WATER QUALITY CRITERIA FOR EUROPEAN FRESHWATER FISH
Report on chromium and freshwater fish
EUROPEAN INLAND FISHERIES ADVISORY COMMISSION (EIFAC)

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WATER QUALITY CRITERIA FOR EUROPEAN FRESHWATER FISH

REPORT ON CHROMIUM AND FRESHWATER FISH

Prepared by

the European Inland Fisheries Advisory Commission Working Party on Water Quality Criteria for European Freshwater Fish

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS
Rome 1983
This is the twelfth review of the EIFAC Working Party on Water Quality Criteria for European Freshwater Fish.

For the preparation of this report, the following experts were appointed to the Working Party:

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The Working Party used the same general basis for their work as that on which they had agreed for the preparation of their first report that:

"Water quality criteria for freshwater fish should ideally permit all stages in the life cycles to be successfully completed and, in addition, should not produce conditions in a river water which would either taint the flesh of the fish or cause them to avoid a stretch of river where they would otherwise be present, or give rise to accumulation of deleterious substances in fish to such a degree that they are potentially harmful when consumed. Indirect factors like those affecting fish-food organisms must also be considered should they prove to be important".

This report was prepared by D. Calamari and J.F. de L.G. Solbé and presented to the Twelfth Session of EIFAC (Budapest, 31 May - 5 June 1982) where it was approved.

The Working Party acknowledges the helpful comments on the draft review provided by J.S. Alabaster, D. Mount and I. van der Putte.

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This report, prepared by the European Inland Fisheries Research Advisory Commission Working Party on Water Quality Criteria for European Freshwater Fish, critically reviews the literature on the occurrence and effects of chromium in fresh water. It lists and discusses the sources of chromium, and its chemistry and analysis in fresh water. The mode of action of chromium and the factors which affect its short- and long-term toxicity to the various life-cycle stages of fish are dealt with in detail. Similar information for plants and invertebrates is considered and evidence for the accumulation of the metal in animals is reviewed.

The tentative water-quality criteria proposed distinguish between salmonid and non-salmonid waters but, in order to protect sensitive species of invertebrates, it is recommended that criteria for salmonid waters should be used to cover the majority of waters. The conditions under which more or less stringent standards may be appropriate are indicated. To protect salmonid waters, the mean aqueous concentration of 'soluble' chromium should not exceed 0.025 mg Cr/l and the 95 percentile should not exceed 0.1 mg Cr/l.
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1. INTRODUCTION

1.1 Sources and Occurrence of Chromium

Chromium is a metal of widespread natural occurrence (but not as the pure metal). In nature it generally occurs in the trivalent state but hexavalent chromium has also been found. The metal is present in spinel-type minerals (iron and magnesium chromo-aluminates). Chromite (FeO.Cr2O3) is the most important mineral for refining purposes and is mined in the Philippines, South Africa, Turkey, USSR and Zimbabwe and is widely used industrially. Several general reviews list the concentrations (Cheremisinoff and Habib, 1972; National Research Council of Canada (NRCC), 1976) and tonnages employed (NRCC, 1976; Van de Velde, 1978) and a number of papers refer to specific industrial uses, for example tanning (Ryder, 1978; Smillie, Hunter and Loutit, 1981; Ziglio and Del Corno, 1979), metal-plating (Pfeiffer, Fiszman and Carbonell, 1980), as mordants in textiles (United States Environmental Protection Agency, 1980) and as an anti-corrosion agent in cooling-water systems (Cranston and Murray, 1980; Landy, 1971; Young, Jan and Moore, 1977). The occurrence (Imhoff, Koppe and Dietz, 1980; Jan, Moore and Young, 1977; Schaefer, 1976) and details of the methods and problems of treating waste waters containing chromium have been reported (Ryder, 1978; Wickliff et al., 1982). Chromium in fly ash (Eggett and Thorpe, 1978) and deposited from the air after a major bush fire (Young and Jan, 1977) has also received attention.

The occurrence of chromium, partly at least of natural origin, in fresh water, sediments and organisms has been the subject of many papers, some of which refer to Europe (Ebner and Gams, 1975 and 1975a; Imhoff, Koppe and Dietz, 1980; Rehwoldt, Karimian-Teherani and Altman, 1975; Teherani et al., 1979; Thomas and Neuland, 1979). A number of collections of data are referred to in reviews (NRCC, 1976; US EPA, 1980).

Train (1979) quoted the results of two extensive surveys of United States surface waters in the United States. These indicated that chromium was usually only present at trace concentrations. In a set of 386 samples the mean concentration was less than 0.01 mg/L and in a similar set of 700 samples only 11 contained more than 0.005 mg/L as hexavalent chromium. Similar results were described by Kopp (1969) for 1577 samples.

1.2 Chemistry of Chromium in Fresh Water

Chromium is a metal which occurs in oxidation states from -2 to +6. However, as far as the protection of the environment is concerned, only the two most stable oxidation states chromium (III) and chromium (VI), need be considered (NRCC, 1976; US EPA, 1978).

The trivalent form has only a low solubility in the pH range of natural waters and can react with water to form the hydrated oxides Cr(OH)2+ and Cr(OH)2+. Moreover, as a positively charged ion tends to form stable complexes with negatively charged organic and inorganic compounds, chromium (III) is also easily adsorbed onto many surfaces and particulate matter (NRCC, 1976).

Hexavalent chromium on the other hand is very soluble in natural waters. In aqueous solution the equilibrium among the different species can be described, in a simplified form, according to the following equations:

$$2\text{CrO}_4^{2-} + 2\text{H}^+ \Leftrightarrow 2\text{HCrO}_4^- \Leftrightarrow \text{Cr}_2\text{O}_7^{2-} + \text{H}_2\text{O}$$

The proportion of each species depends on pH and total concentration of chromium (Stumm and Morgan, 1970). Under relatively alkaline conditions the oxoanion chromate (CrO72-) is dominant while under more acid conditions the proportions of hydrochromate (HCrO4-) and dichromate (Cr2O72-) increase (Van der Putte, Brinkhorst and Koeman, 1981).

Thermodynamic data indicate that, in aqueous solution CrO42- and HCrO4- should be the dominant species in well oxygenated water (pE>12; Eh>120 mV) for the range of pH from 5 to 9, while Cr(OH)2+ and Cr(OH)2+ are the species predicted to occur in water lacking oxygen (Cranston and Murray, 1980).

1/ Throughout this report, concentrations of chromium are expressed as mg Cr/l
Smissaert, Van Bruggen and Thiedens (1977) made calculations on the basis of equilibrium constants from Sillen and Martel (1964) and Cotton and Wilkinson (1972) and demonstrated pH dependence, solubility and distribution of chromium (III) ions in water.

In the presence of reducing agents chromium (VI) has a strong tendency to act as an oxidant, especially in acid solutions, and to form chromium (III). Nevertheless, when the concentration of the reducing agent is low or the pH is not low enough, hexavalent chromium is stable and persistent in natural waters.

In well-oxygenated water trivalent chromium should be transformed, according to thermodynamic data, to hexavalent chromium. However, the data on the kinetics of this transformation are not in full agreement. Cranston and Murray (1980) suggested a first-order oxidation rate with a half-life of chromium (III) of one month, but the observations of Schroeder and Lee (1975) and Fukai and Vas (1969) suggest first-order half-lives of 20 and 2 months respectively.

Therefore, due to this process, to its low solubility at naturally occurring pH values and to the strong tendency for it to be adsorbed, most chromium (III) is not available as dissolved species, but occurs in stable forms with particulate matter or in colloidal forms.

On the other hand chromium (VI) remains in solution and is therefore more mobile in aquatic ecosystems. For these reasons, in developing water quality criteria, most attention has been paid to the hexavalent form.

1.3 Analytical Methods

The main analytical techniques used routinely for determining the concentration of chromium in natural and polluted waters are atomic absorption spectroscopy (AAS) using flame excitation (Burrell, 1974; Welz, 1975) preceded if necessary by solvent extraction and concentration (Chau, Sim and Wong, 1968) flameless AAS using a graphite furnace (Martin, Kopp and Ediger, 1975; Welz and Wiedeking, 1973) and colorimetry (APHA, AWA, WPCF, 1980). Detection limits vary according to the different techniques: flame AAS, 0.01 mg Cr/l, colorimetric methods, 0.1 mg/l (but with a 48% standard deviation), as total chromium. Other techniques have been used including neutron activation, X-ray fluorescence, atomic fluorescence and spark source mass spectrometry.

