscopic slides and incubated in a Chrome azurol S staining solution for 24 hours at room temperature as described by Suzuki et al. (1978). At the end of the staining period, the sections were rinsed in distilled water then dipped into the masking solution for 25 minutes to decolorize the chelate complex of other metals which might be present in the tissue. Because of the weakly acidic level of both staining and masking solutions, the background was stained red while chromium was stained blue so there was no need to use counter staining. The sections, after the masking process was completed, were rinsed in distilled water then dehydrated and mounted in neutral balsam.
A number of adult guppies were exposed to 400 mg $\text{K}_2\text{Cr}_2\text{O}_7 \, 1^{-1}$ (141.40 mg Cr(VI) $1^{-1}$) for 24 hours and 150 mg $\text{K}_2\text{Cr}_2\text{O}_7 \, 1^{-1}$ (53.03 mg Cr(VI) $1^{-1}$) for 96 hours in a similar way as described for acute toxicity test (p.36) except that the chromium test solution was changed every day in the 96 hours experiment. At the end of the test period live fish from both chromium solutions along with the control were anaesthetized in a solution of 150 mg MS-222 $1^{-1}$. Then immediately a small portion from the middle region of the fish body (containing liver and kidney) was chopped off. The chopped portions were frozen in liquid nitrogen. Then they were sectioned and stained following the same procedure described for the rainbow trout (p.51).
RESULTS

1. Acute Toxicity

1.1 Rainbow Trout

The toxic effect of different concentrations of hexavalent chromium on rainbow trout for 24 hour exposure periods is shown in Fig. 1. The toxicity of Cr increases with increasing chromium concentration and the figure is more or less a reversed sigmoid curve. The 24-hr LC$_{50}$ (lethal concentration of chromium causing 50% mortality over 24 hour exposure period) is 40.30 mg Cr l$^{-1}$ as shown in Table 3.

1.2 Minnow

The results of exposure to different concentrations of hexavalent chromium for 24 hours are shown in Fig. 2. It is clear that the toxicity of chromium increases with increasing chromium concentration. The survival curve is a reversed sigmoid. The toxicity increased sharply as the chromium concentration increased from 141.40 to 212.10 mg Cr l$^{-1}$. Then there was a gradual increase until 100% mortality was reached. The 24-hr LC$_{50}$ was 181.35 mg Cr l$^{-1}$ as shown in Table 3.

1.3 Guppy

The survival of adult and juvenile guppies exposed to different concentrations of hexavalent chromium for 24 hours is shown in Fig. 3. Similarly this figure shows that increasing chromium concentration causes
an increase in the toxicity of the metal. At the lower concentrations of chromium adult guppies were more resistant than the juveniles. But at concentrations above 106.05 mg Cr l⁻¹, the survival of both adult and juvenile guppies was more or less similar. At these concentrations the survival of both groups of fish has dropped sharply as shown in Fig. 3.

The 24-hr LC₅₀ for adult and juvenile guppies was 126.38 and 123.73 mg Cr l⁻¹ respectively as given in Table 3. There was a little difference in the toxicity of chromium between adult and juvenile guppies.

1.4 Daphnia magna

The toxicity results of different concentrations of hexavalent chromium for 24 hours are shown in Fig. 4. The survival curve represents a typical reversed sigmoid. The results of a similar test are shown in Fig. 5. When comparing the results of the two experiments it appears that the pattern of survival in both groups is similar. But the survival curve in the second experiment (Fig. 5) is shifted slightly towards the right which indicates that this group of Daphnia was more resistant to chromium than the first group (Fig. 4).

The 24-hr LC₅₀ for the 1st and 2nd experiments were 0.85 and 1.10 mg Cr l⁻¹ respectively as shown in Table 3. These differences seem to be a result of either individual variation or it could be due to the fact that the two experiments were carried out at different times of the year.

2. Chronic Toxicity on Daphnia magna

The survival of groups of Daphnia magna reared in canal water con-
taining 0.00 (control), 0.035, 0.07, 0.11, 0.18 and 0.35 mg Cr \(l^{-1}\) is given in Fig. 6-A, B, C, D, E and F respectively. The results clearly show that the survival at the three highest chromium concentrations (D, E and F) was lower than that of the control (A). The results also show that the survival decreased as the chromium concentration increased as shown in Fig. 6-D, E and F. On the other hand it seems that the survival in the remaining two chromium concentrations (B and C) was higher than that of the control (A) especially after 40 days has elapsed.

The LD\(_{50}\) (days) of the groups of Daphnia in the experiments mentioned are given in Table 4. This also shows that the LD\(_{50}\) of the control was strangely lower than the LD\(_{50}\) of Daphnia reared in 0.035 and 0.07 mg Cr \(l^{-1}\). But the LD\(_{50}\) of the control animals was much higher than the LD\(_{50}\) of Daphnia reared in 0.11, 0.18 and 0.35 mg Cr \(l^{-1}\). This indicates that there was a sharp decrease in the LD\(_{50}\) at these higher concentrations.

A further experiment was carried out to determine any possible recovery of Daphnia magna in Cr-free water after exposure to 0.35 mg Cr \(l^{-1}\) (which was the highest chromium concentration tested in the previous experiment) for 0, 2, 4, 6, and 8 days. The results of this experiment are shown in Fig. 7-A, B, C, D and E. It appears that there was not much difference between the survival of the first four groups (A, B, C and D) throughout all stages of the experiment as there are overlaps in the survival curves of these groups from the beginning until 100% mortality was reached. On the other hand the survival duration of the fifth group (E) was highly reduced when compared with the survival duration of the first four groups. The same could be said about the LD\(_{50}\) (days) of these groups of Daphnia as given in Table 5. In calculating the LD\(_{50}\), the exposure period to chromium before transferring the animals to chromium-
free water was taken into consideration. It is evident that Daphnia were able to recover when exposed to 0.35 mg Cr l⁻¹ for up to 6 days but they fail to recover when they are exposed to chromium for 8 days.

3. Tissue Accumulation

3.1 Rainbow Trout

Analyses of hexavalent chromium in 8 different tissues taken from individuals exposed to different concentrations of chromium for 24 hours along with controls are given in Table 6 and shown in Figs. 8, 9, 10 and 11. The results show that the accumulation of chromium in all tissues increased with increasing the exposure concentrations. Among all tissues analysed, the highest accumulation was found in the gill, kidney and liver. While the lowest accumulation was found in the brain and muscle with the gonad, heart and stomach in between.

The results of the statistical analyses of chromium accumulation in the different tissues are also given in Table 6. In all concentrations of chromium tested, the accumulation of the metal in stomach, liver, gill and kidney was significantly different than that of the control (P<0.05 or P<0.01). But in heart and brain, the difference was significant in all test concentrations (P<0.05 or P<0.01) except at 7.07 mg Cr l⁻¹ (P>0.05). With gonad the difference was significant only in fish exposed to the two highest concentrations (P<0.05 or P<0.01). The chromium content of muscle showed a significant difference from the control at the three highest concentrations (P<0.01).
3.2 Minnow

Accumulation of chromium in 8 different tissues taken from individuals exposed to different concentrations of hexavalent chromium for 24 hours along with controls is given in Table 7 and shown in Figs. 12, 13, 14 and 15. It is obvious from the results that tissues accumulate more chromium as the test chromium concentrations were increased. The curves showing the chromium accumulation in the tissues were more or less exponential in shape. The highest concentrations of chromium were found in gill, stomach, liver and kidney. While the lowest concentration was found in the muscle with brain, gonad and heart in between.

The chromium concentrations in all tissues analysed were significantly different from control tissues (P<0.05 or P<0.01) except in case of brain taken from fish exposed to 106.05 mg Cr l⁻¹ (P>0.05) as shown in Table 7.

3.3 Guppy

Accumulation of chromium in various tissues of adult guppies exposed to different concentrations of hexavalent chromium for 24 hours along with controls is given in Table 8 and shown in Figs. 16, 17, 18 and 19. It is also evident that the metal concentrations in each tissue increased with increasing chromium test concentrations except for tissues taken from fish exposed to 88.38 mg Cr l⁻¹. Here the chromium concentration in the tissues was less than that in tissues taken from fish exposed to lower chromium concentration (70.70 mg Cr l⁻¹) except for heart and liver tissues. This deviation could be due to the fact that the exposure to this chromium concentration was conducted a few months after the original experiment was carried out. The reason for adding this new chromium concentration was due to the high mortalities caused by exposure
to chromium concentrations higher than 106.05 mg Cr l\(^{-1}\) which made it impossible to obtain enough tissue for chromium analyses.

The highest chromium concentrations were found in stomach, gill and liver, and the lowest concentration was found in the muscle with brain, gonad and heart in between. The chromium concentrations in all tissues analysed were significantly different from control tissues (P<0.05 or P<0.01) as shown in Table 8.

Accumulation of chromium in the anterior, middle and posterior body regions of juvenile guppies exposed to different concentrations of hexavalent chromium for 24 hours along with controls is given in Table 9 and shown in Fig. 20. Mostly the chromium content in the different regions increased with increasing the chromium test concentration. In the anterior and middle regions of the body, large amount of chromium accumulated after exposure to the lower concentrations and then increased slightly. But in case of the posterior region the accumulation was increased more or less proportionally.

The chromium content of all regions was significantly different than the control (P<0.01) as shown in Table 9. The highest concentrations of chromium were found in the middle region and the lowest were found in the posterior region.

4. **Oxygen Consumption**

4.1 **Guppy**

The effect of exposure to different concentrations of hexavalent
chromium for 24 hours on the rate of oxygen consumption and the subsequent recovery was followed as shown in Figs. 21 - 26 and in Tables 10, 11 and 12. All the results indicate that the rate of oxygen consumption was dropped after exposure to chromium. In fish exposed to 7.07 mg Cr l\(^{-1}\), the rate of oxygen consumption was not significantly lower than that of the control fish (P>0.05). But with the remaining two chromium concentrations (35.35 and 106.05 mg Cr l\(^{-1}\)) there was a decrease in the oxygen consumption which was significantly different from that of the control fish (P<0.01). The percentage fall in the rate of oxygen consumption was 4.0, 48.7 and 19.7 in fish exposed for 24 hours to 7.07, 35.35 and 106.05 mg Cr l\(^{-1}\) respectively.

It is clearly evident from the results shown in Figs. 23 and 25 and Tables 11 and 12 that the chromium treated fish show recovery in their oxygen uptake after being transferred to chromium-free water. Both groups had nearly recovered after 24 hours in chromium-free water as their oxygen consumption was not significantly different from that of the control fish (P>0.05). This rate of oxygen uptake remained not significantly different (P>0.05) from that of the control fish for a period of 7 days in the recovery conditions. Figs. 24 and 26, on the other hand, show the rate of oxygen consumption of representative individual fish prior to chromium exposure, immediately at the end of the exposure period to chromium and at different intervals in chromium-free water. These figures show more or less the same pattern as mentioned above.

On the other hand, the rate of oxygen consumption in fish exposed to 7.07 mg Cr l\(^{-1}\) continued to decrease even after the fish were transferred to chromium-free water. But, however, the rate of oxygen uptake after the
24 hour recovery period was not significantly different from the control (P>0.05), while it was significantly different from the control after 2 days in the recovery conditions (P<0.01).

4.2 *Daphnia magna*

The rate of oxygen consumption of fed 2-day old and 3-day old fed and starved control animals along with 3-day old fed and starved animals exposed to 0.35 mg Cr l$^{-1}$ for 24 hours is shown in Fig. 27 and given in Tables 13 and 14. It is clear from the results that starvation for 24 hours caused a highly significant decrease (P<0.01) in the rate of oxygen consumption as compared with that of fed animals in both control and chromium treated animals. The values of oxygen consumption in representative individuals of fed 2-day old and 3-day old starved control and chromium treated *Daphnia* are shown in Fig. 28, while Fig. 29 shows the results of a similar experiment using fed animals. These two figures also show that starved animals consumed less oxygen than fed animals in both control and chromium treated *Daphnia*. It is also clear from the results that 3-day old fed control animals consumed far more oxygen than 2-day old control (Figs. 27 - A and 29 - A), while 3-day old starved control animals consumed much less oxygen than the 2-day old control (Figs. 27 - B and 28 - A). But both fed and starved 3-day old chromium treated animals consumed less oxygen than 2-day old control (Fig. 27 - A and B). In order to eliminate any factor other than chromium which might affect the rate of oxygen consumption in *Daphnia*, all the remaining experiments investigating the effect of chromium on oxygen uptake in *Daphnia* were carried out with fed individuals.

Fig. 30 and Tables 14, 15 and 16 show the effect of exposure for 24...
hours to different concentrations of chromium on the rate of oxygen consumption in Daphnia. Exposure to both 0.35 and 0.18 mg Cr l\(^{-1}\) for 24 hours caused a sharp decline in the rate of oxygen consumption when compared with control animals (P<0.01) as shown in Fig. 30 - C and B. It is also clear that the reduction in the oxygen consumption increased with increasing the chromium concentration. On the other hand, the rate of oxygen consumption in animals exposed to 0.035 mg Cr l\(^{-1}\) was more or less similar to that of the control (Fig. 30 - A and Table 16), as there was no significant difference in the rate of oxygen consumption of these two groups (P>0.05). The oxygen consumption of representative control individuals and individuals exposed to different chromium concentrations is shown in Figs. 29, 31 and 32.

A comparison of the rate of oxygen consumption of 3-day old Daphnia exposed to different concentrations of chromium for 24 hours is shown in Fig. 33. This figure indicates that exposure to very low concentration of chromium (0.035 mg Cr l\(^{-1}\)) does not seem to alter the rate of oxygen consumption as there was no significant difference from the control (P>0.05). While the other two higher concentrations tested caused a sharp decrease in the rate of oxygen consumption which was significantly different from that of the control (P<0.01).

5. Reproduction of Daphnia magna

The results of the effect of different concentrations of chromium on some aspects of reproduction in Daphnia magna are shown in Figs. 34, 35, 36 and 37 and are given in Table 17. As shown in Table 17 the time required for the appearance of the 1st group of eggs was significantly
higher in animals reared in 0.035 mg Cr $1^{-1}$ ($P<0.05$) when compared with the control. But it has been found that there was no significant difference between Daphnia reared in 0.07, 0.11 and 0.18 mg Cr $1^{-1}$ and the control ($P>0.05$). On the other hand, this period was significantly longer in Daphnia reared in 0.35 mg Cr $1^{-1}$ ($P<0.01$) when compared with the control.

The time required for the production of the 1st group of young was significantly longer only in the animals reared in 0.35 mg Cr $1^{-1}$ ($P<0.01$), but at the lower concentrations of chromium it seems that chromium has no significant effect ($P>0.05$).

The concentration of 0.035 mg Cr $1^{-1}$ seems to have no effect on the peak number of young produced per one female. But all the remaining chromium concentrations caused a decrease in the peak number of young which was significantly different from that of the control ($P<0.05$ or $P<0.01$) as clearly shown in Table 17 and Fig. 34.

The total number of young produced per one female during its life span was significantly higher in Daphnia reared in 0.035 mg Cr $1^{-1}$ than the control ($P<0.05$) which might suggest that this low concentration of chromium stimulates the production of more young. The production of young in the group reared in 0.07 mg Cr $1^{-1}$ was not significantly different from that of the control ($p>0.05$). However rearing in 0.11, 0.18 and 0.35 mg Cr $1^{-1}$ highly reduced the total number of young produced per female and the difference was highly significant when compared with the control ($P<0.01$). This reduction was increased with increasing the chromium concentration as shown in Table 17 and Fig. 35.

All the chromium concentrations appear to have an effect on the life
span of Daphnia. At the lower concentrations (0.035 and 0.07 mg Cr l\(^{-1}\)), the mean life span was significantly higher than the control (P<0.05) while at the other three higher concentrations (0.11, 0.18 and 0.35 mg Cr l\(^{-1}\)), there was a highly significant reduction in the mean life span from the control (P<0.01). This reduction also increased with increasing the chromium concentration (see Table 17 and Fig. 36).

The mean number of young produced per female per day does not seem to be affected by the chromium concentrations up to 0.07 mg Cr l\(^{-1}\) (P>0.05). But beyond that, there was a highly significant reduction in that mean number compared with the control (P<0.01).

The mean period of egg incubation (the duration required from appearance of eggs to emergence of young) was not affected by chromium up to 0.18 mg Cr l\(^{-1}\). At the higher concentration (0.35 mg Cr l\(^{-1}\)) this period was significantly shorter than the control (P<0.01) as shown in Table 17 and Fig. 37.