Several analytical methods for the determination of chromium in biological samples are described in the literature (NRCC, 1976; US EPA, 1978) (see also Section 4 on Accumulation in Fish). Among the most utilized are those that carry out acid dissolution of the sample and an appropriate method of digestion in an autoclave in closed vessels, in order to avoid any contamination (Julshamin and Braekkan, 1975).

A method that allows the discrimination of chromium (III) and (VI) has been reported by Cranston and Murray (1980). Another method that could differentiate chromium (III) from chromium (VI) is electron spike resonance (Gutierrez, Sarna and Swartz, 1976). However, the application of these techniques to water pollution research is rare.

Because of the difficulty both in measuring chromium in water and particularly in identifying the forms relevant to toxicity to aquatic organisms, many published data, in particular on chromium (III), have to be regarded with caution and interpreted with care. In many cases the concentrations used in laboratory tests have been well above the level of solubility and often the nominal concentrations used have not been checked by chemical analysis.

Chromium adsorbed onto minerals, clays and other kinds of suspended solids cannot be readily available for toxicological processes and water quality criteria for chromium should therefore be expressed as the total soluble chromium (able to pass through a 0.45 μm pore-size filter). Furthermore, considering that all the oxidation states and forms of chromium could be converted to the more thermodynamically probable form chromium (VI) and assuming additivity of the various fractions, it seems reasonable to adopt standards in terms of hexavalent chromium.
2. LETHAL EFFECTS ON FISH

2.1 Mode of Action

Chromium in the hexavalent form, but not in the trivalent form, has been shown to cross biological membranes easily (Gray and Sterling, 1950) and then to be reduced by various enzymes to the more biologically active trivalent form (Grogan, 1958). Cellular damage could be provoked both by chromium (VI) itself, due to its action as an oxidizing agent, and by chromium (III), which is capable of reacting with various organic molecules (Merts, 1969; Sanderson, 1976) and of inhibiting several microsomal and soluble enzymes (Feldman, 1968).

Most of the results referred to here are more fully reported in Section 3, Sub-Lethal Effects on Fish, below.

It has been demonstrated that chromium can enter the tissues of fish and become accumulated to an asymptotic level that is related to the concentrations in the water (see Section 4 on Accumulation in Fish).

Histopathological damage has been observed by various workers in gill (Strik et al., 1975), stomach (Van der Putte, Brinkhorst and Koeman, 1981), intestine (Fromm and Schiffman, 1958) and kidney (Fromm and Schiffman, 1958; Van der Putte, Brinkhorst and Koeman, 1981) but always as a result of exposure to very high concentrations of chromium. Recently, Van der Putte, Van der Galüen and Strik (1982) demonstrated that there were histological alterations increasing in severity in yearling rainbow trout (Salmo gairdneri) exposed for 12 weeks to concentrations which would cause mortality in this period.

High concentrations of chromium have also been shown to cause increases in haematocrit and haemoglobin (Strik et al., 1975; Srivastava, Agrawal and Chaudhry, 1979). In a long-term test Van der Putte, Van der Galüen and Strik (1982) found the same effects, but only at pH 6.5.

Studies have shown that the concentration of sodium in fish blood may decrease in the short term but conversely may increase in the long term when fish are exposed to chromium. A significant decrease in kidney Na⁺/K⁺ ATPase activity was demonstrated by Kuhnert, Kuhnert and Stokes (1976) in a short-term experiment but, again conversely, more recently Van der Putte, Van der Galüen and Strik (1982) found that the activity of the enzyme increased significantly after long-term exposure. Bühler, Stokes and Caldwell (1977) could not find any difference in the activity of several mitochondrial, microsomal or soluble-fraction enzymes between control fish and those exposed to 2.5 mg/l for 22 days.

High concentrations of chromium impaired respiratory and osmoregulatory function (Van der Putte, Laurier and Van Eijk, 1982) and, in chronic exposure, 0.2 mg/l caused an alteration in gill mucus (Arillo et al., 1982).

As a result of these observations Van der Putte, Laurier and Van Eijk (1982) and Van der Putte and Pärt (1982) indicated that respiratory impairment and osmoregulatory dysfunction were part of the mechanisms of chromium toxicity. However, similar effects appear to be quite non-specific, and could be attributed to a number of metals.

At present the mode of action of chromium on fish cannot be precisely defined, although a number of facts suggest that one specific effect of chromium is on glucose metabolism, in which chromium (III) acts under normal conditions as a biologically essential element in mammals. Experiments have been reported which demonstrate both an impairment of glucose transport in trout intestine (Stokas and Fromm, 1965) and an increase in the levels of glucose in the blood (Strik et al., 1975, Van der Putte, Laurier and Van Eijk, 1982). Moreover recent work (Arillo et al., 1982) has described marked decreases in the glucose content of the liver; statistically different relationships between total glycides and protolytic activities were also found. Such an alteration was attributed to an interference on lysosomal proteases which being metallo-enzymes could be inhibited by high concentrations of metals. This phenomenon has been confirmed by in vitro experiments (Arillo et al., 1982). Thus, chromium probably affects deleteriously the complex biochemical mechanisms connecting proteases and gluconeogenesis. Since the physiological role of chromium is concerned with the control of some aspects of glucidic metabolism through the "glucose tolerance factor" (a molecule containing chromium, as co-factor of insulin), the toxic activity of chromium could therefore be exerted at the same metabolic level.
2.2 Factors Affecting Acutely Lethal Levels

2.2.1 Introduction

Much of the work discussed in the following section is based on studies with species of fish which are not indigenous to Europe, although one, the rainbow trout, is now widespread in the region and is of considerable economic importance. Apart from this salmonid, most of the papers are based on the studies with the North American bluegill sunfish (*Lepomis macrochirus*) and fathead minnow (*Pimephales promelas*).

A number of factors seem to influence the toxicity of chromium to fish. These include temperature, pH, hardness, age and size of fish and acclimation. However, the amount of information available is generally small and conclusions drawn from such sparse data should be regarded as extremely tentative.

From this limited information it appears that salinity and the presence of other poisons do not markedly affect the toxicity of chromium to fish. The influence of (in particular) dissolved oxygen and suspended solids on the toxicity of chromium has not been studied but should receive attention. As well as the need for information on European species, more work is required on the effects of hardness at controlled pH values and on the effects of mixtures of pollutants which include chromium.

2.2.2 Temperature

Among the few data from studies designed to examine the effects of temperature on the acute lethal toxicity of chromium to freshwater fish, the US Environmental Protection Agency (1980) reported that the responses of bluegill exposed to hexavalent chromium in soft or hard water were very little affected by temperature. Values for the 96-h LC50 in soft and hard water at 10°C were 113 and 135 mg/l whereas at 30°C the values were 113 and 130 mg/l, respectively.

Ruesink and Smith (1975) examined the toxicity of chromium (VI) to adult fathead minnow at 15° and 25°C. This species, unlike bluegill (US EPA, 1980)(see above), was slightly affected by temperature so that at 15°C the 2 and 4-d LC50 values were 61 and 52 mg/l while at 25°C the 2 and 4-d values were 58 and 37 mg/l. The results should be treated with some caution because many of the fish contained parasites and all were treated with neomycin sulphate before the study.

Interspecific differences in the effect of temperature on the acute lethal toxicity of chromium have been demonstrated by Smith and Heath (1979). Goldfish (*Carassius auratus*) in particular were more sensitive to acutely lethal concentrations in warmer water. At 5°, 15° and 30°C the 24-h LC50 values were about 309, 220 and 111 mg/l respectively. Golden shiner (*Notemigonus crysoleucus*) were less affected: corresponding values were about 150, 111 and 102 mg/l. For other species there was no clear pattern. Bluegill and channel catfish (*Ictalurus punctatus*) at the same temperatures gave results from 211 to 278 and from 52 to 69 mg/l respectively while for rainbow trout at 5, 12 and 18°C the 24-h LC50 values were 54, 111 and 92 mg/l.

The very few data available generally show either an insignificant effect of temperature or a reduction in toxicity in warmer water.

2.2.3 pH

Trama and Benoit (1960), although they used static tests, realized that the pH of test solutions of potassium chromate and dichromate in a fairly soft water (45 mg/l as CaCO₃) varied with concentration and drifted towards neutrality during a 96-hour test. It can be inferred from their data that with potassium dichromate the pH values after 4 days were around 6.8 at a concentration of 155 mg/l and 6.2 at 490 mg/l. Potassium chromate seemed to cause a drift from an initial pH of 8.5 to 7.5 at 320 mg/l and to 7.8 at 870 mg/l. The drift in pH was attributed to the effect of mucus secreted into the water by the fish (bluegill). These authors were among the earliest to identify the species of chromium in tests with fish, as discussed below.
The principal studies on this aspect of the acute lethal toxicity of chromium to freshwater fish have been carried out in the Netherlands. Hogendoorn-Roozemond et al. (1978) exposed young rainbow trout to solutions of sodium chromate in water of total hardness (1.55 meq/1, taken to be 77.5 mg/1 as CaCO₃) and at 12°C. Two sets of experiments were carried out, at pH 7.8–8.0 and at 6.4–7.2. The 48, 72, 96 and 120-h LC₅₀ values were from 50 to 200 times higher at the higher pH (28 to 58 mg/1 compared with 0.22 to 0.57 at the lower pH values). The authors considered that H₂CrO₄ was the most probable toxic species of hexavalent chromium and suggested that differences reported in earlier papers between the toxicities of chromate and dichromate were principally due to the reduction in pH caused by the addition of dichromate to dilution waters.

The definitive study to date on the effect of pH on mortality of rainbow trout is that of Van der Putte, Brinkhorst and Koeman (1981). They exposed fish of different ages and weight classes to solutions of sodium chromate in tap water in a flow-through test conducted at pH 6.5, 7.0 and 7.8. At pH values of 7.8 and 6.5 the 96-h LC₅₀ values to 0.2 g trout were 12.2 and 3.4 mg/1 respectively. For 25 g fish the corresponding values were 65.5 and 20.2 mg/1. At intermediate sizes and at pH 7.0 the LC₅₀ values were also intermediate. The toxicity was approximately four times greater at pH 6.5 than at pH 7.8. The authors discussed the chemical forms of chromium under the conditions of their study and concluded that both HCrO₄⁻ and Cr₂O₇²⁻ may be responsible for the toxic effects observed. They calculated that HCrO₄⁻ was the more toxic of the two species although the period of exposure appeared to affect the ratio, Cr₂O₇²⁻ becoming less toxic as exposure periods lengthened. It was concluded that toxic concentrations of HCrO₄⁻ and Cr₂O₇²⁻ ions in the external solution ultimately lead to common effects in the blood, although different organs were affected.

Pickering and Henderson (1966) studied the effects of chromium potassium sulphate, potassium dichromate and potassium chromate on a number of warm-water species in two types of water, one soft (20 mg/1 as CaCO₃, total hardness; pH 7.5) the other hard (360 mg/1 as CaCO₃, total hardness; pH 8.2). The tests were static and the salts, except potassium chromate, reduced the pH when the higher concentrations were added, though the subsequent changes of pH were not reported in the paper. With fathead minnow and bluegill sunfish a comparison could be made between the toxicities of chromium potassium sulphate and potassium dichromate. The effect of reducing hardness and thus pH was much more marked with the former salt, 96-h LC₅₀ values being reduced from 67.4 to 5.07 mg/1 for the fathead minnow and from 71.9 to 7.46 mg/1 for the bluegill. With potassium dichromate comparable changes were 27.3 to 17.6 and 133 to 118 mg/1 respectively.

Therefore chromium salts are markedly more toxic under conditions of lower pH and this is partly attributable to the species of chromium present in solution, however from the most accurate experimental data it appears that the acute toxicity does not increase by more than one order of magnitude for a change in pH from 7.8 to 6.5.

2.2.4 Hardness

Very few papers have been published in which the effect of hardness alone on the short-term lethal toxicity of chromium to fish was critically examined. Pickering and Henderson (1966) compared the toxicities of potassium chromium sulphate and potassium chromate in waters of two hardness characteristics (total hardness 20 mg/1 and alkalinity 18 mg/1 as CaCO₃; total hardness 360 mg/1 and alkalinity 300 mg/1, all expressed as CaCO₃). Using fathead minnow and bluegill sunfish considerable increases in toxicity were demonstrated in the softer water, especially with potassium chromate sulphate for which the 96-h LC₅₀ values were 5.1 mg/1 (minnow) and 7.5 mg/1 (bluegill) compared with values in hard water of 67.4 and 71.9 mg/1 respectively. However, these differences cannot be attributed to the effects of hardness alone, if at all.

On the other hand, D. Calamari (personal communication) compared the 48-h LC₅₀ of hexavalent chromium at a single pH (7.4) to rainbow trout (100 mm in length) in hard and soft water (total hardness 320 and 20 mg/1 as CaCO₃, respectively). At 15°C in the hard water the results were 79.6 mg/1 and in the soft water 21.3 mg/1.
Inspection of other data (Adelman, Smith and Siesennop, 1976; Benoit, 1976; Broderius and Smith, 1979; Pickering and Henderson, 1966; Ruesink and Smith, 1975; Trama and Benoit, 1960; Van der Putte, Brinkhorst and Koeman, 1981), although not obtained from tests in which comparisons were made, but only from studies at single values for hardness, confirms that chromium is less toxic in harder water. From the quoted data it appears that decreases of up to one order of magnitude may exist in short-term lethal toxicity when increases in hardness from about 20 to about 320 mg/l as CaCO₃ are considered.

2.2.5 Salinity

In experiments with silver (Coho) salmon (Oncorhynchus kisutch) (Washington, State of, 1960) potassium chromate proved to be more toxic in seawater than in fresh water within the first 4 days of exposure. (In 56.3 mg/l, for example, the percentage mortalities were 0 in fresh water and 80 in sea water by Day 4.) However, after 11 days there was no difference in toxicity, the 11-d LC₅₀ lying between 17.8 and 31.8 mg/l in both types of water. Trivalent chromium (added as chromium sulphate) at a concentration of 50 mg/l killed all the test fish in 71 hours in fresh water but none in sea water. The cause of death may have been the rapid fall in pH; within 4 hours of addition of the chromium salt the pH fell from 7.9 to 5.6 in fresh water. When the concentration added to fresh water was 28.4 mg/l the pH did not fall so low, a precipitate formed (of chromous carbonate) and all the fish lived. Thus trivalent chromium appears to be more toxic in fresh water than in sea water, but this may be largely due to the changes in pH as described above.

Sugatt (1980) showed that exposure to chromium could impair the ability of juvenile coho salmon to adapt to saline conditions. When the fish were exposed in fresh water to 0.23 mg/l for four weeks or to 0.5 mg/l for two weeks their survival was significantly decreased when they were subsequently transferred to waters having salinity values of 20 and 30% respectively. If the fish were only exposed to chromium for one week the inability to adapt to saline conditions was not so marked.

In summary, hexavalent chromium is only a little more toxic in sea water than in fresh water in short periods of exposure. Exposure to chromium can impair the ability of fish to adapt to saline waters.

2.2.6 Age and size of fish

Sauter et al. (1976) compared the concentrations of hexavalent chromium which prevented or reduced hatching and reduced survival for up to 60 days following hatching among seven species. The tests were carried out in soft water (range of mean values for each species 33-38.8 mg/l as CaCO₃). For rainbow trout, hatching was prevented at or above 26.4 mg/l as chromium and reduced at 6.1 mg/l. The fry could not survive for 30 days as well as control fish if exposed to 3.2 mg/l or for 60 days at 0.82 mg/l. Comparable data for lake trout (Salvelinus namaycush) were 50.7 (hatching prevented), 24.4 (hatching reduced), 6.0 (30-d survival) and 6.0 mg/l (60-d survival). Hatching of channel catfish was prevented or reduced by an undetermined concentration in excess of 1.29 mg/l but survival was affected in the first 30 days after hatching by 0.3 mg/l and in the next 30 days by 0.57 mg/l. Values for these four characteristics lay above 1.12 mg/l for bluegill and 1.97 mg/l for white sucker (Catostomus commersoni). The tests with northern pike (Esox lucius) were marred by cannibalism among the fry after Day 20. Survival to this point was affected by 0.96 mg/l but effective concentrations concerning hatching were not identified, except that they were shown to be above 1.97 mg/l. For walleye Stizostedion vitreum, concentrations below 2.17 mg/l did not affect hatching or survival to 30 days but effective concentrations were not established.