It could be concluded from the above results that exposure to the two lower concentrations of chromium caused an increase in the total number of young produced by 24.3% and 22.7% (reproductive stimulation) for 0.035 and 0.07 mg Cr l\(^{-1}\) respectively. These two chromium concentrations also seem to cause an unexpected increase in the life span by 22.7% and 23.9% respectively. On the other hand, the remaining three chromium concentrations caused a decrease in the total number of young produced (reproductive impairment) and in the life span. The decrease in the total number of young produced for Daphnia reared in 0.11, 0.18 and 0.35 mg Cr l\(^{-1}\) was 66.1%, 85.3% and 92.8% respectively. While the reduction in the life span for the same chromium concentrations was 60.0%, 71.3% and 82.1% respectively.
It seems that the small number of young produced at higher chromium concentrations was not a result of a slower rate of egg development at these concentrations, as the mean duration of egg incubation at the higher chromium concentrations was similar to or even shorter than that of the control (see table 17). So this reproductive impairment could be attributed to the fact that at higher concentrations large number of eggs failed to complete their development to give young as they are usually eliminated from the body of the female before development is completed.

The results of the recovery in the rate of reproduction in *Daphnia* exposed to 0.35 mg Cr l\(^{-1}\) for 2, 4, 6 and 8 days and then transferred to chromium-free water are shown in Figs. 38, 39, 40 and 41 and given in Table 18. In general, the results seem to indicate that recovery was evident in all groups of *Daphnia* exposed to chromium for up to 6 days as there was no significant difference in the rate of reproduction of these groups when compared with the control (P>0.05). The exceptions were some delay in the appearance of 1st group of eggs, in the production of 1st group of young and in the number of young produced per female per day in *Daphnia* exposed to chromium for 4 and 6 days (P<0.01).

But *Daphnia* exposed to 0.35 mg Cr l\(^{-1}\) for 8 days did not show any recovery in the rate of reproduction. When compared with the control there was a significant difference (P<0.01) in the following aspects observed: a delay in the 1st appearance of eggs and in the production of the 1st group of young; a lower peak number of young produced per female; a lower total number of young produced per female; a shorter life span and a lower number of young produced per female per day.

The reduction in the total number of young produced per female and in the life span of *Daphnia* exposed to 0.35 mg Cr l\(^{-1}\) for 2, 4, 6 and 8
6. **Histological Alterations**

Exposure of minnows to 176.75 mg Cr $\text{l}^{-1}$ and guppies to 141.40 mg Cr $\text{l}^{-1}$ for 24 hours did not cause any alteration in the structure of liver when compared with the control in paraffin sections stained with hematoxylin and eosin. The same has been found for sections of kidney of guppies. But sections from the kidney of chromium treated minnow showed some dilation in the lumen of tubules when compared with the control. However the mean diameter of the lumen of the tubules in sections of control and chromium treated kidneys were 2.85 µm (S.E. = 0.16) and 3.27µm (S.E. = 0.29) respectively. There was no significant difference in the lumen of these two kidney sections (P>0.05).

It is suspected that failure of chromium treated tissue to show any difference in the structure from control tissue was due to the short exposure period (24 hours). So a longer exposure period (96 hours) of guppies to 53.03 mg Cr $\text{l}^{-1}$ was tested to observe any effect on the histology of liver and kidney. Here, too, sections of liver from chromium treated fish showed no difference in histology from control. Chromium treated kidney sections showed only a slight dilation of some tubules when compared with control sections. The mean diameters of tubule lumen of both control and chromium treated sections of kidney were 3.51 µm (S.E. = 0.23) and 4.18 µm (S.E. = 0.27) respectively. This difference in the diameter of tubules from both tissues was not significant (P>0.05).
7. **Histochemical Staining of Chromium**

7.1 **Rainbow Trout**

Histochemical staining of chromium in sections of liver and kidney taken from rainbow trout exposed to 17.68 mg Cr $1^{-1}$ for 24 hours did not show any difference in coloration from control sections. This could be due to the fact that during this exposure period, the amount of chromium accumulated in the tissue was not sufficient to be shown by this technique. As a result of this another group of fish was exposed to chromium for a longer period (96 hours) to allow enough time for accumulation of more chromium in the tissues.

Microphotographs of liver and kidney sections taken from trout exposed to 7.07 mg Cr $1^{-1}$ for 96 hours compared with the control tissues are shown in Plate 1 and 2 respectively. In the liver section taken from chromium treated fish, the blue coloration indicates the presence of chromium (Plate 1 - b). This blue coloration was due to the formation of a chromium - Chrome azurol S chelate complex indicating the presence of chromium (Suzuki et al., 1978). While the control liver section was stained somewhat red which indicates the absence of chromium (Plate 1 - a). On the other hand, it seems that there is no obvious difference in the basic histology between control and chromium treated liver.

When comparing the kidney section from control fish with that of chromium treated fish, it seems that the blue coloration is due to the presence of chromium in the interstitial connective tissue between the tubules and in the glomerulus of chromium treated kidney section (Plate 2 - a & b). This concentration of chromium used does not seem to cause
any pathological alteration in the kidney.

7.2 Guppy

In case of this species, as well, sections containing liver and kidney taken from fish exposed to 141.40 mg Cr l\(^{-1}\) for 24 hours did not show any difference after staining with Chrome azurol S when compared with the control. It also seems that the amount of chromium accumulated in these tissues for 24 hours was not large enough to show up by this staining technique. This gave a reason for trying a longer exposure to chromium in an attempt to allow more time for chromium to accumulate in the tissues.

Plate 3 shows photographs of liver sections taken from control fish (a) and from fish exposed to 53.03 mg Cr l\(^{-1}\) for 96 hours (b). These photographs clearly show a difference in the coloration; the control being reddish and the chromium treated section showing a blue coloration all over. This blue coloration is due to the presence of chromium in the tissue. It could be said also that there is no obvious difference in the histology of liver between control and chromium treated fish.

Photographs of kidney sections taken from control and from fish exposed to 53.03 mg Cr l\(^{-1}\) for 96 hours are shown in Plate 4 - a and b respectively. It is also evident that the section from chromium treated fish is bluish in color while the section from the control fish is more or less red. This blue coloration is distributed in the connective tissue between the tubules. The chromium treated section does not show also any clear alteration in the structure when compared with that of the control.
DISCUSSION

General:

Since potassium dichromate \( \left( \text{K}_2\text{Cr}_2\text{O}_7 \right) \) is an acidic salt of hexavalent chromium, the pH of almost all the test solutions used in the present work was lowered as is shown in Table 2. It is also clear from the Table that the pH of the test solutions increased after a period of 24 hours. Similarly Pickering and Henderson (1966) in their work on the toxicity of heavy metals (including hexavalent chromium) on four species of warm water fishes, found that the pH of most test solutions was lowered and the acidity is increased after adding the metal salt. They also reported that in the bioassays conducted in hard water the pH value of the test solutions usually increased with time. Riva et al. (1981) also found that the pH values of test solutions dropped from 6 ± 0.2 to 4 ± 0.2 with concentrations of \( \text{K}_2\text{Cr}_2\text{O}_7 \) from 110 to 200 mg Cr \( 1^{-1} \). On the other hand, Oshida et al. (1981) reported that the pH of a potassium dichromate solution used in a 7-day bioassay experiment was 7.8 at the beginning and 7.6 after 7 days. No explanation has been given to account for this variation in the pH value with time.

In the present work no adjustment of pH was made in preparing the test solutions containing different concentrations of hexavalent chromium. Grindley (1946) and Riva et al. (1981) also made no adjustment of the pH when determining the toxicity of hexavalent chromium and other heavy metals on some species of fish.
Acute Toxicity

The 24-hr LC$_{50}$ value of hexavalent chromium (as K$_2$Cr$_2$O$_7$) for rainbow trout in dechlorinated tap water with a hardness of 50.8 mg l$^{-1}$ as CaCO$_3$ was 40.3 mg Cr l$^{-1}$ at a pH of about 6.35 and a temperature of 9 ± 0.5°C (Table 3 and Fig. 1). The 24-hr LC$_{50}$ values of hexavalent chromium for rainbow trout have been reported by a number of workers. Schiffman and Fromm (1959) reported a 24-hr LC$_{50}$ value of 100 mg Cr(VI) l$^{-1}$ at 14 ± 1°C (as K$_2$CrO$_4$) for 10-17 cm long rainbow trout in water with hardness of 334 mg l$^{-1}$ as CaCO$_3$ and pH of 8.5 - 8.8. But Cairns et al. (1978) found 24-hr LC$_{50}$ values of 58.9, 141.0 and 95.5 mg Cr(VI) l$^{-1}$ (as K$_2$Cr$_2$O$_7$) at 5°, 12° and 18°C respectively. They used less than 10 cm long rainbow trout in dechlorinated tap water with a hardness of about 40 mg l$^{-1}$ as CaCO$_3$ and a pH range of 4.6 - 7.5. Smith and Heath (1979) using K$_2$Cr$_2$O$_7$ reported that the 24-hr LC$_{50}$ for small rainbow trout (<10 cm long) in water with a hardness of 36 mg l$^{-1}$ as CaCO$_3$ and a mean pH of 5.8 was around 110 mg Cr(VI) l$^{-1}$ at 12°C and about 45 mg Cr l$^{-1}$ at 5°C. Recently Van der Putte et al. (1981 b) showed that the 24-hr LC$_{50}$ values of hexavalent chromium (as Na$_2$CrO$_4$) for 6 g rainbow trout in water with 80 mg l$^{-1}$ total hardness as CaCO$_3$ at 12°C were 180.2, 136.7 and 90.6 mg Cr l$^{-1}$ at pH 7.8, 7.0 and 6.5 respectively. Other studies have been made to demonstrate the toxic action of hexavalent chromium. Rainbow trout failed to recover from a 6-hr exposure to a solution of potassium dichromate containing 35 mg Cr l$^{-1}$ even when returned to fresh water (Rushton, 1922). While Grindley (1946) concluded that concentrations of hexavalent chromium below 20 mg l$^{-1}$ would be non toxic to yearling rainbow trout, he also demonstrated that trout survived for about 60 and 70 hours in distilled water containing 20 mg Cr l$^{-1}$ in the form of potassium chromate and potassium dichromate respectively. While at 50 mg Cr l$^{-1}$,
the average survival time was about 30 hours for both chromium salts.

The 24-hr LC$_{50}$ value obtained in the present work appears to be much lower than those values reported in the previous work except that there is some similarity with the value given by Smith and Heath (1979) in their test conducted on rainbow trout at 5°C. It is obvious that these toxicity results vary from each other. There are variations in the 24-hr LC$_{50}$ values of hexavalent chromium for rainbow trout determined by other workers. These variations could be due to the differences in some of the experimental factors such as pH, temperature, hardness and other environmental factors. There could also be genetic differences between the trout used by the various investigators. So it is quite difficult to compare my results with similar tests conducted by previous workers.

The 24-hr LC$_{50}$ of hexavalent chromium (as K$_2$Cr$_2$O$_7$) for minnows in canal water with a hardness of 291 mg l$^{-1}$ as CaCO$_3$ was 181.35 mg Cr l$^{-1}$ at a pH of about 6.32 and a temperature of 9 ± 0.5°C (Table 3 and Fig. 2). This value seems to be much higher than the 24-hr LC$_{50}$ values of hexavalent chromium (as K$_2$Cr$_2$O$_7$) for 1.5 - 2.5 inch long fathead minnows. These were 39.6 and 63.5 mg Cr l$^{-1}$ in soft water (hardness 20 mg l$^{-1}$) and hard water (hardness 360 mg l$^{-1}$) respectively at 25°C (Pickering and Henderson, 1966). This difference in the toxicity of hexavalent chromium may be due to the difference in pH as Pickering and Henderson (1966) gave no figure for the pH of the test solution or it may be due to the difference in the experimental temperature and it could be due partly to the variation between the different species used.

The 24-hr LC$_{50}$ values of hexavalent chromium (as K$_2$Cr$_2$O$_7$) for adult and juvenile guppies in dechlorinated tap water with a hardness of 50.8 mg l$^{-1}$ as CaCO$_3$ were 126.38 and 123.73 mg Cr l$^{-1}$ respectively at a pH of
about 5.6 and a temperature of 23 ± 0.1°C (Table 3 and Fig. 3). This result seems to agree with that reported by Pickering and Henderson (1966) who found that the 24-hr LC_{50} of hexavalent chromium (as K_{2}Cr_{2}O_{7}) for 0.75-1 inch long guppies (Lebistes reticulatus) in soft water of 20 mg l^{-1} hardness was 113 mg Cr l^{-1} at 25°C. The 24-hr LC_{50} values of hexavalent chromium for a variety of fish species have also been reported. Abegg (1950) showed that the 24-hr LC_{50} values of hexavalent chromium for bluegills (4 - 10 cm long) in a synthetic dilution water having a hardness between 75 - 150 mg l^{-1} as CaCO_{3} were 290 mg Cr l^{-1} for Na_{2}Cr_{2}O_{7} and 300 mg Cr l^{-1} for Na_{2}CrO_{4} at 21°C. Trama and Benoit (1960) reported that the 24-hr LC_{50} values of hexavalent chromium for bluegills (5 - 9 cm long) in water with 45 mg l^{-1} hardness and pH 8.0 at 20°C were 175 mg Cr l^{-1} for K_{2}Cr_{2}O_{7} and 225 mg Cr l^{-1} for K_{2}CrO_{4}. Cairns et al. (1978) found that the 24-hr LC_{50} values of hexavalent chromium (as K_{2}Cr_{2}O_{7}) for goldfish, golden shiner, bluegill and Channel catfish were 213, 109, 280 and 58 mg Cr l^{-1} respectively when tested in water of about 40 mg l^{-1} hardness at 15°C. More recently Bellavere and Gorbi (1981) reported that the 24-hr LC_{50} value of hexavalent chromium (as K_{2}Cr_{2}O_{7}) for the fish Brachydanio rerio was 96 mg Cr l^{-1} in water having a hardness of 100 mg l^{-1} as CaCO_{3} and a pH of 7.8 at 20°C. Riva et al. (1981) stated that the 24-hr LC_{50} of hexavalent chromium (as K_{2}Cr_{2}O_{7}) for the goldfish was 140 mg Cr l^{-1} at pH value under 6.0 and at 22°C. When comparing these results with the 24-hr LC_{50} values of hexavalent chromium for rainbow trout, minnows and guppies obtained in the present work (see Table 3), it seems clear that rainbow trout is the most susceptible species. But when the resistance of rainbow trout to chromium was compared with that of the Channel catfish reported by Cairns et al. (1978), it appears that there is some similarity between these two species. While minnows and guppies in the present work were more
resistant to hexavalent chromium than some of the species mentioned above and less resistant than others, it could not be concluded with certainty that these variations in the resistance between the different species of fish is due entirely to species variations but other factors could play some role in modifying the toxicity of hexavalent chromium. Kaviraj and Konar (1982) suggested that although toxicity of any metal may vary with the species of fish, the wide differences of LC$_{50}$ values of chromium may be due to variation in water quality. Obviously, at low alkalinity, hardness and pH of water, chromium was more toxic to fish than in water with higher values of these factors. The impact of such factors on chromium toxicity will be summarised in the following paragraph.

Despite the considerable amount of work dealing with the toxic action of hexavalent chromium on aquatic organisms, it is difficult to compare the results with each other and to draw a general conclusion regarding the toxicity of this metal. This difficulty is due to the variations among different species, variability of both chemical and physical characteristics of dilution water including the pH, hardness and temperature and the differences in the duration of toxicity test (Ellis, 1937; Trama and Benoit, 1960; Ruesink and Smith, 1975; Cairns et al. 1978; Chapman, 1978; Fales, 1978; Andros and Garton, 1980; Riva et al., 1981; Van der Putte et al., 1981 b; Kaviraj and Konar, 1982 and others). For instance, Pickering and Henderson (1966) found variation in the toxicity of hexavalent chromium (as K$_2$Cr$_2$O$_7$) among 4 species of warm water fishes. They reported that the 24-hr LC$_{50}$ values in soft water (20 mg l$^{-1}$ hardness) at 25°C were 39.6, 284, 122 and 113 mg Cr l$^{-1}$ for fathead minnows, bluegills, goldfish and guppies respectively. Similarly Cairns et al. (1978) and Smith and Heath (1979) reported considerable variation in the 24-hr LC$_{50}$ values of hexavalent chromium among the following species of fish;
goldfish (Carrassius auratus), golden shiner (Notemigonus crysoleucus), bluegill (Lepomis macrochirus), Channel catfish (Ictalurus punctatus) and rainbow trout (Salmo gairdneri). It has been well established that lowering the pH causes an increase in the toxicity of hexavalent chromium to fish (Grindley, 1946; Hogendoorn - Roozemond et al., 1978; Van der Putte et al., 1981 b). Toxicity of hexavalent chromium has been reported to decrease when the hardness is increased (Ellis, 1937; Cairns and Scheier, 1959; Pickering and Henderson, 1966). Increasing the experimental temperature has been found to increase the toxicity of hexavalent chromium on fish (Cairns and Scheier, 1959; Ruesink and Smith, 1975; Cairns et al., 1978). Finally the acute toxicity of hexavalent chromium on fish has been determined using different exposure periods. For example; some workers used the 24-hr exposure period (Schiffman and Fromm, 1959; Trama and Benoit, 1960; Pickering and Henderson, 1966; Cairns et al., 1978; Smith and Heath, 1979). Others used a 48-hr exposure period (Ruesink and Smith, 1975; Hogendoorn-Roozemond et al., 1978; Van de Putte et al., 1981 b). While others used a 96-hr exposure period (Pickering and Henderson, 1966; Benoit, 1976; Broderius and Smith, 1979; Van der Putte et al., 1981 b; Kaviraj and Konar, 1982).