The study by Van der Putte, Brinkhorst and Koeman (1981) into the effect of pH can also be used to examine the effect of size of fish on susceptibility to chromium poisoning. At any of the three pH values tested, 6.5, 7.0 and 7.8, the tolerance of hexavalent chromium by rainbow trout increased with increasing weight of fish. Taking the 96-h LC₅₀ values as an example, differences of at least 5-fold were observed over the size range 0.2 to 25 g. At pH 7.8 the 96-h LC₅₀ values were 12.2 to 65.5 mg/l, at pH 7.0 the range was 7.6 to 45 mg/l and at pH 6.5 it was 3.4 to 20.2 mg/l.

No other papers have been found with which this work can be compared.
In summary larger fish of a given species may be more tolerant of chromium than smaller fish.

2.2.7 Acclimation

No specific investigations of acclimation to chromium have been found. However, in one experiment (Adelman et al., 1976) an opportunity for acclimation was given. At the start of one series of tests, instead of the full test concentrations required being attained immediately, an error resulted in only 35% of the chromium being present at first, and only after 3-4 hours were the correct levels achieved. The 11-d LC50 to goldfish was increased by this accident from 11 ± 2 mg/l to 18 ± 2 mg/l.

Thus, limited evidence would suggest that some fish may be able to acclimate to chromium to a certain extent.

2.2.8 Joint effects with non-metals

Bills, Marking and Olson (1977) studied the effect of previous exposure of rainbow trout fry for 30 days to a PCB (Aroclor 1254) on the subsequent response of the fry to trivalent chromium; a control (no PCB), a low-level (0.00001 mg/l Aroclor) and high-level (0.0001 mg/l Aroclor) were used. The 96-h LC50 values derived from these three groups of fish were 11.2, 9.0 and 7.05 mg/l respectively and the difference between the first and last of these figures was statistically significant.

Broderius and Smith (1979) found that mixtures of chromium and cyanide produced responses indicating slightly less-than-additive toxicity to fathead minnow over a 4-day period of exposure. This was not thought to be due to the formation of less toxic complexes. A median kill in 4 days required 1.31 times more of the two constituents of the mixture than predicted from a simple concentration additive model (EIFAC, 1980).

The effects of the sodium salts of the complexants nitrilotriacetic acid (NTA) and ethylenediaminetetraacetic acid (EDTA) on the toxicity of trivalent chromium to carp (Cyprinus carpio) were studied by Muramoto (1981). Under static-test conditions and at 14.5-16.5°C carp were exposed to chromium sulphate or chloride. At 20 mg/l 60% of fish died in the sulphate solution within 48 h compared with 100% mortality in chromium chloride. However, the addition of 317 mg NTA/l or 522 mg EDTA/l prevented any mortality at 20 mg/l.

2.2.9 Effects on disease and parasitism in fish

Draggan (1977) exposed the fungal (Saprolegniales) parasite of carp eggs to hexavalent and trivalent chromium in the range of 0.1 to 30.0 mg/l. He found at low chromium concentrations (<0.3 mg/l for chromium (VI) and <1 mg/l for chromium (III)) that there was increased egg mortality due to increased fungal growth, allowed because the chromium inhibited the other micro-organisms present. At high concentrations of chromium, in excess of 0.3 mg/l for chromium (IV) and 10.0 mg/l for chromium (III), the opposite happened and the fungi were unaffected, allowing development of the carp eggs uninhibited by fungal parasites.

Sugatt (1980) exposed juvenile coho salmon to dichromate in fresh water, finding that it decreased their resistance to Vibrio anguillarum. The fish, exposed to 0.0 and 0.5 mg/l for 14 days (temperature 13 ± 2°C) were injected with the bacteria, and kept for one week in dechlorinated tap water at 15 ± 1°C, 56 mg/l CaCO₃ hardness and a pH of 7.3. The bacterial dose of 3.06 x 10⁶ bacterial/ml caused 90% and 100% mortality to those fish exposed to chromium, but only 50% and 30% in the controls.

2.2.10 Summary of acute toxicity data

In short-term exposure chromium is not very toxic to freshwater fish. Even the salmonid species were able to survive 4 days in concentrations of chromium from 11 to 65 mg/l except in certain circumstances. In soft water and/or at low pH (6.5) 96-h LC50 values as low as 3.4 mg/l were reported for 0.2 g rainbow trout, about 4 times lower than at pH 7.8. Fathead minnow are a little less sensitive and bluegill sunfish much less so, except in very soft water where 96-h LC50 values of 7.5 mg/l have been reported. Among
European species the 7-d LC50 of chromium to perch (Perca fluviatilis) in hard water is about 26 mg/l and to roach (Rutilus rutilus) >80 mg/l (J.F. de L.G. Solbë and V.A. Cooper, personal communication).

From the sparse data on the factors affecting the toxicity of chromium an increase in temperature either has no effect, or reduces toxicity over the range 5° to 30°C by a factor of up to 3 (goldfish) or 1.5 (golden shiner). The pH of the water has a more marked effect, toxicity increasing by a factor of about 3 for rainbow trout as pH falls from 7.8 to 6.5. A decrease in hardness from 320 to 20 mg/l as CaCO3 can reduce the 96-h LC50 by a factor of 4, from 80 to 21 mg/l for rainbow trout. Very limited data suggest that salinity does not affect toxicity but that exposure to chromium reduces the ability of a fish to adapt to saline water. As fish grow they become less sensitive to the presence of chromium, at least as far as rainbow trout is concerned, so that at pH 7.8 the 96-h LC50 of a 0.2 g fish may be 12.2 mg/l and that of a 25 g fish, 65.5 mg/l.

No short-term tests have been reported using mixtures of metals and no general tendency can be seen in the few studies of joint effects with non-metals. The presence of chromium can make fish more prone to disease, either directly or indirectly (by selectively killing the micro-organisms which were protecting fish eggs from fungal infection).

2.3 Long-Term Lethal Toxicity

Very few data are available from studies of the lethal toxicity of chromium of more than one week's duration.

Adelman et al. (1976) established 11-d LC50 values of hexavalent chromium for 11-week old fathead minnow and 5 to 18-month old goldfish to be 18 ± 2 and 33 ± 7 mg/l respectively. The tests were made in a hard water (210 mg/l as CaCO3) at 23.6-26.2°C, pH 7.5-7.9 and 5-7.6 mg/l DO. At 11 days a threshold LC50 had not been achieved.

Benoit (1976) exposed brook trout (Salvelinus fontinalis) and rainbow trout to concentrations of hexavalent chromium in a water of total hardness 45 mg/l as CaCO3. The studies lasted 88 and 32 weeks respectively. Median lethal concentrations were not calculated for those periods but seem to lie between 0.2 and 0.35 mg/l for brook trout and 0.2 and 0.34 mg/l for rainbow trout. Mortalities among control fish (apparently 32.5% for alevin to adult brook trout and 15% for rainbow trout) make more precise interpretation difficult.

As part of a study of mixtures, Broderius and Smith (1979) defined the 10, 20 and 30-day LC50 values for fathead minnow exposed to hexavalent chromium in hard water (alkalinity 235 mg/l, total hardness 220 mg/l, as CaCO3), at 25°C. The LC50 values were: 10-d, 12.4 mg/l; 20-d, 5.99 mg/l; 30-d, 4.36 mg/l. The 95% confidence limits about the 10-d LC50 were 11-14 mg/l.

The 12-week LC50 of hexavalent chromium to rainbow trout in water of total hardness 250 mg/l as CaCO3 and pH 7.5 was approximately 3 mg/l (Water Research Centre, 1976), very much lower than the 48-h LC50 value (70 mg/l) for this water.

For roach the 6-week LC50 (probably the threshold LC50) of hexavalent chromium was 32.5 mg/l in hard water (J.F.de L.G.Solbë and V.A. Cooper, personal communication). For perch the 1-week LC50 (probably also the median lethal threshold concentration) was 26 mg/l. Both tests were carried out under flow-through conditions in water having the following characteristics: total hardness 262 mg/l and bicarbonate alkalinity 230 mg/l (both as CaCO3); temperature 15.9 ± 0.15°C; dissolved oxygen concentration >90% of the air saturation value (ASV). The pH values were affected by the presence of potassium dichromate so that in the highest concentration values were about 7.3 and in the control aquaria 7.6.

Chromium at a concentration of 0.4 mg/l caused more than 30% mortality among adult rainbow trout in 60 days (D. Calamari, personal communication) but 0.2 mg/l did not cause any deaths in 180 days (Calamari, Caggino and Pacchetti, 1982). However in the latter concentration animals showed a significant depletion of liver total glucides and of sialic acid in the gill (Arillo et al., 1982) (see Section 2.1, Mode of Action).
Van der Putte, Van der Galin and Strik (1982) in a 32-week test on alevin to juvenile rainbow trout found neither retarded growth nor mortality at 0.2 mg/l at pH 6.5 and 7.8 but 100% and 56% mortality respectively at 2 mg/l. However, in those concentrations in which mortality occurred it was observed that smaller fish died first (I. Van der Putte, personal communication).

2.4 Long-Term Joint Effects of Chromium with Other Metals

Only mixtures of chromium and nickel have received attention (Water Research Centre, 1977). In a study with rainbow trout in hard water (about 250 mg/l as CaCO₃) the estimated asymptotic LC₅₀ values, based on 10 weeks exposure, for chromium and nickel were 2.9 mg/l and 1.08 mg/l respectively. When half this concentration of nickel was mixed with as little as 0.21 mg/l chromium (giving a sum of fractions of the predicted threshold LC₅₀ values of only 0.57) more than 50% of the fish were killed in 15 weeks.

On the other hand, Calamari, Gaggino and Pacchetti, (1982) exposed rainbow trout to a mixture of 0.01 mg Cd/l, 0.20 mg Cr/l and 1.0 mg Ni/l in a hard water (320 mg/l as CaCO₃) for 6 months and found that there was no mortality. Although the water was a little harder than in the previous study, a crucial difference may lie in the volume of the test aquaria. Calamari, Gaggino and Pacchetti used 70 fish each of 150-200 g in tanks of 4000 l capacity whereas in the British work 10 yearling fish were held in 40 l tanks, a much more crowded situation.

In a recently completed study at the Water Research Centre the toxicity of mixtures of nickel and chromium to roach was examined (J.F. de L.G. Solbø and Miss C.A. Willis, personal communication). Using ratios of the fractions of the median asymptotic lethal concentrations of the metals from 0.5 to 2.0 and exposure periods of up to 13 weeks, the toxicity of the mixture proved to be additive.

Thus more-than-additive effects between nickel and chromium have been reported but these have not been confirmed by more recent data.

2.5 Developmental Stages and Life Cycle Studies

Hatch of rainbow trout was not affected and embryos were normal, as revealed by histological examination, after exposure from the time of fertilization to a concentration of 0.5 mg/l at pH 7.4 (D. Calamari, personal communication).

Hatching was also successful at 2 mg/l at pH 6.5 and 7.8, but alevins died after 32 weeks exposure in both the treatments, mortality being 100% and 68% respectively. At pH 6.5 also, 0.2 mg/l caused a mortality of 60% in alevins (Van der Putte et al., 1982).

Benoit (1976) found that the hatch of brook trout was not affected at 0.35 mg/l, but the same concentration caused 22% less survival than the control. A similar concentration (0.34 mg/l) completely killed alevins of rainbow trout in about 12 weeks whereas 0.20 mg/l killed only 20%.

Olson and Foster (1956) reported that in long-term tests on chinook salmon (Oncorhynchus tshawytscha) and on rainbow trout, exposure to 0.08 mg/l increased mortality and a concentration as low as 0.02 mg/l affected growth.

Birge et al. (1978) reported that the 28-d LC₅₀ for rainbow trout embryos was 0.180 mg/l.

Benoit (1976) demonstrated that growth of rainbow and brook trout was retarded at all test concentrations during tests lasting 8 months. However in a life-cycle test, over 22 months, exposure of brook trout to 0.35 mg/l produced growth comparable with that of the control fish even if in the presence of a high level of mortality. He concluded that retardation of growth was only a transient effect. In all these tests 0.2 mg/l was the non-effect concentration observed.
Van der Putte, Van der Galiën and Strik (1982) substantially confirmed these data, not finding any effect on length and weight after 32 weeks of exposure even in the presence of lethal concentrations (but see Section 2.3), the observed no effect concentrations being 0.2 mg/1 at pH 7.8 and 0.02 mg/1 at pH 6.5.

Pickering (1980) in a life-cycle test observed retarded growth for a limited period of 9 weeks in fathead minnow exposed to 0.018 mg/1, but by the end of the experiments the final weight of the fish was exactly the same as that of the controls. The highest concentration which caused no effect was 1.0 mg/1 at pH 7.5-8.2 in hard water.

Sauter et al. (1976), working on seven species of fish, reported that survival and growth of alevins or fry were influenced at lower concentrations than those impairing hatchability, the maximum acceptable concentration being in the range 0.05 to 0.10 mg/1 for the most sensitive species and about 2 mg/1 for less sensitive fish.

3. SUB-LETHAL EFFECTS ON FISH

Knoll and Fromm (1960) demonstrated that chromium could enter fish by passive diffusion across the gill membranes and could accumulate in gill, kidney, liver, spleen and intestinal tract. However, after exposure, release was quite slow from certain organs such as kidney and liver (see Section 4, Accumulation in Fish).

Histopathological damage has been found, in general after exposure to high concentrations of chromium (VI), but the results are somewhat conflicting. For example Strik et al. (1975) found that rainbow trout exposed to 10 mg/1 developed hypertrophy and hyperplasia in gill lamellae, whereas Fromm and Schiffman (1958) and Hughes, Perry and Brown (1979) used the same species of fish but exposed them for shorter periods and to higher concentrations.

Van der Putte, Brinkhorst and Koeman (1981) clarified the question, showing that in rainbow trout slight gill damage can be caused at pH 7.8 only by treating the fish at very high concentrations of chromium. At lower concentrations at pH 6.5 severe damage has been described by the same authors and shown also using the scanning electron microscope (Van der Putte and Prânt, 1982), demonstrating the strong influence of pH on the intoxication processes.

Fromm and Schiffman (1958) described severe pathological changes in the intestine immediately behind the pyloric caeca, dilation of the kidney tubules and increase in the nucleus: cytoplasm ratio in the tubular epithelium. Strik et al. (1975) also found necrosis of the kidney tubules. Van der Putte, Brinkhorst and Koeman (1981) too, exposing trout to very high concentration of the metal, showed kidney damage similar to that described above, as well as extensive hyperaemia and necrotic changes in the gastric mucosa at pH 7.8 but not at pH 6.5.

However Van der Putte, Van der Galiën and Strik (1982) in long-term exposure to 2 mg/1 at pH 7.8 found no histological alterations in yearling trout while fish exposed to the same concentration but at pH 6.5, suffered damage to the gill epithelium.

Schiffman and Fromm (1959) showed a significant increase in the hematocrit of trout after exposure to 2-4 mg/1. Strik et al. (1975) found that haematocrit, haemoglobin, red and white blood cells did not differ in roach exposed for 32 days to concentrations up to 10 mg/1, although the same level provoked significant increases in haematocrit and haemoglobin content and a decrease of concentration of Na⁺ in blood of rainbow trout.

Srivastava, Agrawal and Chaudhry (1979) found increased erythrocytes and haematocrit in Colisa fasciatus, a freshwater teleost, after exposure to 35 mg/1.

Van der Putte, Laurier and Van Eijk (1982) confirmed these effects in short-term experiments. In the already quoted long-term tests (Van der Putte, Van der Galiën and Strik, 1982) of 12 weeks duration, similar results were obtained, except for a decrease in Na⁺, which occurred only for the treatment with 2 mg/1 at pH 6.5 and not for pH 7.8. (However a transient increase in haematocrit value was observed at pH 7.8.)
Buhler, Stokes and Caldwell (1977) after exposing fish for 22 days to 2.5 mg/l were not able to find difference among treated and control fish when examining the activities of representative mitochondrial, microsomal, and soluble enzymes. They also observed that chromium (VI) was not particularly active in \textit{in vitro} tests.