Upon observing the results obtained in this work on the toxicity of hexavalent chromium on the three species studied, it could be concluded that rainbow trout proved to be the most sensitive species to chromium especially when compared with guppies as the same dilution water (dechlorinated tap water) was used for tests conducted on both species. This seems in agreement with Grindley (1946) who concluded that rainbow trout are notably more sensitive to adverse physical and chemical conditions than most other fish. But minnows were found to be the most resistant of the three species to chromium. This increase in resistance
of minnows to chromium may be partly due to the fact that toxicity tests on minnow were carried out in hard water (291 mg l\(^{-1}\) as CaCO\(_3\)) as compared to soft water (50.8 mg l\(^{-1}\) as CaCO\(_3\)) used in tests for the other two species. The effect of hardness has been reported previously when Trama and Benoit (1960) stated that increased alkalinity and hardness significantly reduce the toxicity of solutions containing hexavalent chromium on fish.

Some effort has been made to explain the toxic action of chromium and other heavy metals on fish. Doudoroff and Katz (1953) indicated that hexavalent chromium behaves toxicologically in a manner quite different from most heavy metals. Carpenter (1927, 1930) stated that the death of fish in dilute solutions of heavy metals is due to asphyxia resulting from precipitation of mucus on the gill filaments, thus preventing their movement and impeding gas exchange. Ellis (1937) suggested that precipitation of the gill mucus by heavy metal salts interferes with its excretory process which is a contributory cause of death. Similarly Jones (1939) concluded that the death of sticklebacks after exposure to salts of heavy metals including chromium is due to the precipitation of the gill secretions, thus causing asphyxiation. The same author in 1947 stated that in the case of sticklebacks exposed to salts of heavy metals respiration is obstructed at the gill surface; the combined effect of oxygen deprivation and carbon dioxide retention results in an increase in the rate and depth of breathing. The fish is soon breathing at the maximum rate of which it is capable, but with continuing fall in the oxygen intake the animal becomes exhausted; the ventilation rate cannot be maintained and death results. Also Fromm and Schiffman (1958) cited that it is generally believed that the death of fish in solutions of heavy metals such as Zn, Cu and Pb is caused by precipitation of mucus secreted by the
gills or by damage to gill tissues. Similarly, precipitation of mucus on the gills of all fish treated with chromium was also observed in the present work which might lead to death by affecting the main function of the gills. However Fromm & Schiffman (1958) found a gradual decrease in the general metabolism of tissues in largemouth bass exposed to chromium which might reflect a gradual decrease in cellular metabolism caused by the accumulation of chromium in various tissues. The same authors showed a direct evidence of an internal site for chromate toxicity. They observed severe pathological changes in the intestine immediately posterior to the pyloric caeca in juvenile largemouth bass exposed to 96 mg Cr(VI) l⁻¹. In rainbow trout, lower environmental hexavalent chromium concentrations (2 - 4 mg l⁻¹) produced a significant increase in hematocrit values (Schiffman and Fromm, 1959). Also Stokes and Fromm (1965) detected a reduced transport of glucose by caecal and midgut sections obtained from chromium treated rainbow trout.

Skidmore (1970) concluded that the loss of osmotic control may be ruled out as the cause of death in trout exposed to a rapidly lethal solution of zinc sulphate because the changes in blood osmotic concentration were too slight to exceed the normal range. Similarly, the small changes in blood concentrations of sodium, potassium, calcium, magnesium and zinc were unlikely to cause a general physiological imbalance. Generally fish exposed to zinc had to work increasingly hard to maintain an adequate rate of oxygen uptake. Failure to do so resulted fairly quickly in respiratory failure.

Smissaert et al. (1975) reported that the specific activity of the gill AChE (Acetylcholinesterase) of chromium exposed rainbow trout was significantly lower than that of the control. They suggested that a lower
AChE level may cause higher acetylcholine concentrations to occur which resulted in muscle contraction, thus narrowing the blood vessels and hampering oxygen uptake with the usual sublethal and lethal effects. They stated, however, that the experiment should be repeated before definite conclusions could be drawn. Kuhnert et al. (1976) found that Na⁺, K⁺-ATPase activity in kidney, liver and intestine of rainbow trout exposed to 2.5 mg Cr(VI) l⁻¹ was lower than that of control tissues except for gill. They stated that this decrease in the activity of Na⁺, K⁺-ATPase may partially explain the toxic effect of hexavalent chromium on fish. Previous workers have shown that the level of Na⁺, K⁺-ATPase activity parallels the level of active sodium transport in those teleost organs having major osmoregulatory roles; i.e. gill and intestine in marine fish and gill and kidney in fresh water fish (Oide, 1967; Jampol and Epstein, 1970). More recently Van der Putte et al. (1981 b) confirmed the hypothesis that acute hexavalent chromium toxicity is attributable to hydrochromate (HCrO₄⁻) and chromate (CrO₄²⁻) ions which was suggested by Trama and Benoît (1960), Becker and Thatcher (1973), Van der Putte et al. (1981 a). Despite this work, the actual mechanism by which chromium exerts its lethal effect on fish is not yet fully understood.

The 24-hr LC₅₀ value of hexavalent chromium for 6 - 12 hr old *Daphnia magna* in canal water having a hardness of 291 mg l⁻¹ as CaCO₃ at pH of about 8.2 at 23 ± 0.1°C was 0.85 mg Cr l⁻¹ in one test and it was 1.10 mg Cr l⁻¹ in a replicate test (Table 3 and Figs. 4 and 5). The variation in the resistance of *D. magna* between the two tests is probably due to the fact that these tests were carried out at different times of the year. These results seem to be in agreement with the 24-hr LC₅₀ of hexavalent chromium for *Daphnia magna* reported by Cairns et al. (1978) which was 1.0 mg Cr l⁻¹ (as K₂Cr₂O₇) at pH 7.5 and temperature of
20°C in soft water (40 mg l⁻¹ total hardness). But they are higher than
that value reported by these authors on the same species at temperature
25°C (24-hr LC₅₀ = 0.76 mg Cr l⁻¹). It should be kept in mind that the
tests reported by Cairns et al. (1978) were conducted in soft water and at
a lower pH value. Moreover the 24-hr LC₅₀ values for D. magna in the
present work were lower than the 24-hr LC₅₀ value for D. magna reported
by Bellavere and Gorbi (1981) which was 1.57 mg Cr l⁻¹ (as K₂Cr₂O₇) in
water with a hardness of 200 mg l⁻¹ as CaCO₃ and pH 7.8 at 20°C. But
my data were more or less similar to the 24-hr LC₅₀ value for the same
species in water having a hardness of 100 mg l⁻¹ as CaCO₃ and a pH of
7.8 at 20°C which was 0.83 mg Cr(VI) l⁻¹ (Bellavere and Gorbi, 1981).
These results could not be fully related to each other because of
differences in temperature and hardness of dilution water used in these
different tests which resulted in variation of the 24-hr LC₅₀ of
hexavalent chromium for this species. This variability in acute toxicity
tests among Daphnia has also been mentioned by Winner (1976). The 24-hr
LC₅₀ value of hexavalent chromium has also been determined for D. pulex.
Sherr and Armitage (1971) reported that concentrations of 1.0 and 0.1 mg
l⁻¹ sodium dichromate caused 100% mortality for D. pulex in 24 hours at
21°C. While Batac-Catalan and Cairns (1977) reported that the 24-hr
LC₅₀ of hexavalent chromium for D. pulex in water having a hardness of
171 mg l⁻¹ as CaCO₃ at 21°C was 0.68 mg l⁻¹ potassium chromate. Finally
Cairns et al. (1978) found that the 24-hr LC₅₀ values of hexavalent
chromium for D. pulex in soft water (40 mg l⁻¹ total hardness) at pH
range of 4.6-7.5 were 0.8 and 0.56 mg Cr l⁻¹ at 20°C and 25°C
respectively. When compared with the present work, D. pulex showed more
sensitivity to hexavalent chromium than D. magna. The same has been
concluded by Cairns et al. (1978) who found that D. pulex are more
sensitive to hexavalent chromium than D. magna.
A lot of work has been done on the acute toxicity of hexavalent chromium on a variety of invertebrate species (see p.11). The results of such work seems to be variable because it has been conducted on different species and under different chemical and physical conditions. For these reasons it is not possible to compare the results and draw a general conclusion.

In the present work, D. magna was more sensitive to hexavalent chromium than the three species of fish used. This agrees with the finding of Anderson (1948) who compared the 64-hr apparent - threshold metal concentrations for Daphnia with acute values for fish and concluded that "in general, the Daphnia and related forms, are more susceptible to cations than are fish". Recently Bellavere and Gorbi (1981) also found that D. magna was far more sensitive to hexavalent chromium than the fish Brachydanio rerio.

The mechanism of the toxic action of heavy metals on aquatic invertebrates is not fully known. Danielli and Davis (1951) suggested that the metal ions exert their toxic influence by covalent binding at cell surfaces, and that the difference in electronegativity of the various ions is a toxicity - determining factor. The study of Somers (1959) showed a correlation between toxicity of the metals to Botrytis fabae and the electronegativity of the metals, since the site of action between the metal and the organic ligand appeared to involve nitrogen, sulfur or other electronegative functional groups. Shaw and Grushkin (1957) and Shaw (1961) related the toxicity for many organisms to metal sulfide solubility and found a positive correlation. Biesinger and Christensen (1972) worked on the effects of various metals on Daphnia magna. They found that the glutamic oxalacetic transaminase (GOT) activity was stimulated appreciably
by addition of some metals or it was inhibited by others while others caused only slight alterations in GOT activity. These metals had a variable effect on body protein and certain enzyme (GOT). They suggested that in order to clarify the mechanisms of toxic action of pollutants on aquatic organisms, further concurrent studies on whole animals and on specific molecular processes should be encouraged.

2. Chronic Toxicity on Daphnia magna

The LD$_{50}$ (duration in days required to cause 50% mortality) values of different sublethal concentrations of hexavalent chromium ranging between 0.035 - 0.35 mg Cr l$^{-1}$ for Daphnia magna are given in Table 4. At the lower concentrations of chromium (0.035 and 0.07 mg l$^{-1}$) it appears that the LD$_{50}$ values were higher than that of control animals. This result seems to be peculiar. But, however, Sherr and Armitage (1971) reported that in some cases a low concentration of dichromate (0.01 mg l$^{-1}$) may even have a slight positive effect (though not significant) on D. pulex survival. While at chromium concentrations of 0.11 mg l$^{-1}$ and above, there was a sharp decline in LD$_{50}$ values when compared with control animals. So it could be said that chromium concentrations below 0.07 mg l$^{-1}$ has no adverse effect on the survival of D. magna in long term exposures.

Upon exposure of D. magna to 0.35 mg Cr(VI) l$^{-1}$ (the highest concentration tested for chronic toxicity) for 2, 4, and 6 days, the animals appear to recover after they have been transferred to chromium-free water. This is clearly observed in the survival of these groups in which the LD$_{50}$ for animals exposed to chromium for 2, 4, and 6
days did not differ much from control animals (Table 5 and Fig. 7). This suggests that the animals have more or less recovered from exposure to chromium. But at 8 days exposure to 0.35 mg Cr(VI) l\(^{-1}\), the animals failed to recover from chromium exposure as their LD\(_{50}\) was highly reduced when compared with control animals (Table 5 and Fig. 7). The chronic toxicity values in this work seem to be lower than the 3-week LC\(_{50}\) of trivalent chromium for D. magna which was 2 mg l\(^{-1}\) in Lake Superior water having a hardness of 45 mg l\(^{-1}\) (Biesinger and Christensen, 1972). This difference could be due to the fact that Biesinger and Christensen used trivalent chromium which has been proven to be less toxic than hexavalent chromium used in the present work.

There are a number of papers concerned with the chronic toxicity of chromium on other species of aquatic animals. Olson and Foster (1956) exposed young chinook salmon (Oncorhynchus tshawytscha) and rainbow trout (Salmo gairdneri) continuously to various concentrations of sodium dichromate in running water for periods of up to 7 months. They found that survival was impaired at 0.08 mg Cr(VI) l\(^{-1}\) and growth was inhibited at only 0.013 - 0.022 mg Cr(VI) l\(^{-1}\). Raymont and Shields (1964) confirmed a threshold level of hexavalent chromium (as Na\(_2\)CrO\(_4\)) for Nereis virens of just below 1 mg Cr l\(^{-1}\). At 0.05 mg Cr l\(^{-1}\), they found that no death occurred in these worms for a period up to 5 weeks. But at a concentration of chromium of 1 mg l\(^{-1}\) 50% mortality occurred after approximately 3 weeks. For the shore crab (Carcinus maenas), these authors showed that survival in animals exposed to 20 and 40 mg Cr(VI) l\(^{-1}\) was similar to that of controls. But at a concentration of 60 mg Cr(VI) l\(^{-1}\) 60% mortality occurred after 2 weeks. In addition, they reported that the threshold toxicity level of hexavalent chromium for small prawns (Leander squilla) was around 5 mg Cr l\(^{-1}\). While Pringle
et al. (1968) showed that chromium concentrations of 0.1 and 0.2 mg l⁻¹ (as K₂Cr₂O₇) produced the same mortality with molluscs as control animals. Benoit (1976) found that exposing brook trout (Salvelinus fontinalis) to various concentrations of hexavalent chromium for up to 22 months significantly increased alevin mortality at 0.35 mg Cr l⁻¹. Eight months exposure of rainbow trout (S. gairdneri) significantly increased alevin mortality at 0.34 mg Cr l⁻¹. The 28-day LC₅₀ values of hexavalent chromium (as CrO₃) at pH 7.8 for the polychaete Neanthes arenaceodentata were 0.55 and 0.7 mg Cr l⁻¹ for adult and juvenile worms respectively. But for the adult polychaete Capitella capitata this value was only 0.28 mg Cr l⁻¹ (Reish et al., 1976). More recently Oshida et al. (1981) reported that exposure of the polychaete Neanthes arenaceodentata to 0.2 mg Cr(VI) l⁻¹ caused 50% mortality by day 59. While exposure of the same species to 50.4 mg Cr(III) l⁻¹ for 293 days caused no significant mortality. It is clear from the above results that chronic toxicity of chromium varies with the type of the salt, with the species tested and with the experimental period used.

3. Tissue Accumulation

In the present work, the accumulation of hexavalent chromium after 24 hour exposure to different chromium concentrations has been determined in various tissues of rainbow trout (Table 6 and Figs. 8, 9, 10 and 11), minnow (Table 7 and Figs. 12, 13, 14 and 15), adult guppies (Table 8 and Figs. 16, 17, 18 and 19) and in three body regions of juvenile guppies (Table 9 and Fig. 20). In the three species of fish it could be said that tissue concentrations of chromium increased with increasing chromium test concentrations. The exception to this has been observed only in adult
guppies exposed to 88.38 mg Cr l⁻¹ in which tissue concentrations of chromium was lower than those observed in tissues of fish exposed to 70.7 mg Cr l⁻¹. This deviation in the pattern of chromium accumulation could be due to the fact that testing guppies at 88.38 mg Cr l⁻¹ has been carried out sometime later than the tests of other concentrations. Low concentrations of chromium have been detected in all tissues of control rainbow trout, in some tissues of control minnow and in control juvenile guppies. While no chromium could be detected in some tissues of control minnow and in all tissues of adult control guppies. The absence of chromium in tissues of these control fishes may be because the chromium levels in tissues were beyond the detection limit of the analytical method. Presence of low concentrations of chromium in tissues of control fish could be due to the presence of trace amounts of this metal in the natural environment of these fishes. The presence of chromium in control tissues of fish has been also reported by other workers. For example, Kuhnert et al. (1976) found low concentrations of hexavalent chromium in liver, gill, intestine and kidney tissues of control rainbow trout which were 0.114, 0.156, 0.181 and 0.218 µg Cr per gram wet weight respectively. Similarly Buhler et al. (1977) also reported low concentrations of chromium in various tissues of 2-year old control rainbow trout. While Van Hoof and Van San (1981) found small amounts of Cu, Zn and Cd in muscle, gill, opercle, liver and kidney tissues of the control fish Scardinius erythrophthalmus. Most recently, Calamari et al., (1982) found levels of chromium in muscle, liver and kidney tissues ranging from 0.287 - 0.609 µg Cr per gram wet weight in control rainbow trout.

In this work it has been found that accumulation of chromium differs from one tissue to another. For rainbow trout it appears that gill and kidney accumulated the highest concentrations of chromium followed by
liver, stomach, heart, gonad, brain and muscle. However there is some deviation in this order of chromium accumulation in some of the tissues at certain chromium test concentrations. For the minnows, the order of chromium accumulation in various tissues from the highest to the lowest levels was as follows: gill, stomach, liver, kidney, heart, gonad, brain and muscle. For the adult guppies this order was stomach, gill, liver, heart, gonad, brain and muscle. While for juvenile guppies the middle portion of the body showed the highest levels of chromium followed by the anterior portion of the body while the posterior portion of the body, which consists entirely of muscles, accumulated the lowest levels of chromium. These various tissues, hence, could be divided into 3 groups. The 1st group includes kidney, gill, liver and stomach which appears to accumulate high concentrations of chromium. These concentrations, however, may vary from one species to another. The 2nd group includes heart and gonad which accumulated moderate levels of chromium. Whereas the 3rd group including brain and muscle accumulated low levels of chromium. Generally these findings agree to some extent with other data reported by a number of workers. Knoll and Fromm (1960) worked on the accumulation of chromium in rainbow trout exposed to 2.5 mg Cr(VI) l⁻¹ (as K₂CrO₄) for 12 days at 14°C. They found that all tissues studied (pyloric caeca, gut, stomach, kidney, spleen and liver) except muscle accumulated chromium in concentrations exceeding that of the environment. They also observed that the rate of accumulation of chromium by the spleen and stomach were of a similar magnitude but less than that of the liver, gut, kidney and pyloric caeca. The pyloric caeca accumulated chromium more rapidly than any other tissue studied. While the muscle tissue did not accumulate chromium during the exposure period. Kuhnert et al. (1976) exposed 2-year old rainbow trout to 2.5 mg Cr(VI) l⁻¹ (as chromate) for 48 hours at 14°C, they reported the chromium levels of 2.164, 2.141, 0.579
and 0.544 µg Cr per gram wet weight for kidney, gill, intestine and liver respectively. Buhler et al. (1977) determined chromium levels in various tissues of 2-year old Hanford rainbow trout after exposure to 2.5 mg Cr(VI) \(\text{L}^{-1}\) (as \(\text{Na}_2\text{Cr}_2\text{O}_7\)) for 24 hours at 15°C. They found that chromium levels in brain, posterior kidney, gill, anterior kidney, heart, liver, stomach and white muscle were 14.1, 7.09, 5.86, 4.04, 2.52, 2.04, 0.91 and 0.54 µg Cr per gram wet weight respectively. The high level of chromium in the brain was unusual since this level was dropped sharply after longer periods of exposure to chromium up to 22 days. Van Hoof and Van San (1981) after exposing 10 - 15 cm long rudd to acute concentrations of hexavalent chromium (as \(\text{K}_2\text{Cr}_2\text{O}_7\)) (20, 80 and 145 mg Cr \(\text{L}^{-1}\)) for 24 hours in water having a hardness of 200 mg \(\text{L}^{-1}\) as \(\text{CaCO}_3\) and pH value of 8.0 at 20°C. They reported chromium levels in gill, kidney, liver and muscles in fish exposed to 20 mg Cr \(\text{L}^{-1}\) were 4.9, 10.3, 5.6 and 0.5 µg Cr per gram wet weight respectively; in fish exposed to 80 mg Cr \(\text{L}^{-1}\) these levels were 48.2, 23.8, 18.4, 0.8 µg Cr per gram wet weight respectively, but in fish exposed to 145 mg Cr \(\text{L}^{-1}\) these values were 30.6, 27.8, 15.2 and 0.6 µg Cr per gram wet weight respectively. They concluded that in all acute concentrations of chromium the fish accumulated a measurable chromium levels in muscle, gill, liver and kidney and most elevated values were detected in gill tissues. They also stated that in all tissues except kidney, chromium levels found in the highest chromium concentration tested (145 mg Cr \(\text{L}^{-1}\)) were lower than those found after exposure to 80 mg Cr \(\text{L}^{-1}\) probably because of the shorter exposure time before death. This finding does not agree with the results obtained in the present work for the three species of fish in which chromium levels in tissues increased consistently with increasing chromium test concentrations. Van der Putte et al. (1981 a) exposed yearling rainbow trout to \(^{51}\text{CrO}_4^{2-}\) - containing \(\text{Na}_2\text{CrO}_4\) solution having a chromium
concentration of 40 mg Cr l\(^{-1}\) for 2 and 4 days at pH values of 7.8 and 6.5. For the 2-day exposure experiment at pH 6.5 the chromium levels in gill, kidney, liver, stomach, heart, gonad, brain and white muscle were 67.1, 37.4, 24.7, 19.3, 4.0, 2.6, 1.0 and 0.5 µg Cr per gram wet weight respectively. This pattern of chromium accumulation by various tissues agrees totally with the pattern of chromium accumulation by similar tissues of rainbow trout obtained in the present work in which the pH range was between 6.1 - 6.9. However, Van der Putte et al. (1981 a) reported, in trout exposed to 40 mg Cr l\(^{-1}\) for 2 days at a higher pH (7.8), similar pattern as that at the lower pH (except that kidney accumulated more chromium than gill). Most recently, Calamari et al. (1982) reported that tissue chromium of rainbow trout exposed to 0.2 mg Cr l\(^{-1}\) (as K\(_2\)Cr\(_2\)O\(_7\)) for 30, 90 and 180 days were higher than control. They found that kidney accumulated the highest concentration of chromium followed by liver and muscle. They also noticed in all tissues that the level of chromium increased with increasing exposure period. Starý et al. (1982) reported that when guppies (Poecilia reticulata) were kept in labelled Cr(III) solutions (5X10\(^{-8}\) mol. l\(^{-1}\) and 2X10\(^{-7}\) mol. l\(^{-1}\)) they showed after 1-2 hours an appreciable radioactivity which was caused mostly by the absorption of Cr(III) on the surface of the fish. While fish kept in labelled Cr(VI) solutions (10\(^{-7}\) mol. l\(^{-1}\) and 2X10\(^{-7}\) mol. l\(^{-1}\)) showed after a short exposure time only low radioactivity which indicates that the absorption of Cr(VI) on the surface of the fish is small.

From these results it could be said that there is a total agreement that muscle always accumulates the lowest chromium levels when compared with other tissues of fish. On the other hand, other tissues of fish seem to show variable tendencies to accumulate chromium in different species and under different experimental conditions. It has been concluded by
Calamari et al. (1982) that despite the presence of a certain amount of
data in the literature concerning determinations of metal accumulation in
fish, comparison of these data is quite difficult. The literature
considers a wide range of species, in field and in laboratory, variation
in the test procedures and in environmental conditions, with samples
analysed by different methods and using different digestion procedures on
various tissues or whole body. Thus variations between chromium
concentration in various tissues of rainbow trout, minnows and guppies in
the present work may be due to differences in the behaviour of these
fishes to chromium and/or the variations in experimental conditions such
as chromium concentrations, and physico-chemical properties of dilution
water. pH is one factor which has been found to affect the pattern and rate
of chromium accumulation in fish. Riva et al. (1981) found that chromium
levels in gills of goldfish exposed to 110, 150 and 170 mg Cr(VI) $1^{-1}$
were significantly higher when pH was not controlled (acidic conditions)
than in those in which the pH was maintained close to neutral. Van der
Putte et al. (1981 a) found, in fingerlings of rainbow trout exposed to
chromium concentrations of 2 and 5 mg $1^{-1}$ at pH 6.5, that the whole fish
and the gill tissues accumulated significantly more chromium than at pH
7.8. In addition, they reported that chromium contents in the gills of
the exposed fish at the lower pH far exceeded those in kidney, liver and
digestive tract. They also observed higher concentrations of chromium in
gills of yearling trout exposed to 40 mg Cr(VI) $1^{-1}$ at the lower pH than
at the higher pH.

In fish three avenues of entry are available to heavy metals including
hexavalent chromium: (1) absorption through the skin, (2) ingestion into
the stomach, or (3) entry across the gill and mucus membranes. The
possibility of chromium gaining entrance via the skin would tend to be
discredited due to a consistently low concentration of chromium found in muscle samples of chromium treated fish (Knoll and Fromm, 1960; Van Hoof and Van San, 1981; Van der Putte et al., 1981a; Calamari et al., 1982 and in the present work). The small absorption of labelled hexavalent chromium through the surface of the guppy has been confirmed by Starý et al. (1982) who observed that fish kept in labelled hexavalent chromium solution showed only low radioactivity after a short exposure time. This avenue of entry cannot be ruled out entirely, however, since muscle may not bind chromium even if it is present (Knoll and Fromm, 1960). In a freshwater environment, the assumption that chromium from the environmental media reaches the gut as a result of drinking is somewhat doubtful, since freshwater fish supposedly drink very little water. Fromm and Schiffman (1958) suggested that chromium accumulated in the gut as a result of excretion by liver via the bile. Knoll and Fromm (1960) showed that radioactive chromate placed directly into the stomach of trout failed to be absorbed in significant amounts within 24 hours, thus tending to minimize the importance of chromium uptake via the digestive system. They also showed that tissue distribution of chromium in the oesophageal occluded trout compared favorably with those of normal fish exposed to hexavalent chromium. With these observations in mind, it is postulated that the gill is the most likely path for the entrance of hexavalent chromium and other heavy metals. When fish are exposed to hexavalent chromium, or other heavy metals dissolved in the water, the gill functions as the major route for uptake of these compounds (Knoll and Fromm, 1960; Olson et al., 1973). The gill tissue rapidly accumulates most heavy metals in such a manner that its content, at least initially, far exceed that in other tissues. This has been shown for copper (Sellers et al., 1975), Cadmium (Sangalang and Freeman, 1979) and mercury (Olson et al., 1973; Lock, 1979). Following uptake by the gills of fish, the transport
of the metals to other tissues is undoubtedly by the way of the circulatory system (Van der Putte and Pärt, 1982).

4. **Oxygen Consumption**

4.1 **Guppy**

The rate of oxygen consumption in guppies exposed to 7.07, 35.35 and 106.05 mg Cr $\text{L}^{-1}$ for 24 hours and the subsequent recovery of this rate upon returning the chromium treated fish to chromium-free water are given in Tables 10, 11 and 12 and in Figs. 21 - 26 respectively. In the lower chromium concentration tested (7.07 mg Cr $\text{L}^{-1}$) no significant effect on oxygen uptake is noticed. But the remaining two chromium concentrations caused a significant drop in the rate of oxygen consumption in chromium treated fish when compared with control fish ($P<0.01$). The unaffected rate of oxygen consumption in guppies exposed to 7.07 mg Cr $\text{L}^{-1}$ for 24 hours is similar to some results by others. Fromm and Stokes (1962) demonstrated that in vitro respiration of pyloric caeca, liver and kidney sections from rainbow trout exposed to 1.0 mg Cr(VI) $\text{L}^{-1}$ for as long as 39 days was not different from that of control fish. Recently Van der Putte et al. (1982) also found that exposure of 1 - 2 year old rainbow trout to different concentrations of hexavalent chromium ranging from 0 - 50 mg Cr $\text{L}^{-1}$ at pH 7.8 for 4 days did not have any significant effect on the rate of oxygen uptake. They suggested that any alteration in the rate of oxygen consumption as a result of exposure to chromium may be masked by variations in spontaneous activity of the fish. However, the decreased rate of oxygen consumption observed in the present work in guppies exposed to the remaining two chromium concentrations (35.35 and
106.05 mg Cr $l^{-1}$) agrees with the results of other work reported in the literature. Hingorani and Diwan (1979) showed that industrial effluents containing heavy metals caused a decrease in the rate of oxygen consumption of the freshwater fish *Labeo rohita*. This decrease in the rate of oxygen consumption was more pronounced as the concentration of the effluents increased.

While other species of fish when exposed to heavy metals showed an increase in the rate of oxygen consumption at first but after sometime in the test solution, the rate of oxygen consumption began to drop. For instance, Jones (1947) exposed sticklebacks to low concentrations of mercuric chloride, copper sulphate and lead nitrate. He found that these heavy metals produced at first (10 - 20 minutes) an increase in the respiration rate. But after 20 minute exposure period the rate of respiration began to decline. Fromm and Schiffman (1958) reported that exposure of largemouth bass to 94 mg Cr(VI) $l^{-1}$ caused an increase in the rate of oxygen consumption after up to 23.5 hours of exposure. At 49 hour exposure, the rate of oxygen consumption was lower than that of the control and after 68 hour exposure, it gradually declined to 27% below normal. O'Hara (1971) found in juvenile bluegills exposed to different concentrations of copper ranging from 0.5 - 5.0 mg $l^{-1}$ that there was a maximum increase in the rate of oxygen consumption between 3 - 6 hours after copper was added. At the end of 24 hr exposure period, all the fish in the copper concentrations that caused a respiratory increase, had oxygen consumption rates below that of the control.

The decline in the rate of oxygen uptake by fish exposed to heavy metals has been explained in different ways. Some authors suggested that this decrease in the rate of oxygen consumption is due to the accumulation
of mucus which cover the gills of fish, thus impairing the function of gills as the site for gaseous exchange (Fromm and Schiffman, 1958; Hingorani and Diwan, 1979; Kaviraj and Konar, 1982). In addition Fromm and Schiffman (1958) suggested that the gradual decrease in the general metabolism which they found in largemouth bass exposed to chromium, reflects the gradual decrease in cellular metabolism caused by the accumulation of chromium in various tissues. It has been suggested that the decline in the rate of oxygen consumption in fish exposed to heavy metals could be due to the lower oxygen content in the test solution (Hingorani and Diwan, 1979). It is doubtful that the decreased oxygen consumption noted in this work was caused by variations in the oxygen level during the test since the oxygen level in the test solutions has been kept near saturation by continuous aeration throughout the experiments. In rainbow trout exposed to hexavalent chromium, Van der Putte et al. (1982) stated that gill epithelial damage and increased levels of plasma lactate suggest a blockage of oxygen uptake in the gills and a shift to some anaerobic metabolism. They added that elevated levels in ventilation frequency, hematocrit values, plasma glucose and lactate, which they have observed in their experiments, have also been found in fish subjected to severe hypoxic conditions. Also Van der Putte and Pärt (1982) found that gill preparations of trout which had been pre-exposed in vivo for 4 days to 10 mg Cr(VI) l⁻¹ at pH 6.5, exhibited an impaired oxygen transfer. This could be well explained by the structural alterations seen after histological examination of the perfused gills. On the other hand, it has been shown by Skidmore (1970) that rainbow trout with a reduced arterial oxygen tension caused by exposure to zinc, exhibited a normal rate of oxygen uptake. From these results, it could be said that the decrease in the rate of oxygen consumption in fish species upon exposure to heavy metals may be due to one or more of several causes.
However, some fish species have been found to exhibit an increase in their respiration rate after exposure to heavy metals. Skidmore (1970) observed an increase in the rate of oxygen consumption of unanaesthetized rainbow trout exposed to 40 mg l$^{-1}$ zinc, but slightly sedated fish respired at rates similar to that of control fish after exposure to the same concentration of zinc. Calabrese et al. (1975) also found that the oxygen consumption of gill tissue in winter flounder exposed to 0.01 mg l$^{-1}$ mercury for 60 days was significantly higher than that of the control. Jones (1947) and Skidmore (1970) suggested that this increase in the rate of oxygen consumption may be due to increase in the activity of the exposed fish. This has been contradicted by O'Hara (1971) who stated that bluegills, which have an increased rate of oxygen uptake after exposure to copper, did not show any increase in the activity other than in the rate of opercular movement that would normally be associated with a greater respiratory demand.