Kuhnert, Kuhnert and Stokes (1976) have shown a significant decrease in Na\(^+\)/K\(^+\) ATPase activity of rainbow trout kidneys and intestine, but not in gill and liver, after 2 days exposure to 2.5 mg/l. Van der Putte, Van der Galiën and Strik (1982), in contrast to these results, observed a significant increase of Na\(^+\)/K\(^+\) ATPase activity after 12 weeks treatment at pH 6.5 and 7.8 with 0.2 and 2 mg/l (lower pH) and 2 mg/l (higher pH) respectively.

Ellgaard, Tusa and Maliziz (1978) observed that locomotory activity of the bluegill was comparable with that of the control at 0.5 mg/l but was increased 3.6-fold at 2.4 mg/l.

Impairment of respiratory function has also been reported (Van der Putte, Laurier and Van Eijk, 1982). In the presence of more than 10 mg/l at pH 7.8 and more than 2 mg/l at pH 6.5 ventilation frequency and coughing rate increased in proportion to increase of metal concentrations.

Van der Putte and Prt (1982) using \(^{51}\)chromium examined the transfer of oxygen and chromium in perfused gills at a concentration of 10 mg/l and at pH 6.5 and 8.1. Transfer of chromium was directly coupled with that of oxygen but transfer of the metal was significantly more effective at pH 6.5 than at pH 8.1 and, as previously reported (Van der Putte, Lubbers and Kolar, 1981) gill tissue accumulated more chromium at the lower pH. Oxygen transfer at the gill was impaired after fish had been exposed for 4 days to 10 mg/l and there seemed good reason for this impairment, to judge from histological examination of the perfused gills.

A component of gill mucus, the N-acetyleneuraminic acid (a sialic acid) was found to decrease significantly in rainbow trout after six months treatment with 0.2 mg/l. This can be considered as an expression of quantitative alteration of the gill mucus and impairment of osmoregulation and respiration processes (Arillo \textit{et al.}, 1982).

Sugatt (1980) found that survival was significantly decreased in coho salmon exposed in fresh water to 0.23 mg/l for four weeks and then transferred to 20\% saline water. He attributed death to the impairment in osmoregulation causing excessive dehydration when the fish were placed in sea water.

Stokes and Fromm (1965) reported that glucose transport by caecal and midgut sections from trout exposed to 2.5 mg/l for one week was about 40\% that of the control. They suggested that the decrease was primarily the result of inhibition of the entry of glucose into epithelial cells caused by the presence of chromium in the tissue.

Strik \textit{et al.} (1975) explained the high level of glucose found in the blood of roach as an effect of the impaired uptake of glucose by cells (anti-insulin action) or increased metabolism.

Van der Putte, Van der Galiën and Strik (1982) also found an increase of glucose and lactate in the blood plasma of trout and suggested that this phenomenon could be attributed to the blockage of oxygen uptake in gills and a shifting towards anaerobic metabolism, as a general response to stress. Trout exposed to chromium (0.2 mg/l for 6 months) had a liver glucidic content significantly lower than the control (Arillo \textit{et al.}, 1982). These findings are coherent with decrease of intestinal glucose uptake and blood hyperglycemia already referred to, and could demonstrate an effect of chromium on glucose metabolism, as already discussed in Section 2.1, Mode of Action.
4. ACCUMULATION IN FISH

4.1 Laboratory Data

All the experiments quoted below have been performed exclusively on rainbow trout. Fromm and Stokes (1962) showed that chromium uptake was a passive process. The amount of chromium accumulated was proportional to the concentrations in the water and the asymptotic level was attained after 10 days of exposure.

Van der Putte and Pârt (1982), working on isolated gills, showed that transfer of chromium and oxygen were positively correlated and therefore it could be inferred that both could cross the epithelium of the secondary lamellae by passive diffusion with the same mechanism, in which case the transfer of chromium would be 1.6 times more efficient at pH 6.5 than at 8.1.

Van der Putte, Lubbers and Kolar (1981) found that gill, liver, kidney and digestive tract accumulated most of the chromium and that when fish were exposed to high concentrations considerably more was accumulated at pH 6.5 (where no equilibrium was reached) than at pH 7.8. However at lower concentrations of chromium (2 and 5 mg/l) the differences in accumulation between the two pH values were small; equilibrium was reached in about four days and the release of chromium was quite slow from a number of organs.

This rapid uptake and slow release have been found also by Ten Holder et al. (1977) who found that after stopping exposure to the metal 34% of the total chromium was retained for a period of 1 day while the remainder had a half-life in the fish of 26 days.

Buhler, Stokes and Caldwell (1977), analyzing several organs and tissues, found the highest chromium levels in opercular bone, gall bladder, kidney and spleen. Equilibrium occurred rapidly and release was rapid from most of the organs and tissues except from gill, liver, gall bladder, bile and kidney, for which more than 17 days were necessary for the elimination of the metal. The bioconcentration factors (BCF) in all these tests were quite low, generally less than 10.

Fromm and Stokes (1962) working at low concentration (0.05 mg/l) did not establish an asymptotic level of uptake in a 28-day test.

This observation is comparable with the results obtained by Calamari, Gaggino and Pacchetti (1982), who found, in long-term exposure to 0.2 mg/l, a continuous uptake for 180 days. They also found, by means of a toxico-kinetic model, that the theoretical asymptotic values for liver, kidney and muscle should be reached in about 300 days. However the BCFs defined in this last paper were comparable with those previously reported being 20, 10 and 3 for kidney, liver and muscle respectively. Release was rather slow, the residual chromium after 90 days was 35, 60 and 60% in kidney, liver and muscle respectively. The $K_2$ (constant of release) established during, and used in, the uptake phase was adequate to describe the pattern of release. This fact could confirm the two-phase excretion-process equation proposed by Ten Holder et al. (1977) and the hypothesis advanced by Buhler, Stokes and Caldwell (1977) of rapid and slow turnover pools. The first would be constituted by the hexavalent form and the second by the trivalent form of the metal.

Both the contents of chromium in the control fish and the levels obtained as asymptotic values in the most recent papers are comparable and approximately in the same range. For example (in terms of weight of chromium per net weight of tissue) Buhler, Stokes and Caldwell (1977) found, as asymptotic levels, 6.4 and 9.4 mg/kg for anterior and posterior kidney, Van der Putte, Lubbers and Kolar (1981), 5-6 mg/kg and Calamari, Gaggino and Pacchetti (1982), 3.5 mg/kg. For the liver the concentrations were in the range of 0.1 to 1.7 mg/kg and for kidneys 0.04 to 0.4 mg/kg. Muscle was found to have little capacity for accumulating chromium (Calamari, Gaggino and Pacchetti, 1982) the BCFs being around 3 with a background content around 0.3 mg/kg. The lowest value was found by Buhler, Stokes and Caldwell (1977), with 0.01 mg/kg in white muscle.

Buhler, Stokes and Caldwell (1977) also examined the subcellular distribution of chromium in brain, gill, posterior kidney and liver. Most of the metal was found in the soluble and microsomal fraction from gill and liver and in the soluble fraction of kidney. Accumulation did not occur in the nuclear fraction and the lack of differences in brain subcellular fractions did not lead to any effect: the authors found no significant changes in the activities of a number of mitochondrial, microsomal and soluble enzymes tested.
Van der Putte, Lubbers and Kolar (1981) exposed rainbow trout to very high concentrations of chromium and found that most of the metal occurred in the nuclear fraction of gill, and soluble fractions of the liver and kidney.

Singh and Ferns (1978) observed a significant, but not very great, increase in the chromium content of the whole body of rainbow trout fed for 10 weeks with a diet contaminated by metals containing activated sludge.

Muramoto (1981) exposed carp to trivalent chromium (chromium chloride and sulphate), apparently under static-test conditions. Concentrations of chromium in the visceras, gills and remaining tissues of fish which survived exposure to ambient concentrations of 5, 10 and 20 mg/l were found to increase with ambient concentration. Furthermore, the gills of fish which survived 48 h exposure to 20 mg/l contained markedly lower concentrations of chromium than the gills of fish which died after such an exposure. The concentrations of chromium in the gills, expressed as mg Cr/kg ashed tissue, were about 5000 in survivors and just under 10000 (100000 in paper taken to be an error) in cadavers. Concentrations in the visceras did not show this phenomenon.