Recovery was evident in the rate of oxygen consumption of guppies exposed to 35.35 and 106.05 mg Cr l$^{-1}$ for 24 hours after returning the treated fish to chromium-free water (Tables 11 and 12 and Figs. 23 and 25). The depressed rate of oxygen consumption in fish, which resulted from exposure to chromium, returned to the normal rate after only 24 hours in chromium-free water. This recovered rate remained at a steady level for 7 days in the recovery conditions. Very little work has been done on the recovery of oxygen consumption rate in fish exposed to heavy metals. Jones (1947) reported that the rate of oxygen consumption in sticklebacks was depressed when the fish were exposed to 0.0001 N mercuric chloride. But when the treated fish were kept in clean well-aerated tap water, the oxygen consumption began a slow upward climb. After 24 hours in the mercury-free water, the fish appeared to have recovered completely.
Similarly, O'Hara (1971) also reported that the rate of oxygen consumption of juvenile bluegills after 7 day exposure to different concentrations of copper (0.5 - 3 mg l⁻¹) was dropped. But he found that when copper treated fish were kept in copper-free water for 7 days, they had either recovered their base oxygen consumption rate or showed strong evidence of recovery.

On the other hand, guppies which have been exposed to 7.07 mg Cr l⁻¹ for 24 hours in the present work, showed no effect on the rate of oxygen consumption (Table 10 and Fig. 21). This unaffected rate continued for one day after the fish were transferred to chromium-free water. But on the second day in the recovery condition this rate exhibited a significant drop. No explanation could be offered for this decrease in the rate of oxygen consumption.

The decrease in the rate of oxygen consumption which has been observed in the present work in guppies exposed to chromium may be due to reason(s) other than structural damage to the gills which has been reported by other workers to occur in some fish species exposed to heavy metals. This could be said because recovery in the rate of oxygen consumption in chromium treated guppies was complete after only 24 hours in chromium-free water. If such damage has occurred as a result of exposure to chromium, the gills might require a longer period to reconstitute their normal structure.

4.2 Daphnia magna

In the present work, the rate of oxygen consumption has been
found to decrease significantly in both 3-day old control Daphnia and in those exposed to 0.35 mg Cr l\(^{-1}\) upon starvation for 24 hours when compared with similar groups of fed Daphnia (Tables 13 and 14 and Fig. 27). It appears that food is very important to Daphnia during this stage in their life cycle because of the active growth which requires energy. So lack of food even for 24 hours would have a depressing effect on the metabolic activities of these animals. To eliminate this effect of starvation, the remaining experiments were carried out using fed Daphnia to evaluate the effect of hexavalent chromium on their rate of oxygen uptake. Exposure of Daphnia to both 0.35 and 0.18 mg Cr l\(^{-1}\) for 24 hours produced a significant reduction in their rate of oxygen consumption when compared with controls (Table 14 and 15 and Fig. 30 - C and B). This reduction increased with chromium concentration. The chromium content of the food was very small (p.38) so it is unlikely that death was due to chromium being taken in from this source in significant quantities. A concentration of 0.035 mg Cr l\(^{-1}\) did not seem to cause any significant alteration in the rate of oxygen consumption of Daphnia after 24 hour exposure when compared with that of the control (Table 16 and Fig. 30-A).

Very few papers could be found in the literature in which the effect of heavy metals on metabolic activity of invertebrate animals has been investigated. Some workers have reported a reduction in the rate of oxygen consumption in certain aquatic animals as a result of exposure to heavy metals which support some of my findings. Brown and Newell (1972) demonstrated that exposure of the mussel (Mytilus edulis) to copper in the form of copper sodium citrate at a concentration of 500 mg l\(^{-1}\) caused 50% depression in the respiratory rate of the whole animals as compared to unexposed controls and sodium citrate-exposed controls. Similarly Scott and Major (1972) reported a reduction in the rate of oxygen consumption of
**M. edulis** exposed to copper. Chaisemartin and Chaisemartin (1976) found that there was a reduction in the respiration rate of two species of crabs *Macropodia rostrata* and *Pachygrapsus marmoratus* exposed to both 1.0 and 0.5 mg Cr(VI) l⁻¹ for 1 day. They also reported that further reduction in the rate of respiration was observed in both species after 10 day exposure to 0.5 mg Cr l⁻¹ and after 5 - 7 day exposure to 1.0 mg Cr l⁻¹ at 24°C. They observed a good correlation between the level of bioaccumulation of chromium and the metabolic depletion of the tissues.

Capuzzo and Sasner (1977) also reported that excised gill tissue of *M. edulis* exposed to 10 mg Cr l⁻¹ and 1.0 mg Cr g⁻¹ clay, exhibited a reduction in their rate of oxygen consumption. They concluded that the decline in oxygen uptake of excised gill tissue suggests that chromium interferes with an energy supplying metabolic process, resulting in an inhibition of ciliary activity or vice versa.

Other data in the literature agrees with the result obtained in the present work in which low concentration of chromium (0.035 mg Cr l⁻¹) did not have a significant effect on the rate of oxygen consumption. Reeve et al. (1976) showed that respiration and excretion in *Calanus plumchrus* and *Metridia pacifica* were not significantly changed at 5 - 10 μg Cu l⁻¹. But Engel (unpublished data; in Engel and Fowler, 1979) found that the rate of oxygen consumption in excised clam gill tissue has been little affected following acute cadmium exposure (0.1 - 1.0 mg l⁻¹).

Contrary to the results obtained in this work, Sherr and Armitage (1971) measured oxygen consumption for 40 hours of both 6-day old controls *Daphnia pulex* and *Daphnia* exposed to 0.01 mg l⁻¹ sodium dichromate solution. They found that the rate of oxygen consumption of chromium
exposed *D. pulex* was twice as high as that for control animals. Because of the long period of measurement, a high percentage of *Daphnia* in the dichromate solution have either produced young or were containing well formed young in the brood pouches, so they concluded that it was not possible to state with certainty that the oxygen consumption rate is increased by exposure to dichromate. Thus, the difference from my results may be due to the formation of young which increased the rate of oxygen consumption or it was as a result of using a very low concentration of chromium compared with those used in the present work.

It could be concluded that *Daphnia magna* is much more sensitive to hexavalent chromium than the guppies used in this work as the concentrations of chromium which affected the rate of oxygen consumption in *Daphnia* were much lower than those found to affect the rate of oxygen consumption in fish.

5. **Reproduction of *Daphnia magna***

In the present work hexavalent chromium caused an effect on the reproductive activity of *Daphnia magna* when present in low concentrations. There was an increase in the total number of young produced by 24.3% and 22.7% (reproductive stimulation) in chromium concentrations of 0.035 and 0.07 mg l\(^{-1}\) respectively. At these concentrations it has been observed that there was an unexpected increase in the life span by 22.7% and 23.9% respectively. Similar reproductive enhancement by some heavy metals has been reported by other workers. Biesinger and Christensen (1972) reported that reproduction in *D. magna* was stimulated by small metal addition including trivalent chromium. The percentage of hatching of eggs in zebra
fish actually increased at 10 µg l\(^{-1}\) phenylmercuric acetate (Kihlström and Hulth, 1972). They concluded that this improved hatching at the low phenylmercuric acetate concentration was attributed to its bactericidal and fungicidal activity. Similarly Reish and Carr (1978) observed an enhancement of reproduction at lower concentrations of cadmium, copper and mercury in the polychaete Ctenodrilus serratus and at lower concentrations of cadmium, copper, mercury, lead and zinc in the polychaete Ophryotrocha diadema. However, they offer no explanation to account for this reproductive enhancement. More recently Soni and Abbasi (1981) found that the incidence of sexual reproduction (juvenile formation) in the earthworm Pheretima posthuma was higher in the case of worms treated with hexavalent chromium compared with control worms. Similar to the increased life span of D. magna observed in this work, Sherr and Armitage (1971) reported that in some cases a low concentration of sodium dichromate (0.01 mg \(l^{-1}\)) may even have a slight (though nonsignificant) positive effect on D. pulex survival.

On the other hand, the higher concentrations of chromium tested in the present work, caused a decrease in the total number of young produced (reproductive impairment) and in the life span. The decrease in the total number of young produced by D. magna reared in 0.11, 0.18 and 0.35 mg Cr \(l^{-1}\) was 66.1%, 85.3% and 92.8% respectively. While the reduction in the life span for the same chromium concentrations was 60%, 71.3% and 82.1% respectively. It seems that the reduction in the number of young observed at the higher concentrations of chromium did not result from a slower rate of eggs development at these concentrations, because the mean duration of egg incubation (from appearance of eggs to emergence of young) at the higher chromium concentrations was similar to or even shorter than that of the control animals. Such shortening in the duration of eggs development
and emergence of young has also been observed by Sherr and Armitage (1971) in *D. pulex*. So the reproductive impairment noticed in the present work could be due to failure of large number of eggs to complete their development and to give young at higher chromium concentrations as they were usually aborted from the body of females, and/or it could be due to reduction in the life span of females at the higher chromium concentrations. These results agree with the findings of Verriopoulos and Moraftou-Apostolopoulou (1981) who investigated the impact of 0.5, 1.0 and 2.0 mg Cr(VI) l\(^{-1}\) as Na\(_2\)CrO\(_4\) on the copepod *Tisbe holothuriae*. They found that all tested chromium concentrations affect the longevity of both the *F*\(_2\) and *F*\(_3\) generations, the latter being much more sensitive. No inhibition of the ability of egg sac formation has been noticed. The females of *F*\(_2\) generation, in various chromium concentrations, produced egg sacs throughout their life and at the same frequency as the control animals. The observed decrease in the number of egg sacs produced with increasing chromium concentrations was due to analogous shortening of life of the *F*\(_2\) generation. They also found that the development of egg sacs was strongly influenced by chromium and an increased percentage of abortion was observed in direct relationship with chromium concentrations. Moreover, they reported a decrease in the number of *F*\(_3\) offspring with increasing chromium concentrations.

Other workers have also reported a reduction in the rate of reproduction in a variety of aquatic animals as a result of exposure to heavy metals. Biesinger and Christensen (1972) found that the concentrations of trivalent chromium causing 50% and 16% reproductive impairment in *Daphnia magna* were 0.6 and 0.33 mg l\(^{-1}\) respectively. These higher values compared with my data in the present work, suggest that trivalent chromium is less toxic than hexavalent chromium. Similarly Oshida et al.
(1981) studied the effect of both trivalent and hexavalent chromium on reproduction of the polychaete *Neanthes arenaceodentata* and found that trivalent chromium is less toxic than hexavalent chromium. In tests conducted with hexavalent chromium, they observed that the polychaete reproduction ceased at 0.1 mg l\(^{-1}\) and the number of young produced was reduced at the 0.0125 and 0.05 mg l\(^{-1}\) levels and above. However, they added that the polychaetes which have lived in trivalent chromium showed no adverse effect in a concentration of 50.4 mg l\(^{-1}\). Most recently Oshida and Word (1982) tested the effect of different concentrations of hexavalent chromium ranging from less than 1 to 38.2 \(\mu\)g l\(^{-1}\) on *N. arenaceodentata* for two generations (309 days). They observed that there were no significant changes in time to spawning in any chromium concentration. There were no significant reductions in brood size among the parental (P) generation polychaetes exposed to hexavalent chromium; however, the number of young per brood produced in 16.6 \(\mu\)g l\(^{-1}\) was significantly higher than that produced by control polychaetes. F\(_1\) polychaetes that had spawned in 38.2 \(\mu\)g l\(^{-1}\) showed significantly reduced brood sizes when compared with the controls, while polychaetes in the lower chromium concentrations did not show significant changes in brood size. Finally they were unable to establish a direct relationship between tissue chromium concentration and reduced number of offspring.

In addition, it has been found in the present work that groups of *D. magna* were able to recover their normal rate of reproduction when returned to chromium-free water after exposure to 0.35 mg Cr l\(^{-1}\) for up to 6 days (Table 18). These groups showed a rate of reproduction more or less similar to that of control animals. The exceptions were some delay in the appearance of the first group of eggs, in the production of the first group of young and in the number of young produced per female per
day in *Daphnia* exposed to 0.35 mg Cr \(1^{-1}\) for 4 and 6 days. On the other hand, when the exposure period to 0.35 mg Cr \(1^{-1}\) was extended to 8 days, *Daphnia* failed to recover as their subsequent rate of reproduction and survival was significantly reduced when compared with control animals. From this, it could be concluded that *Daphnia* were able to regain normal activity when returned to chromium-free water after exposure to chromium for 6 days. But when the exposure period was extended to 8 days, the animals were unable to recover from the damage caused by chromium which resulted in subsequent increase in mortality and in a reduction of the reproductive ability. Studies similar to this could not be found in the literature, and this makes it impossible to compare these results with those of other workers.

Reproductive impairment is a more sensitive measure of toxicity by heavy metals, since a particular concentration of a heavy metal may not be lethal to an organism, but still it may cause a stress on its physiological process(es). Thus, in attempting to set up water quality standards in connection with a specific heavy metal, reproductive impairment measure could provide valuable information. Such measures are also more accurate in determining the safe concentration of a specific pollutant for a particular organism or ecosystem. In the present work, concentrations of hexavalent chromium below 0.07 mg \(1^{-1}\) appear to have no adverse effect on *D. magna* in long-term exposures, which might suggest that such concentrations could be considered safe for this animal in somewhat hard waters.

6. Histological Alterations

Exposure of minnows and guppies, in the present work, to 176.75 and
to 141.40 mg Cr l\(^{-1}\) respectively for 24 hours, failed to produce any histological alterations in the structure of liver in both species and in the kidney of guppies. However, kidney sections taken from chromium treated minnows showed some inconsistent dilation in the lumen of the tubules. But the difference in the diameter of the lumen of the tubules was not significant between sections of chromium treated and control fishes. It was suspected that the absence of any histological alterations in liver and kidney may be due to the short period of exposure to chromium (24 hours). But, in longer exposure (96 hours) of guppies to 53.03 mg Cr l\(^{-1}\), no alterations in the histology of both liver and kidney sections were observed.

Other studies on the histological alterations in liver and kidney tissues of fishes exposed to heavy metals are limited and variable. Baker (1969) found no noticeable difference in kidney sections taken from the winter flounder (Pseudopleuronectes americanus) exposed to 0.56 mg Cu l\(^{-1}\) for 700 hours. But in kidney sections of fish exposed to 1 and 3.2 mg Cu l\(^{-1}\), he observed a considerable change. The hemopoetic tissue was necrotic and very much reduced in volume. The tubule cells themselves were vacuolated and reduced in size. The apical portion of the tubule cells seemed to disintegrate and the lumen of the tubules showed considerable dilation and contained much dense material. Strik et al. (1975) exposed 2 year old rainbow trout to 10 mg Cr(VI) l\(^{-1}\) for 15 - 22 days and observed that kidney sections of chromium treated trout showed severe necrosis of the tubules. In the proximal part of the nephron, the tubular epithelium was completely disrupted and there was a loss of nuclei. The cytoplasm of these necrotic cells was fractionated into hyalin globules. In less severely affected cells, a hydropic degeneration was noticed. Recently, Van der Putte et al. (1981 b)
reported alterations in the histology of kidney in rainbow trout exposed to 44.8 mg Cr(VI) l⁻¹ for 96 hours at pH 7.8. These alterations included; dilation of lumen of the tubules and an increased ratio of the nucleus-to-cytoplasm in the tubular epithelium. Results on liver studies are also variable. Baker (1969) observed no appreciable alteration in liver sections of winter flounder exposed for 700 hours to 0.56 mg Cu l⁻¹. But liver sections of fish exposed to 1 and 3.2 mg Cu l⁻¹ showed the presence of fat in the cells around the central vein. While Van der Putte et al. (1981 b) found no alteration in the histology of liver sections of rainbow trout exposed for 4 days to 100, 25 and 0% (control) of the 96-hr LC₅₀ values at pH 7.8 and 6.5.

Since that the result of the present work showed no histological alterations in both liver and kidney of fish exposed to chromium. It could be concluded, in this case, that hexavalent chromium caused death in the fishes studied by means other than structural damage to liver and kidney.