4.2 Field Data

Despite the number of surveys on metal-content in fish the number of papers analysing chromium is quite limited for Europe. Field data reported by North American authors are scarce and somewhat contradictory. For example Tong et al. (1974) found that the concentrations of chromium in lake trout from which the head, digestive tract and fins had been removed, increased with age. In the first four years, concentrations of 0.002-0.005 mg/kg wet weight might be expected, from 5 to 10 years concentrations of 0.005-0.013 mg/kg and in 11 and 12 year-old fish 0.032 and 0.090 mg/kg respectively.

On the other hand Giesy and Wiener (1977) did not find a significant relationship between the whole-body chromium concentrations of chain pickerel (Esox niger) and body length. They studied the frequency distribution of chromium in whole-body concentrations from five species of fish in an uncontaminated pond (concentrations in water 0.00035 mg/l) and found that the concentrations were often lognormally distributed and ranged from 0.01 to 2.02 mg/kg dry weight. The species were generally not significantly different in their chromium contents. Lucus, Edington and Colby (1970) found values for three species from 0.9 to 1.6 mg/kg (whole fish, fresh weight), while Uthe and Bligh (1971) reported data from 0.017 to 0.065 mg/kg for seven species.

Elwood, Beauchamp and Allen (1980) examined fish from a lake contaminated by cooling-water discharges and which contained about 0.1 mg/l. (The bottom sediments in the lake contained about 15 times higher concentrations of chromium than a nearby uncontaminated lake in which concentrations of dissolved chromium were about 0.003 mg/l.) However, there was no difference in the chromium concentrations in bluegill or largemouth bass (Micropterus salmoides), both of which feed on insects and small fish, between contaminated and uncontaminated waters, although the concentrations of chromium differed by almost two orders of magnitude. Furthermore, goldfish feeding on the contaminated sediments and benthic organisms, could not be distinguished in their chromium contents from the other two species. The authors concluded that chromium was relatively labile in fish tissue so that short-term changes in ambient concentration would be rapidly mirrored in fish tissues. In some species of fish there was a decrease in chromium concentration of the tissues with an increase in body weight.

In the Illinois River (Mathis and Cummings, 1973), the muscle of carnivorous fish (mean 0.12 mg/kg wet weight) contained significantly less chromium than muscle from omnivorous (0.22 mg/kg). Samples of the river water in this case contained 5 to 38 mg/kg and the sediments 17 mg/kg, compared with sediments in clean tributary streams (6 mg/kg).

European data are also scarce. Filipovic, Vukovic and Knezvevic (1980) found in the muscle of four cyprinid fish species from a Yugoslavian lake a chromium content from 0.1 to 0.5 mg/kg (fresh weight).

Gaggino (1982) analysing the muscle of bleak (Alburnus alburnus) and chub (Leuciscus cephalus) from the River Po found mean values from 0.17 to 0.36 and from 0.117 to 0.79 mg/kg respectively with peak concentrations in the most polluted areas.
Carp in the Danube River and Canal contained lowest concentrations of chromium in bone (0.032 mg/kg dry weight) and highest in the gills (53 mg/kg), which, it was suggested, were contaminated with solids (sediments were found to contain 67 mg/kg) (Rehwoldt, Karimian-Teherani and Altman, 1976). The liver contained almost half the body burden of chromium and muscle about 25%.

Fish in rivers from Upper Austria contained concentrations of chromium in the range of 0.02-0.21 mg/kg dry weight (Teherani et al., 1979).

Chromium concentrations in the overlying water may commonly be reflected in the degree of increase of concentrations in the sediments, but only to a limited extent in fish muscle. There is no suggestion of biomagnification from prey to carnivorous fish. Fish may accumulate chromium in various organs but BCF values are relatively low and regulatory mechanisms are also apparent.

5. EFFECTS ON FRESHWATER INVERTEBRATES

The effects of chromium on invertebrates are discussed in this section, under the same series of headings as used for freshwater fish.

5.1 Introduction

Very few species of invertebrates, from only a few Phyla, have been used in laboratory studies on the effects of chromium. Chief among these have been the crustacea, particularly Daphnia (presumably because of its role in international standard testing procedures), but rarely or never, unfortunately, the slightly larger invertebrates Heelius and Gammarus, featuring in many biotic index systems and in the diets of many fish. A few studies with rotifers and tubificid worms have been reported. The effects on toxicity of temperature, pH hardness, salinity, age and the presence of complexants have received limited attention, but Daphnia has featured in almost all these areas of study.

Limited work on sub-lethal toxicity has been described, covering aspects of growth, reproduction, metabolism and behaviour.

Field observations have been concerned, particularly, with accumulation of chromium in tissues and with benthic community structure.

5.2 Temperature

When Daphnia pulex were exposed to potassium chromate in water of total hardness 171 mg/l, alkalinity 117 mg/l (both as CaCO₃), dissolved oxygen 10 mg/l and at 21°C the 24 and 48-h LC50 values were about 0.69 and 0.26 mg/l (Batac-Catalan and Cairns, 1977). By exposing Daphnia for one day to 0.52 mg/l 'chromate' at 21°C and then transferring the animals to water held at 31°C the average of a series of determinations of mean survival time (2.9 h) was found to be almost double that of Daphnia exposed to the elevated temperature without prior exposure to chromium. A number of areas of confusion make this paper difficult to interpret usefully.

On the other hand the work of Schaefer and Pipes (1973) clearly showed that at higher temperatures the toxicity of hexavalent chromium (as sodium chromate) to the relatively tolerant rotifer Philodina roseola increased. For example the 24-h LC50 at 30°C was 65 mg/l and at 35°C it was 18 mg/l. The 96-h LC50 was 12 mg/l at 5°C and 4.4 mg/l at 30°C. As far as the concentration permitting completion of the normal life-span was concerned however (28 days at 15°C and 3.7 days at 30°C for example) there was little difference with temperature. In the range 15 to 35°C the median threshold concentrations for an effect on life span were from 3.5 to 4.6 mg/l. The hardness of the dilution water was not given but was likely to be fairly high, in view of the method for preparing the infusion medium from distilled water, lettuce and calcium carbonate.

The sparse data suggest that as temperature increases so does the toxicity of chromium.

5.3 pH

Müller (1980) examined the effect of water quality on the 24-h LC50 of potassium dichromate to Daphnia magna. Test waters were prepared by adding salts to deionized water...
to give a range of alkalinities from 0 to nearly 3 mmol/1 (taken to be 0 to 300 mg/1 as CaCO₃). As alkalinity increased from 0 (deionized water) to about 0.7 mmol/1 the pH rapidly increased from about 6.1 to 7.9 and the 24-h LC50 from about 0.01 to 0.7 mg/1. With an increase in alkalinity from 0.7 to 1.9 mmol/1 pH only rose from 7.9 to 8.2 but the 24-h LC50 increased from 0.7 to 2.2 mg/1. Further increases in alkalinity did not give rise to much higher values of pH or 24-h LC50.

Chromium is more toxic at low pH values and alkalinities than in more alkaline waters at least to Daphnia. Trivalent chromium is not necessarily non-toxic when in the form of a precipitate.

5.4 Hardness

Buikema, Cairns and Sullivan (1974) found that for potassium dichromate in soft water (total hardness 25 mg/1, alkalinity 24 mg/1, both as CaCO₃, pH 7.4-7.9) the 48- and 96-h LC50 values to a rotifer, Philodinia acuticornis, were 31.2 and 3.1 mg/1 respectively. In hard water (total hardness 81 mg/1, alkalinity 54-67 mg/1, both as CaCO₃, pH 7.4-7.8) the corresponding values were 21 and 15 mg/1. The Ca:Mg ratio in the dilution waters (which were prepared artificially) was about 2:1.

For Daphnia magna, Muller (1980) found that, provided the Ca:Mg ratio was 4:1, an increase in total hardness from about 0.8 mmol/1 (80 mg/1 as CaCO₃) to about 2.4 mmol/1 (240 mg/1 as CaCO₃) caused an increase in 24-h LC50 from around 0.45 to 1.0 mg/1. Daphnia would not survive if calcium was absent from the dilution water, and sensitivity to chromium was increased if only calcium was present.