7. Histochemical Staining of Chromium

Sections of liver and kidney taken from rainbow trout and guppies exposed to 17.68 and 141.40 mg Cr l⁻¹ respectively for 24 hours and stained with Chrome-azurol S (CAS) showed no clear difference of coloration from control sections. This indicates that the amount of chromium accumulated in these tissues during the 24 hour exposure period was not enough to be shown by this technique. However, when sections of liver and kidney of rainbow trout exposed to 7.07 mg Cr l⁻¹ for 4 days were stained with CAS, a clear difference in coloration was observed from
that of control sections. The blue coloration (which indicates the presence of chromium due to formation of Chromium-Chrome azurol S chelate complex, Cr-CAS) was distributed all over the liver section of chromium treated fish. But control liver sections showed no blue coloration. The kidney sections of chromium treated trout also showed the Cr-blue coloration which was concentrated in the interstitial cells between the tubules and in the glomeruli, while this coloration was not observed in control kidney sections. Similar differences in the coloration have also been observed in liver and kidney sections of guppies exposed to 53.03 mg Cr l⁻¹ for 4 days when compared with control sections. This pattern of chromium coloration agrees with the findings of Suzuki et al. (1978) who were succeeded for the first time to stain chromium in liver and kidney tissues of rat injected with 0.05 M aqueous solution of CrCl₃. They found that in the liver sections of chromium injected rats, the Cr-CAS complexes (blue color) filled the sinusoids. Cr-blue infiltrated the hepatic cell cytoplasm except for the nuclear area and the central vein was seen as red spot. But, they found that in the kidney sections of chromium injected rats, the Cr-blue appears in the capillary wall of the glomerulus and the interstitials. The proximal tubules and erythrocytes were stained red which is the original color of CAS. This technique has not yet been used to stain chromium in fish tissues as no work on this subject could be found in the literature.

In addition, the results obtained by this technique in the present work showed no appreciable alterations in the histology of both liver and kidney in chromium treated trout and guppies. This finding supports the results on the histology of liver and kidney of chromium exposed fish which have been discussed in the previous section.
General Conclusions

The present work mainly deals with the toxic action of hexavalent chromium on survival and on some physiological aspects of fish and crustacea. Among fish, chromium was more toxic to rainbow trout than to the other two species, and minnows were the most resistant to chromium. On the other hand, it has been found that Daphnia was far more sensitive to chromium than the fish.

The cause of death in fish is probably due to the precipitation of mucus on the gills of chromium treated fish. This layer of mucus could affect the gaseous exchange thus resulting in decreasing the amount of oxygen reaching the blood and causing asphyxiation. A similar conclusion has also been reported by other workers. Since chromium penetrated the gill membrane and accumulated in internal organs such as liver and kidney in large concentrations, death may in part result from the interference of chromium with the main biochemical function of these organs as no obvious histological alterations were observed in these tissues after exposure to chromium. Some workers have suggested that hexavalent chromium could elicit its toxic effect at some internal site. So it can be concluded that the cause of death is probably multiple in fish exposed to chromium. More detailed studies are needed to gain a better understanding of the toxic action of chromium on fish and other organisms.

The ability of chromium treated fish to recover their normal rate of oxygen consumption on returning to clean water could prove quite important in reducing the toxicity of this metal. This is because discharge of effluents from industry, for example a tannary factory, which contains chromium, is not usually continuous but there are some times during part
of the day or night in which the factory will be closed. This break in
discharging chromium into the environment could give the fish a chance to
recover before the next effluent is discharged. The same could be said
about the importance of recovery of Daphnia magna from any adverse effect
caused by exposure to chromium when these animals are no longer exposed to
this metal.

The higher sensitivity of Daphnia to chromium and their significant
recovery which is observed in the present work could make this organism
useful, in practical circumstances, as a test organism in studies
concerned with pollution of the aquatic environment by chromium. Further
work is suggested to develop this idea.
SUMMARY

1. The toxicity of hexavalent chromium on three fish species; rainbow trout (Salmo gairdneri), minnow (Phoxinus phoxinus) & guppy (Poecilia reticulata), and on the crustacean Daphnia magna was investigated.

2. Hexavalent chromium (as K$_2$Cr$_2$O$_7$) has been found to be toxic to the three species of fish, and its toxicity increased with increasing concentration. Precipitation of mucus was observed on the gills in all fishes exposed to chromium. When the 24-hr LC$_{50}$ values for chromium were determined, it was found that rainbow trout was the most susceptible and the minnow was the most resistant.

3. The acute and chronic toxicity of chromium on Daphnia magna has been found to increase with increasing chromium concentration. In general D. magna was far more sensitive to chromium than fish.

4. Recovery in the survival of D. magna exposed to 0.35 mg Cr l$^{-1}$ for up to 6 days was evident as their survival was more or less similar to that of control animals. But for 8 days exposure to chromium, most Daphnia failed to recover as their survival was highly reduced when compared with control animals.

5. All fish species were able to accumulate measurable concentrations of chromium in their tissues following exposure to chromium for 24 hours. Generally, the levels of chromium in various tissues increased with increasing chromium concentrations in the external
medium. A few exceptions to this were found in some tissues.

6. The accumulation of chromium in all fish species varies among different tissues. In the case of rainbow trout, it was found that gill and kidney tissues accumulated the highest concentrations of chromium followed by liver, stomach, heart, gonad, brain and muscle. For minnows, the order of chromium accumulation by various tissues from the highest to the lowest levels was as follows: gill, stomach, liver, kidney, heart, gonad, brain and muscle. This order for adult guppies was: stomach, gill, liver, heart, gonad, brain and muscle. While for juvenile guppies the highest level of chromium was found in the middle region of the body (which contains the viscera), followed by the anterior region, and the posterior region (which consists of muscle) accumulated the lowest level.

7. Exposure to 7.07 mg Cr l\(^{-1}\) for 24 hours had no effect on the rate of oxygen consumption of male guppies. But this rate was very significantly reduced when male guppies were exposed to 35.35 and 106.05 mg Cr l\(^{-1}\) for 24 hours.

8. Guppies exposed to 35.35 and 106.05 mg Cr l\(^{-1}\) for 24 hours were able to fully recover their normal rate of oxygen consumption in 24 hours after returning to chromium-free water.

9. Both control and chromium treated D. magna respired at a significantly lower rate when they were not given food for 24 hours compared with the respiration rate of corresponding fed groups.

10. Exposure of 3-day old D. magna to 0.035 mg Cr l\(^{-1}\) for 24 hours
(between 2nd and 3rd days) did not seem to cause a significant effect on the rate of oxygen consumption when compared with control animals (P>0.05).

11. The rate of oxygen consumption of 3-day old D. magna exposed to both 0.18 and 0.35 mg Cr l⁻¹ for 24 hours (between 2nd and 3rd days) was significantly reduced compared with that of control animals (P<0.01).

12. Different concentrations of chromium has been found to show variable effects on various aspects of the reproduction in D. magna. Some low concentrations appeared to cause reproductive enhancement while higher concentrations caused reproductive impairment.

13. The total number of young produced per female was significantly higher in D. magna reared in water containing 0.035 mg Cr l⁻¹ than that of control Daphnia (P<0.05). But at 0.07 mg Cr l⁻¹ the total number of young produced per female was not significantly different from that of control (P>0.05). On the other hand, the mean life span of females at these chromium concentrations was unexpectedly significantly longer than in control animals (P<0.05).

14. Higher concentrations of chromium (0.11, 0.18 and 0.35 mg l⁻¹) caused a significant reduction in the total number of young produced per female and in the mean life span of females when compared with control animals (P<0.01).

15. The peak number of young produced per female at 0.035 mg Cr l⁻¹ was not significantly different from that of control animals (P>0.05).
While at the higher concentrations of chromium (0.07, 0.11, 0.18 and 0.35 mg L$^{-1}$) there was a significant reduction in the peak number of young produced per female ($P<0.05$ or $P<0.01$).

16. The mean duration of egg incubation (from egg formation to emergence of young) was not affected by chromium at concentrations up to 0.18 mg L$^{-1}$. But at 0.35 mg Cr L$^{-1}$, this duration was significantly shorter than that of control animals ($p<0.01$).

17. Individuals of D. magna were able to recover from exposure for up to 6 days to 0.35 mg Cr L$^{-1}$ when they were returned to chromium-free water. This recovery was evident as their rate of reproduction and mean life span was not significantly different from control animals. However, when Daphnia were exposed to 0.35 mg Cr L$^{-1}$ for 8 days, recovery was greatly impaired when they were returned to chromium-free water. Their subsequent rate of reproduction was highly reduced and their mean life span was significantly shorter when compared with control animals ($P<0.01$).

18. 24 hours exposure of minnows and guppies to 176.75 and 141.40 mg Cr L$^{-1}$ respectively failed to produce pronounced structural alterations in both liver and kidney tissues of both species. Similarly, guppies exposed to 53.03 mg Cr L$^{-1}$ for 96 hours showed no significant difference in the histology of liver and kidney tissues from that of control fish tissues.

19. Using Chrome-azurol S, it was possible to stain chromium in liver and kidney sections of rainbow trout and guppies exposed for 96 hours to 7.07 and 53.03 mg Cr L$^{-1}$ respectively. In both species, the stained
chromium was found all over the liver sections of chromium treated fish. In kidney sections, however, stained chromium was restricted to the interstitial tissues between the tubules and in the glomeruli. On the other hand, this technique gave negative results in staining chromium in liver and kidney sections taken from rainbow trout and guppies exposed for 24 hours to 17.68 and 141.40 mg Cr $\text{l}^{-1}$ respectively. This was probably because the amount of chromium accumulated in the tissues during this exposure period was not high enough to be detected by this method.

20. The mode of action of hexavalent chromium on the survival and other physiological aspects of fish and Daphnia are discussed.
ACKNOWLEDGEMENTS

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* seen in abstract only
# TABLE 1  Some properties of tap water and canal water used in the present work.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Tap water</th>
<th>Canal water</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.01</td>
<td>8.04</td>
</tr>
<tr>
<td>Alkalinity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mg CaCO₃. l⁻¹)</td>
<td>26</td>
<td>238</td>
</tr>
<tr>
<td>Hardness</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mg CaCO₃. l⁻¹)</td>
<td>50.8</td>
<td>291</td>
</tr>
<tr>
<td>Conductivity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(µmhos.cm⁻¹)</td>
<td>110</td>
<td>540</td>
</tr>
<tr>
<td>Chromium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(µg. l⁻¹)</td>
<td>2.0</td>
<td>2.75</td>
</tr>
</tbody>
</table>
TABLE 2  

The effect of different concentrations of hexavalent chromium on the pH values of both tap water and canal water during a period of 24 hours in the various experiments with no fish present.

<table>
<thead>
<tr>
<th>Cr concentration (mg l⁻¹)</th>
<th>pH value during 24 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Start</td>
</tr>
</tbody>
</table>

| 1 Tap water               | 7.01        | 7.25      |
| a. Rainbow trout          | 6.93        | 7.42      |
| 7.07                      | 6.64        | 7.13      |
| 17.68                     | 6.39        | 6.91      |
| 35.35                     | 6.23        | 6.73      |
| 53.03                     | 6.16        | 6.68      |
| 70.70                     | 6.11        | 6.60      |
| 88.38                     |             |           |
| b. Guppy                  | 6.07        | 6.54      |
| 35.35                     | 5.92        | 6.38      |
| 70.70                     | 5.77        | 6.20      |
| 88.38                     | 5.63        | 5.95      |
| 106.05                    | 5.52        | 5.82      |
| 141.40                    | 5.44        | 5.72      |
| 176.75                    |             |           |

| 2 Canal water             | 8.04        | 8.20      |
| a. Minnow                | 6.52        | 7.42      |
| 106.05                   | 6.42        | 7.20      |
| 141.40                   | 6.34        | 7.08      |
| 176.75                   | 6.28        | 7.01      |
| 212.10                   | 6.22        | 6.87      |
| 247.45                   |             |           |
| b. Daphnia magna         | 8.25        | 8.28      |
| 0.35                     | 8.17        | 8.20      |
| 1.41                     |             |           |

Different tap waters were used in the trout and guppy experiments.
TABLE 3 Concentrations of hexavalent chromium causing 50% mortality after 24 hour exposure period in rainbow trout, minnow, guppy and Daphnia magna.

<table>
<thead>
<tr>
<th>Species</th>
<th>24-hr LC\textsubscript{50} (mg Cr \textsuperscript{-1})</th>
<th>24-hr LC\textsubscript{50} (mg Cr \textsuperscript{-1})</th>
<th>24-hr LC\textsubscript{50} (mg Cr \textsuperscript{-1})</th>
<th>24-hr LC\textsubscript{50} (mg Cr \textsuperscript{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rainbow trout</td>
<td>40.30</td>
<td>33</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minnow</td>
<td>181.35</td>
<td>185</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Guppy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adult</td>
<td>126.38</td>
<td>127</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Juvenile</td>
<td>123.73</td>
<td>125</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daphnia magna</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st Expt.</td>
<td>0.85</td>
<td>0.84</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2nd Expt.</td>
<td>1.10</td>
<td>1.07</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* by log probit method
TABLE 4  Time required for 50% mortality (LD$_{50}$) to occur in groups of Daphnia magna (30 individuals each) reared in different concentrations of hexavalent chromium.

<table>
<thead>
<tr>
<th>Cr concentration (mg l$^{-1}$)</th>
<th>0</th>
<th>0.035</th>
<th>0.07</th>
<th>0.11</th>
<th>0.18</th>
<th>0.35</th>
</tr>
</thead>
<tbody>
<tr>
<td>LD$_{50}$ (days)</td>
<td>45</td>
<td>55</td>
<td>59</td>
<td>17.5</td>
<td>12.75</td>
<td>7.75</td>
</tr>
<tr>
<td>LD$_{50}$ (days)</td>
<td>44</td>
<td>55</td>
<td>58</td>
<td>17</td>
<td>13.2</td>
<td>8.2</td>
</tr>
</tbody>
</table>

TABLE 5  Time required for 50% mortality (LD$_{50}$) to occur in groups of Daphnia magna (30 individuals each) exposed to 0.35 mg Cr l$^{-1}$ for different periods then reared in chromium free canal water.

<table>
<thead>
<tr>
<th>Exposure time to 0.35 mg Cr l$^{-1}$ (days)</th>
<th>0</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>LD$_{50}$ (days)</td>
<td>46.67</td>
<td>39.34</td>
<td>46.00</td>
<td>45.34</td>
<td>8.67</td>
</tr>
<tr>
<td>* LD$_{50}$ (days)</td>
<td>45.0</td>
<td>38.8</td>
<td>47.5</td>
<td>47.0</td>
<td>8.4</td>
</tr>
</tbody>
</table>

*by log probit method
## TABLE 6

Accumulation of chromium in various tissues of rainbow trout in µg Cr. g dry wt⁻¹ following exposure to different concentrations of hexavalent chromium for 24 hours.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Control</th>
<th>Chromium concentration (mg l⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7.07</td>
<td>17.68</td>
</tr>
<tr>
<td>Brain</td>
<td>0.25</td>
<td>1.01*</td>
</tr>
<tr>
<td></td>
<td>±0.25</td>
<td>±0.77</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.14</td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td>±0.10</td>
<td>±0.06</td>
</tr>
<tr>
<td>Gonad</td>
<td>0.30</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>±0.18</td>
<td>±0.00</td>
</tr>
<tr>
<td>Heart</td>
<td>0.62</td>
<td>2.91</td>
</tr>
<tr>
<td></td>
<td>±0.39</td>
<td>±1.03</td>
</tr>
<tr>
<td>Stomach</td>
<td>0.71</td>
<td>5.68**</td>
</tr>
<tr>
<td></td>
<td>±0.38</td>
<td>±1.25</td>
</tr>
<tr>
<td>Liver</td>
<td>0.13</td>
<td>6.79*</td>
</tr>
<tr>
<td></td>
<td>±0.09</td>
<td>±2.68</td>
</tr>
<tr>
<td>Gill</td>
<td>0.71</td>
<td>59.10**</td>
</tr>
<tr>
<td></td>
<td>±0.45</td>
<td>±7.67</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.20</td>
<td>21.94*</td>
</tr>
<tr>
<td></td>
<td>±0.13</td>
<td>±6.97</td>
</tr>
</tbody>
</table>

Values represent mean of five samples ±1 standard error.

**P>0.05**

*P<0.05

**P<0.01
TABLE 7  Accumulation of chromium in various tissues of minnow in μg Cr. g dry wt⁻¹ following exposure to different concentrations of hexavalent chromium for 24 hours.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Control</th>
<th>Chromium concentration (mg l⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>106.05</td>
<td>141.40</td>
</tr>
<tr>
<td>Brain</td>
<td>0.00</td>
<td>4.69</td>
</tr>
<tr>
<td></td>
<td>±0.00</td>
<td>±2.37</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.38</td>
<td>2.64*</td>
</tr>
<tr>
<td></td>
<td>±0.22</td>
<td>±0.81</td>
</tr>
<tr>
<td>Gonad</td>
<td>0.35</td>
<td>6.89**</td>
</tr>
<tr>
<td></td>
<td>±0.27</td>
<td>±1.20</td>
</tr>
<tr>
<td>Heart</td>
<td>0.00</td>
<td>11.70**</td>
</tr>
<tr>
<td></td>
<td>±0.00</td>
<td>±1.12</td>
</tr>
<tr>
<td>Stomach</td>
<td>0.17</td>
<td>64.99**</td>
</tr>
<tr>
<td></td>
<td>±0.13</td>
<td>±13.86</td>
</tr>
<tr>
<td>Liver</td>
<td>0.13</td>
<td>56.38**</td>
</tr>
<tr>
<td></td>
<td>±0.09</td>
<td>±13.28</td>
</tr>
<tr>
<td>Gill</td>
<td>1.13</td>
<td>97.11*</td>
</tr>
<tr>
<td></td>
<td>±0.75</td>
<td>±34.48</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.00</td>
<td>34.89*</td>
</tr>
<tr>
<td></td>
<td>±0.00</td>
<td>±11.74</td>
</tr>
</tbody>
</table>

Values represent mean of five samples ± 1 standard error.

P>0.05  
*P<0.05  
**P<0.01
TABLE 8  Accumulation of chromium in various tissues of adult guppies in 
μg Cr. g dry wt⁻¹ following exposure to different concentrations of 
hexavalent chromium for 24 hours.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Control</th>
<th>Chromium concentration (mg 1⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>35.35</td>
<td>70.70</td>
</tr>
<tr>
<td></td>
<td>88.38</td>
<td>106.05</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Control</th>
<th>Brain</th>
<th>Muscle</th>
<th>Gonad</th>
<th>Heart</th>
<th>Stomach</th>
<th>Liver</th>
<th>Gill</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.00</td>
<td>±0.00</td>
<td>0.00</td>
<td>±0.00</td>
<td>0.00</td>
<td>±0.00</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9.62**</td>
<td>±2.39</td>
<td>4.01*</td>
<td>±1.25</td>
<td>14.60**</td>
<td>±3.73</td>
<td>68.41**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20.13**</td>
<td>±2.18</td>
<td>8.63**</td>
<td>±1.16</td>
<td>22.49**</td>
<td>±2.58</td>
<td>128.44**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>17.62**</td>
<td>±4.02</td>
<td>6.63**</td>
<td>±0.45</td>
<td>11.37*</td>
<td>±4.32</td>
<td>98.97**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>29.18**</td>
<td>±5.40</td>
<td>8.88**</td>
<td>±0.88</td>
<td>36.67**</td>
<td>±6.72</td>
<td>180.10**</td>
</tr>
</tbody>
</table>

Values represent mean of five samples ± 1 standard error.

*P<0.05
**P<0.01
TABLE 9  Accumulation of chromium in various tissues of juvenile guppies in μg Cr. g dry wt⁻¹ following exposure to different concentrations of hexavalent chromium for 24 hours.

<table>
<thead>
<tr>
<th>Body region</th>
<th>Control Chromium concentration (mg l⁻¹)</th>
<th>17.68</th>
<th>35.35</th>
<th>70.70</th>
<th>106.05</th>
<th>141.40</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anterior</td>
<td>1.65</td>
<td>±0.63</td>
<td>±3.54</td>
<td>±3.83</td>
<td>±8.90</td>
<td>±9.89</td>
</tr>
<tr>
<td></td>
<td>34.20**</td>
<td>±3.54</td>
<td></td>
<td>±3.83</td>
<td>±8.90</td>
<td>±9.89</td>
</tr>
<tr>
<td></td>
<td>45.72**</td>
<td>±3.54</td>
<td></td>
<td>±3.83</td>
<td>±8.90</td>
<td>±9.89</td>
</tr>
<tr>
<td></td>
<td>59.60**</td>
<td>±3.54</td>
<td></td>
<td>±3.83</td>
<td>±8.90</td>
<td>±9.89</td>
</tr>
<tr>
<td></td>
<td>84.57**</td>
<td>±3.54</td>
<td></td>
<td>±3.83</td>
<td>±8.90</td>
<td>±9.89</td>
</tr>
<tr>
<td></td>
<td>81.84**</td>
<td>±3.54</td>
<td></td>
<td>±3.83</td>
<td>±8.90</td>
<td>±9.89</td>
</tr>
<tr>
<td>Middle</td>
<td>2.57</td>
<td>±1.03</td>
<td>±3.63</td>
<td>±5.86</td>
<td>±5.26</td>
<td>±5.32</td>
</tr>
<tr>
<td></td>
<td>48.89**</td>
<td>±1.03</td>
<td>±3.63</td>
<td>±5.86</td>
<td>±5.26</td>
<td>±5.32</td>
</tr>
<tr>
<td></td>
<td>72.50**</td>
<td>±1.03</td>
<td>±3.63</td>
<td>±5.86</td>
<td>±5.26</td>
<td>±5.32</td>
</tr>
<tr>
<td></td>
<td>74.82**</td>
<td>±1.03</td>
<td>±3.63</td>
<td>±5.86</td>
<td>±5.26</td>
<td>±5.32</td>
</tr>
<tr>
<td></td>
<td>82.66**</td>
<td>±1.03</td>
<td>±3.63</td>
<td>±5.86</td>
<td>±5.26</td>
<td>±5.32</td>
</tr>
<tr>
<td></td>
<td>86.49**</td>
<td>±1.03</td>
<td>±3.63</td>
<td>±5.86</td>
<td>±5.26</td>
<td>±5.32</td>
</tr>
<tr>
<td>Posterior</td>
<td>1.80</td>
<td>±1.11</td>
<td>±3.71</td>
<td>±3.01</td>
<td>±6.06</td>
<td>±5.79</td>
</tr>
<tr>
<td></td>
<td>20.10**</td>
<td>±1.11</td>
<td>±3.71</td>
<td>±3.01</td>
<td>±6.06</td>
<td>±5.79</td>
</tr>
<tr>
<td></td>
<td>23.13**</td>
<td>±1.11</td>
<td>±3.71</td>
<td>±3.01</td>
<td>±6.06</td>
<td>±5.79</td>
</tr>
<tr>
<td></td>
<td>29.55**</td>
<td>±1.11</td>
<td>±3.71</td>
<td>±3.01</td>
<td>±6.06</td>
<td>±5.79</td>
</tr>
<tr>
<td></td>
<td>31.29**</td>
<td>±1.11</td>
<td>±3.71</td>
<td>±3.01</td>
<td>±6.06</td>
<td>±5.79</td>
</tr>
<tr>
<td></td>
<td>40.84**</td>
<td>±1.11</td>
<td>±3.71</td>
<td>±3.01</td>
<td>±6.06</td>
<td>±5.79</td>
</tr>
</tbody>
</table>

Values represent mean of five samples ± 1 standard error.

**P<0.01
TABLE 10  Oxygen consumption of guppies exposed to 7.07 mg l⁻¹ hexavalent chromium for 24 hours, then transferred to chromium free water for different periods.

<table>
<thead>
<tr>
<th>Time in Cr free water(days)</th>
<th>Control</th>
<th>0</th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>369.56</td>
<td>300.66</td>
<td>281.87</td>
<td>250.55</td>
</tr>
<tr>
<td>2</td>
<td>292.19</td>
<td>419.43</td>
<td>282.76</td>
<td>144.21</td>
</tr>
<tr>
<td>3</td>
<td>368.06</td>
<td>341.77</td>
<td>315.48</td>
<td>252.38</td>
</tr>
<tr>
<td>4</td>
<td>358.39</td>
<td>310.95</td>
<td>321.49</td>
<td>358.39</td>
</tr>
<tr>
<td>5</td>
<td>255.53</td>
<td>297.25</td>
<td>323.32</td>
<td>208.59</td>
</tr>
<tr>
<td>6</td>
<td>297.16</td>
<td>222.87</td>
<td>238.51</td>
<td>192.37</td>
</tr>
<tr>
<td>7</td>
<td>375.49</td>
<td>296.90</td>
<td>288.17</td>
<td>185.99</td>
</tr>
<tr>
<td>8</td>
<td>282.57</td>
<td>306.45</td>
<td>322.37</td>
<td>191.03</td>
</tr>
<tr>
<td>$\bar{x}$</td>
<td>324.87</td>
<td>312.04</td>
<td>296.75</td>
<td>222.94</td>
</tr>
<tr>
<td>S.E.</td>
<td>16.90</td>
<td>19.36</td>
<td>10.53</td>
<td>23.03</td>
</tr>
</tbody>
</table>

$\bar{x} = $ Mean, S.E. = 1 standard error

In Tables 10, 11 and 12 each individual was its own control.
TABLE 11  Oxygen consumption of guppies exposed to 35.35 mg l\(^{-1}\) hexavalent chromium for 24 hours, then transferred to chromium free water for different periods.

<table>
<thead>
<tr>
<th>Time in Cr free water (days)</th>
<th>Control</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>231.66</td>
<td>193.63</td>
<td>313.27</td>
<td>255.87</td>
<td>231.66</td>
<td>242.04</td>
</tr>
<tr>
<td>2</td>
<td>311.18</td>
<td>157.61</td>
<td>252.98</td>
<td>250.56</td>
<td>250.56</td>
<td>222.27</td>
</tr>
<tr>
<td>3</td>
<td>255.26</td>
<td>124.92</td>
<td>288.93</td>
<td>282.42</td>
<td>325.87</td>
<td>353.02</td>
</tr>
<tr>
<td>4</td>
<td>384.51</td>
<td>169.01</td>
<td>447.67</td>
<td>266.20</td>
<td>291.55</td>
<td>350.70</td>
</tr>
<tr>
<td>5</td>
<td>325.52</td>
<td>122.66</td>
<td>542.53</td>
<td>358.54</td>
<td>344.39</td>
<td>320.80</td>
</tr>
<tr>
<td>6</td>
<td>295.69</td>
<td>145.57</td>
<td>254.75</td>
<td>232.00</td>
<td>232.00</td>
<td>236.55</td>
</tr>
<tr>
<td>7</td>
<td>301.70</td>
<td>139.59</td>
<td>319.72</td>
<td>306.21</td>
<td>265.68</td>
<td>279.19</td>
</tr>
<tr>
<td>8</td>
<td>255.33</td>
<td>157.12</td>
<td>441.91</td>
<td>338.80</td>
<td>274.97</td>
<td>314.25</td>
</tr>
<tr>
<td>(\bar{x})</td>
<td>295.11</td>
<td>151.26</td>
<td>357.72</td>
<td>286.33</td>
<td>277.09</td>
<td>289.85</td>
</tr>
<tr>
<td>S.E.</td>
<td>17.12</td>
<td>8.31</td>
<td>37.57</td>
<td>15.78</td>
<td>14.67</td>
<td>18.45</td>
</tr>
</tbody>
</table>

\(\bar{x}\) = Mean, S.E. = 1 standard error
TABLE 12 Oxygen consumption of guppies exposed to 106.05 mg l\(^{-1}\) hexavalent chromium for 24 hours, then transferred to chromium free water for different periods.

<table>
<thead>
<tr>
<th>Time in Cr free water (days)</th>
<th>Control</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>253.68</td>
<td>179.71</td>
<td>336.96</td>
<td>297.65</td>
<td>303.26</td>
<td>297.65</td>
</tr>
<tr>
<td>2</td>
<td>231.95</td>
<td>205.05</td>
<td>243.50</td>
<td>237.09</td>
<td>224.28</td>
<td>224.28</td>
</tr>
<tr>
<td>3</td>
<td>230.92</td>
<td>219.43</td>
<td>304.21</td>
<td>304.21</td>
<td>264.32</td>
<td>339.12</td>
</tr>
<tr>
<td>4</td>
<td>220.80</td>
<td>175.10</td>
<td>206.61</td>
<td>244.87</td>
<td>275.47</td>
<td>451.47</td>
</tr>
<tr>
<td>5</td>
<td>196.34</td>
<td>231.53</td>
<td>249.69</td>
<td>190.67</td>
<td>254.23</td>
<td>299.62</td>
</tr>
<tr>
<td>6</td>
<td>287.82</td>
<td>191.50</td>
<td>160.33</td>
<td>164.78</td>
<td>240.49</td>
<td>195.95</td>
</tr>
<tr>
<td>7</td>
<td>281.87</td>
<td>213.52</td>
<td>208.44</td>
<td>208.44</td>
<td>259.28</td>
<td>305.03</td>
</tr>
<tr>
<td>8</td>
<td>320.94</td>
<td>207.02</td>
<td>196.92</td>
<td>207.02</td>
<td>257.52</td>
<td>318.11</td>
</tr>
<tr>
<td>(\bar{x})</td>
<td>253.04</td>
<td>202.86</td>
<td>238.33</td>
<td>231.84</td>
<td>259.86</td>
<td>303.90</td>
</tr>
<tr>
<td>S.E.</td>
<td>14.54</td>
<td>6.90</td>
<td>20.67</td>
<td>17.49</td>
<td>8.27</td>
<td>27.15</td>
</tr>
</tbody>
</table>

\(\bar{x}\) = Mean, S.E. = 1 standard error
TABLE 13  Oxygen consumption of 2-day and 3-day old starved *Daphnia magna* in chromium free water compared with that of 3-day old *Daphnia* after being exposed to 0.35 mg Cr(VI) l⁻¹ for 24 hours between 2nd & 3rd days.

<table>
<thead>
<tr>
<th></th>
<th>O₂ consumption in µl. animal⁻¹. hr⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2-day old in Cr free water</td>
</tr>
<tr>
<td>1</td>
<td>0.035</td>
</tr>
<tr>
<td>2</td>
<td>0.038</td>
</tr>
<tr>
<td>3</td>
<td>0.040</td>
</tr>
<tr>
<td>4</td>
<td>0.038</td>
</tr>
<tr>
<td>5</td>
<td>0.023</td>
</tr>
<tr>
<td>6</td>
<td>0.024</td>
</tr>
<tr>
<td>7</td>
<td>0.022</td>
</tr>
<tr>
<td>8</td>
<td>0.030</td>
</tr>
<tr>
<td>9</td>
<td>0.033</td>
</tr>
<tr>
<td>10</td>
<td>0.032</td>
</tr>
<tr>
<td>11</td>
<td>0.031</td>
</tr>
<tr>
<td>12</td>
<td>0.032</td>
</tr>
<tr>
<td>13</td>
<td>0.032</td>
</tr>
<tr>
<td>14</td>
<td>0.022</td>
</tr>
<tr>
<td>15</td>
<td>0.033</td>
</tr>
<tr>
<td>16</td>
<td>0.027</td>
</tr>
<tr>
<td>x</td>
<td>0.031</td>
</tr>
<tr>
<td>S.E.</td>
<td>0.001</td>
</tr>
</tbody>
</table>

*x* = Mean, S.E. = 1 standard error
TABLE 14  Oxygen consumption of 2-day and 3-day old fed *Daphnia magna* in chromium free water compared with that of 3-day old *Daphnia* after being exposed to 0.35 mg Cr(VI) l⁻¹ for 24 hours between 2nd & 3rd days.

<table>
<thead>
<tr>
<th></th>
<th>2-day old in Cr free Water</th>
<th>3-day old Cr free water</th>
<th>Cr exposed</th>
</tr>
</thead>
<tbody>
<tr>
<td>0₂ consumption in µl. animal⁻¹. hr⁻¹</td>
<td>0.038</td>
<td>0.054</td>
<td>-</td>
</tr>
<tr>
<td>1</td>
<td>0.033</td>
<td>0.057</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>0.033</td>
<td>0.058</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>0.032</td>
<td>0.051</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>0.033</td>
<td>0.058</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>0.033</td>
<td>0.043</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>0.031</td>
<td>0.042</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>0.033</td>
<td>0.062</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>0.034</td>
<td>-</td>
<td>0.019</td>
</tr>
<tr>
<td>9</td>
<td>0.033</td>
<td>-</td>
<td>0.024</td>
</tr>
<tr>
<td>10</td>
<td>0.033</td>
<td>-</td>
<td>0.027</td>
</tr>
<tr>
<td>11</td>
<td>0.038</td>
<td>-</td>
<td>0.022</td>
</tr>
<tr>
<td>12</td>
<td>0.033</td>
<td>-</td>
<td>0.018</td>
</tr>
<tr>
<td>13</td>
<td>0.032</td>
<td>-</td>
<td>0.027</td>
</tr>
<tr>
<td>14</td>
<td>0.029</td>
<td>-</td>
<td>0.024</td>
</tr>
<tr>
<td>15</td>
<td>0.038</td>
<td>-</td>
<td>0.030</td>
</tr>
<tr>
<td>16</td>
<td>0.034</td>
<td>0.053</td>
<td>0.024</td>
</tr>
<tr>
<td>x</td>
<td>0.001</td>
<td>0.003</td>
<td>0.001</td>
</tr>
</tbody>
</table>

ᵯ = Mean, S.E. = 1 standard error.
TABLE 15  Oxygen consumption of 2-day and 3-day old fed *Daphnia magna* in chromium free water compared with that of 3-day old *Daphnia* after being exposed to 0.18 mg Cr(VI) l⁻¹ for 24 hours between 2nd and 3rd days.

<table>
<thead>
<tr>
<th></th>
<th>2-day old in Cr free water</th>
<th>3-day old Cr free water</th>
<th>Cr exposed</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.025</td>
<td>0.044</td>
<td>–</td>
</tr>
<tr>
<td>2</td>
<td>0.030</td>
<td>0.042</td>
<td>–</td>
</tr>
<tr>
<td>3</td>
<td>0.029</td>
<td>0.043</td>
<td>–</td>
</tr>
<tr>
<td>4</td>
<td>0.031</td>
<td>0.052</td>
<td>–</td>
</tr>
<tr>
<td>5</td>
<td>0.032</td>
<td>0.049</td>
<td>–</td>
</tr>
<tr>
<td>6</td>
<td>0.035</td>
<td>0.051</td>
<td>–</td>
</tr>
<tr>
<td>7</td>
<td>0.033</td>
<td>0.050</td>
<td>–</td>
</tr>
<tr>
<td>8</td>
<td>0.032</td>
<td>0.054</td>
<td>–</td>
</tr>
<tr>
<td>9</td>
<td>0.035</td>
<td>–</td>
<td>0.035</td>
</tr>
<tr>
<td>10</td>
<td>0.031</td>
<td>–</td>
<td>0.031</td>
</tr>
<tr>
<td>11</td>
<td>0.032</td>
<td>–</td>
<td>0.030</td>
</tr>
<tr>
<td>12</td>
<td>0.033</td>
<td>–</td>
<td>0.033</td>
</tr>
<tr>
<td>13</td>
<td>0.038</td>
<td>–</td>
<td>0.035</td>
</tr>
<tr>
<td>14</td>
<td>0.033</td>
<td>–</td>
<td>0.031</td>
</tr>
<tr>
<td>15</td>
<td>0.035</td>
<td>–</td>
<td>0.040</td>
</tr>
<tr>
<td>16</td>
<td>0.036</td>
<td>–</td>
<td>0.033</td>
</tr>
<tr>
<td>x</td>
<td>0.033</td>
<td>0.048</td>
<td>0.034</td>
</tr>
<tr>
<td>S.E.</td>
<td>0.001</td>
<td>0.002</td>
<td>0.001</td>
</tr>
</tbody>
</table>

\( \overline{x} = \text{Mean}, \ S.E. = \text{1 standard error.} \)
Table 16: Oxygen consumption of 2-day and 3-day old fed Daphnia magna in chromium free water compared with that of 3-day old Daphnia after being exposed to 0.035 mg Cr(VI) l⁻¹ for 24 hours between 2nd and 3rd days.

<table>
<thead>
<tr>
<th></th>
<th>2-day old</th>
<th>3-day old</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cr free water</td>
<td>Cr free water</td>
</tr>
<tr>
<td>1</td>
<td>0.032</td>
<td>0.049</td>
</tr>
<tr>
<td>2</td>
<td>0.035</td>
<td>0.045</td>
</tr>
<tr>
<td>3</td>
<td>0.032</td>
<td>0.049</td>
</tr>
<tr>
<td>4</td>
<td>0.035</td>
<td>0.047</td>
</tr>
<tr>
<td>5</td>
<td>0.035</td>
<td>0.054</td>
</tr>
<tr>
<td>6</td>
<td>0.035</td>
<td>0.051</td>
</tr>
<tr>
<td>7</td>
<td>0.036</td>
<td>0.055</td>
</tr>
<tr>
<td>8</td>
<td>0.035</td>
<td>0.052</td>
</tr>
<tr>
<td>9</td>
<td>0.038</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>0.033</td>
<td>-</td>
</tr>
<tr>
<td>11</td>
<td>0.035</td>
<td>-</td>
</tr>
<tr>
<td>12</td>
<td>0.036</td>
<td>-</td>
</tr>
<tr>
<td>13</td>
<td>0.035</td>
<td>-</td>
</tr>
<tr>
<td>14</td>
<td>0.036</td>
<td>-</td>
</tr>
<tr>
<td>15</td>
<td>0.038</td>
<td>-</td>
</tr>
<tr>
<td>16</td>
<td>0.036</td>
<td>-</td>
</tr>
<tr>
<td>x</td>
<td>0.035</td>
<td>0.050</td>
</tr>
<tr>
<td>S.E.</td>
<td>0.0005</td>
<td>0.001</td>
</tr>
</tbody>
</table>

x = Mean, S.E. = 1 standard error.
TABLE 17  Effect of various concentrations of hexavalent chromium on the reproduction of *Daphnia magna*.

<table>
<thead>
<tr>
<th>Control</th>
<th>Chromium concentration (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.035</td>
</tr>
<tr>
<td>1st-appearance of eggs (days)</td>
<td>4.57 ± 0.09</td>
</tr>
<tr>
<td>lst young produced (days)</td>
<td>7.03 ± 0.03</td>
</tr>
<tr>
<td>Peak no. of young produced /female</td>
<td>10.33 ± 0.44</td>
</tr>
<tr>
<td>Total no. of young produced /female</td>
<td>67.20 ± 4.65</td>
</tr>
<tr>
<td>Life span (days)</td>
<td>48.53 ± 2.63</td>
</tr>
<tr>
<td>Number of young produced /female/day</td>
<td>1.35 ± 0.05</td>
</tr>
<tr>
<td>Period of egg incubation (days)</td>
<td>2.73 ± 0.03</td>
</tr>
</tbody>
</table>

Values represent mean of 30 individuals ±1 standard error.

\*P<0.05  
\*\*P<0.01
TABLE 18  Effect of exposure to 0.35 mg Cr(VI) l−1 for 2, 4, 6 and 8 days on subsequent reproduction of Daphnia magna in chromium free canal water.

<table>
<thead>
<tr>
<th>Exposure time to chromium (days)</th>
<th>0</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st - appearance of eggs (days)</td>
<td>4.53</td>
<td>4.73</td>
<td>5.33**</td>
<td>7.35**</td>
<td>5.37**</td>
</tr>
<tr>
<td>±0.09</td>
<td>±0.10</td>
<td>±0.11</td>
<td>±0.17</td>
<td>±0.10</td>
<td></td>
</tr>
<tr>
<td>1st young produced (days)</td>
<td>6.93</td>
<td>7.03</td>
<td>8.03**</td>
<td>9.90**</td>
<td>8.53**</td>
</tr>
<tr>
<td>±0.05</td>
<td>±0.03</td>
<td>±0.11</td>
<td>±0.18</td>
<td>±0.34</td>
<td></td>
</tr>
<tr>
<td>Peak no. of young produced /female</td>
<td>10.80</td>
<td>10.50</td>
<td>11.50</td>
<td>10.17</td>
<td>3.57**</td>
</tr>
<tr>
<td>±0.38</td>
<td>±0.38</td>
<td>±0.48</td>
<td>±0.51</td>
<td>±0.94</td>
<td></td>
</tr>
<tr>
<td>Total no. of young produced /female</td>
<td>83.60</td>
<td>80.20</td>
<td>76.67</td>
<td>79.71</td>
<td>19.57**</td>
</tr>
<tr>
<td>±8.94</td>
<td>±8.10</td>
<td>±7.01</td>
<td>±9.55</td>
<td>±8.53</td>
<td></td>
</tr>
<tr>
<td>Life span (days)</td>
<td>60.20</td>
<td>57.10</td>
<td>53.27</td>
<td>65.90</td>
<td>19.07**</td>
</tr>
<tr>
<td>±6.14</td>
<td>±5.3</td>
<td>±4.77</td>
<td>±6.70</td>
<td>±5.28</td>
<td></td>
</tr>
<tr>
<td>Number of young produced /female/day</td>
<td>1.36</td>
<td>1.35</td>
<td>1.44</td>
<td>1.13**</td>
<td>0.41**</td>
</tr>
<tr>
<td>±0.03</td>
<td>±0.05</td>
<td>±0.05</td>
<td>±0.04</td>
<td>±0.10</td>
<td></td>
</tr>
<tr>
<td>Period of egg incubation (days)</td>
<td>2.63</td>
<td>2.60</td>
<td>2.60</td>
<td>2.64</td>
<td>2.77</td>
</tr>
<tr>
<td>±0.03</td>
<td>±0.04</td>
<td>±0.03</td>
<td>±0.04</td>
<td>±0.08</td>
<td></td>
</tr>
</tbody>
</table>

Values represent mean of 30 individuals ±1 standard error. 

P>0.05

**P<0.01
FIGURE 1

Percent survival of rainbow trout exposed to different concentrations of chromium for 24 hours.
FIGURE 2

Percent survival of minnows exposed to different concentrations of chromium for 24 hours.
FIGURE 3

Percent survival of adult (●) and juvenile (○) guppies exposed to different concentrations of chromium for 24 hours.
FIGURE 4

Percent survival of *Daphnia magna* exposed to different concentrations of chromium for 24 hours (first experiment).
FIGURE 5

Percent survival of *Daphnia magna* exposed to different concentrations of chromium for 24 hours (second experiment).
Percent survival of *Daphnia magna* reared in various concentrations of chromium; (A) control, (B) 0.035, (C) 0.07, (D) 0.11, (E) 0.18 and (F) 0.35 mg Cr. l⁻¹ until 100% mortality is reached.
FIGURE 7

Percent survival of *Daphnia magna* reared in chromium free water after being exposed to 0.35 mg Cr. l\(^{-1}\) for 0 day (A), 2 days (B), 4 days (C), 6 days (D) and 8 days (E) until 100% mortality is reached.
Accumulation of chromium in Brain (●) and Muscle (○) of rainbow trout exposed to different concentrations of chromium for 24 hours.
Accumulation of chromium in Gonad (●) and Heart (○) of rainbow trout exposed to different concentrations of chromium for 24 hours.
Accumulation of chromium in Stomach (●) and Liver (○) of rainbow trout exposed to different concentrations of chromium for 24 hours.
Accumulation of chromium in Gills (●) and Kidney (○) of rainbow trout exposed to different concentrations of chromium for 24 hours.
FIGURE 12

Accumulation of chromium in Brain (●) and Muscle (○) of minnow exposed to different concentrations of chromium for 24 hours.
FIGURE 13

Accumulation of chromium in Gonad (●) and Heart (○) of minnow exposed to different concentrations of chromium for 24 hours.
Accumulation of chromium in Stomach (●) and Liver (○) of minnow exposed to different concentrations of chromium for 24 hours.
Accumulation of chromium in Gills (●) and Kidney (○) of minnow exposed to different concentrations of chromium for 24 hours.
CHROMIUM CONC. IN TISSUE (µg Cr g dry wt⁻¹)

mg K₂Cr₂O₇ l⁻¹

mg Cr l⁻¹
Accumulation of chromium in Brain (●) and Muscle (○) of adult guppy exposed to different concentrations of chromium for 24 hours.
FIGURE 17

Accumulation of chromium in Gonad (●) and Heart (○) of adult guppy exposed to different concentrations of chromium for 24 hours.
Accumulation of chromium in Stomach (●) and Liver (○) of adult guppy exposed to different concentrations of chromium for 24 hours.
FIGURE 19

Accumulation of chromium in Gills of adult guppy exposed to different concentrations of chromium for 24 hours.
Accumulation of chromium in different regions of the body of juvenile guppies exposed to various concentrations of chromium for 24 hours; (A) anterior, (B) middle and (C) posterior regions.
FIGURE 21

Mean oxygen consumption of male guppies exposed to 7.07 mg Cr. 1\(^{-1}\) for 24 hours, and then transferred to chromium free water for up to 2 days.
FIGURE 22

Oxygen consumption of representative individual guppies exposed to 7.07 mg Cr. l\(^{-1}\) for 24 hours, and then transferred to chromium free water for 2 days.
FIGURE 23

Mean oxygen consumption of male guppies exposed to 35.35 mg Cr. l\(^{-1}\) for 24 hours, and then transferred to chromium free water for 7 days.
FIGURE 24

Oxygen consumption of representative individual guppies exposed to 35.35 mg Cr. l\(^{-1}\) for 24 hours, and then transferred to chromium free water for 7 days.
FIGURE 25

Mean oxygen consumption of male guppies exposed to 106.05 mg Cr. 1⁻¹ for 24 hours, and then transferred to chromium free water for 7 days.
FIGURE 26

Oxygen consumption of representative individual guppies exposed to 106.05 mg Cr. 1⁻¹ for 24 hours, and then transferred to chromium free water for 7 days.
Comparison of oxygen consumption between fed (A) and starved (B) *Daphnia magna* after exposure to 0.35 mg Cr. l$^{-1}$ for 24 hours; control (●) and chromium exposed (○).
Oxygen consumption of representative individuals of starved *Daphnia magna*. (A) control (B) exposed to 0.35 mg Cr. l\(^{-1}\) for 24 hours between day 2 and 3.
Oxygen consumption of representative individuals of fed *Daphnia magna*. (A) control (B) exposed to 0.35 mg Cr L⁻¹ for 24 hours between day 2 and 3.
Mean oxygen consumption of fed Daphnia magna. (●) control, (○) exposed for 24 hours between day 2 and 3 to (A) 0.035, (B) 0.18, (C) 0.35 mg Cr. 1\(^{-1}\).
FIGURE 31

Oxygen consumption of representative individuals of fed Daphnia magna. (A) control, (B) exposed to 0.18 mg Cr. l⁻¹ for 24 hours between day 2 and 3.
FIGURE 32

Oxygen consumption of representative individuals of fed Daphnia magna. (A) control, (B) exposed to 0.035 mg Cr. l$^{-1}$ for 24 hours between day 2 and 3.
FIGURE 33

Mean oxygen consumption of 3-day old fed *Daphnia magna* exposed to different concentrations of chromium for 24 hours between second and third days.
FIGURE 34

Mean peak number of young produced per female in groups (30 individuals each) of *Daphnia magna* reared in various concentrations of chromium.
MEAN PEAK NO. OF YOUNG PRODUCED, FEMALE\(^{-1}\)

mg K\(_2\)Cr\(_2\)O\(_7\) l\(^{-1}\)

mg Cr l\(^{-1}\)
FIGURE 35

Mean total number of young produced per female in groups (30 individuals each) of *Daphnia magna* reared in various concentrations of chromium.
Mean life span in groups (30 individuals each) of *Daphnia magna* reared in various concentrations of chromium.
MEAN LIFE SPAN (days)

mg $K_2Cr_2O_7. l^{-1}$

mg Cr. l$^{-1}$
FIGURE 37

Mean period of egg incubation, from egg formation to emergence of young, in groups of *Daphnia magna* (30 individuals each) reared in various concentrations of chromium.
FIGURE 38

Mean peak number of young produced per female in groups (30 individuals each) of *Daphnia magna* exposed to 0.35 mg Cr. l⁻¹ for 2, 4, 6 and 8 days and then transferred to chromium free water.
Mean peak no. of young produced, female⁻¹

Exposure time to chromium (days)
Mean total number of young produced per female in groups (30 individuals each) of *Daphnia magna* exposed to 0.35 mg Cr. l^{-1} for 2, 4, 6 and 8 days and then transferred to chromium free water.
Mean life span of groups (30 individuals each) of *Daphnia magna* exposed to 0.35 mg Cr. 1⁻¹ for 2, 4, 6 and 8 days and then transferred to chromium free water.
Mean period of egg incubation, from egg formation to emergence of young, in groups (30 individuals each) of Daphnia magna exposed to 0.35 mg Cr. l\(^{-1}\) for 2, 4, 6 and 8 days and then transferred to chromium free water.
MEAN PERIOD OF EGG INCUBATION (days)

EXPOSURE TIME TO CHROMIUM (days)
PLATE 1

Liver of rainbow trout stained with Chrome-azurol S. (A) Control fish. (B) Fish exposed to 7.07 mg Cr. l$^{-1}$ for 96 hours (X400). Chromium staining indicated by arrow.
Kidney of rainbow trout stained with Chrome-azurol S. (A)
Control fish. (B) Fish exposed to 7.07 mg Cr. l\(^{-1}\) for 96 hours (X400).
Chromium staining indicated by arrow.
PLATE 3

Liver of guppies stained with Chrome-azurol S. (A) Control fish. (B) Fish exposed to 53.03 mg Cr. l$^{-1}$ for 96 hours (X400). Chromium staining indicated by arrow.
Kidney of guppies stained with Chrome-azurol S. (A) Control fish. (B) Fish exposed to 53.03 mg Cr. 1\(^{-1}\) for 96 hours (X400). Chromium staining indicated by arrow.