Brković-Popović and Popović (1977) prepared four different dilution waters for tests using Tubifex tubifex. The ranges of pH, alkalinity and hardness (both the latter as mg/1 CaCO₃) were 6.3-7.3, 0.1-234 and 0.1-261 respectively. Chromium proved very toxic in the softest (deionized water) but in more relevant soft waters the data indicated that hardness had a greater effect on toxicity than either pH or alkalinity. For example at a hardness of 34 mg/1 but pH values of 6.85 and 7.2, associated with alkalinity values of 7.5 and 22.5 mg/1 respectively the 95% confidence limits of the 48-h LC50 overlapped, being 1.23-1.62 mg/1 in the lower pH/alkalinity and 1.24-1.87 mg/1 in the more alkaline water. In contrast, at pH 7.3, alkalinity 234 mg/1 and hardness 261 mg/1, the 48-h LC50 was 3.66-5.71 mg/1.

From these few examples it seems that in soft water chromium is more toxic than in hard water.

5.5 Salinity

Two studies using brackishwater species are of interest here. They both showed that chromium was more toxic in fresh water than in saline water.

Frank and Robertson (1979) found that, after careful acclimation to the relevant salinity, the blue crab Callinectes sapidus responded to potassium dichromate as follows. The 96-h LC50 values at 20-22°C and salinities of 1, 15 and 35% were, respectively 34.2, 89 and 98 mg/1. (Data were also presented for the 24, 48 and 72-h LC50 values.) As can be seen for the 96-h data the results at salinities of 15 and 35% were not very different.

The same kind of data are presented by Olson and Harrel (1973) for the clam Rangia cuneata. At 24°C and at salinities of <1, 5.5 and 22%, the 96-h LC50 data for potassium dichromate were 0.21, 14 and 35 mg/1.

With increasing salinity, the toxicity of chromium to a crab and a clam decreased, especially with increases from fresh water to half-strength sea water.

5.6 Age of Organism

Trabalka and Gehrs (1977) examined the differences in sensitivity and fecundity between juvenile Daphnia magna (0-24 h from release) and adults ( >7 d from release). The 96-h LC50 at 21°C and pH 8-8.5 was 0.05 mg/1 for both age groups but their ability to produce young differed. If a juvenile was exposed to chromium from the first day of its
free-living existence, it could not produce young at concentrations of chromium in excess of 0.01 mg/l. An adult however, first exposed to chromium on or after the 7th day could produce young, although at greatly reduced rates, in the presence of 0.05 and 0.1 mg/l.

Invertebrates which moult may be particularly sensitive to chromium in the period immediately following a moult. *Daphnia* produce fewer young if first exposed to chromium soon after release from the brood pouch rather than later in life.

5.7 Effects of Complexans

Ryder (1978), in a detailed study of the treatment and conditions for discharge of a tannery waste water, showed that the addition of 1 mg EDTA/1 slightly reduced the toxicity of the waste, which had been diluted to contain 0.04, 0.2 (0.02 in text), 0.5 and 1 mg Cr/1. At 1 mg Cr/1 no adult *Daphnia* survived and no young were produced in the absence of EDTA whereas 5% survived in the presence of EDTA and some reproduction occurred within the exposure period (28 young produced in three weeks compared with 108 young in the controls). Using the same concentrations of chromium, but added as chromium potassium sulphate dodecahydrate to an artificial dilution water rather than achieving the concentrations by diluting the tannery effluent, the effect of adding EDTA was less marked.

Thus, the only study found showed that EDTA was capable of reducing the toxicity of chromium to *Daphnia*.

5.8 Sub-Lethal Responses of Invertebrates

Biesinger and Christensen (1972) examined the effects of chromium on the survival, growth, reproduction and metabolism of *Daphnia magna*. Metabolism was assessed using glutamic oxalacetic transaminase (GOT) activity and by determining the protein content of the *Daphnia*. In Lake Superior water (hardness: 45.3; alkalinity: 42.3 mg/l as CaCO₃; pH 7.4; calcium and magnesium concentrations 13.7 and 3.1 mg/l respectively), the 3-week LC₅₀ was 2 mg/l. Reproduction could be reduced to 50 and 84% of control levels by 0.6 and 0.33 mg/l. After 3 weeks, animals exposed to 0.62 mg/l were 11% lighter than control animals and had lost a little protein and GOT activity.

The work of Trabalka and Gehrs (1977) on reproduction of *Daphnia magna* has already been discussed above.

Concentrations much lower than those proving directly lethal have been shown to reduce growth, inhibit reproduction and reduce the storage of food.

5.9 Other Data

Pardue and Wood (1980) determined the 96-h LC₅₀ values of chromium to three species of Bryozoa - *Lophopodena canteri, Pectinatella magnifica* and *Plumatena emerginata* - in hard water (190-220 mg/l as CaCO₃). The values found were 1.56, 1.44 and 0.65 mg/l respectively.

Warnick and Bell (1969) found that trivalent chromium had a relatively low toxicity to the stonefly *Aoroneuria tygorias* (50% survived for a week in 32 mg/l) and to the caddis fly *Hydropsyche bettini* (96-h LC₅₀: 64 mg/l) but was rather more toxic to the mayfly *Ephemera simulans* (96-h LC₅₀: 2 mg/l). The tests were carried out at 18.5°C in a fairly soft water (total hardness 42-50 mg/l, pH 6.0-6.8). These results may be compared with those of Rehwoldt et al. (1973), who studied the toxicity of trivalent chromium to species typical of one reach of the Hudson River. In water of hardness 50 mg/l as CaCO₃, temperature 17°C, pH 7.6 and dissolved oxygen 6.2 mg/l, they established 96-h LC₅₀ values to a species of worm (*Nais*), 9.3 mg/l; *Gammarus*, 3.2 mg/l; an unidentified caddis, 50 mg/l; a damselfly, 43.1 mg/l; a species of *Chironomus*, 11 mg/l; and the adults and eggs of the snail *Amnicola sp.* (For adult snails the 96-h LC₅₀ was 8.4 mg/l and for eggs, 12.4 mg/l.) The concentrations of trivalent chromium in the river were at least 16 800 times lower than these toxic levels.

Jousny, Vasseur and Perard (1982), in a study of algae and *Daphnia*, established a 24-h LC₅₀ of 0.435 mg/l for the crustacean, but found that mortality among the *Daphnia* was reduced in the presence of the alga *Chlorella vulgaris*, whether or not the algae had been previously contaminated with hexavalent chromium.
5.10 Accumulation in Invertebrates

5.10.1 Laboratory data

Although a number of studies of metal content in the field have been completed (see 5.10.2 below) little work on the accumulation of chromium by freshwater invertebrates under laboratory conditions has been reported. However, Patrick and Loutit (1978), as part of a study of the pathways of metals into a tropical fish (*Hyphessobrycon serrae*), fed juvenile tubificid worms on bacteria grown with or without six heavy metals, each at a concentration of 1 mg metal/litre of medium. Tubificids naturally seem to contain high concentrations of heavy metals but those fed the contaminated diet showed increases in chromium content to 32 mg/kg from 15 mg/kg dry weight. The authors do not mention whether the worms were starved before the analysis for chromium. Although this would have influenced the result as far as the worms were concerned (as Hall and Merlini (1979b) showed), the fish obviously consumed whole worms with their gut contents present.

5.10.2 Field data

Cowgill (1973) catalogued the presence and concentrations of fifty-four elements in water-lilies (*Nymphaea odorata*), aphids (*Rhopalosiphum nymphaeae*) feeding on the water-lily leaves, the uncontaminated waters containing these organisms and soils, rocks and sediments connected with the waters. Two water-bodies, a pond and a lake, were studied in detail. Both contained concentrations of chromium in the outlet water of just below 0.02 mg/l whereas the water-lilies contained 0.5-0.76 mg/kg dry weight and the aphids 0.88 mg/kg.

Cowgill (1976) also looked at the chemical composition of *Daphnia magna* and *D. pullex* as well as their algal food and their environment. Concentrations of chromium were, on average, $2.2 \times 10^{-5}$ mg/l in the water, 1.3 mg/kg dry weight in *D. pullex*, 0.6 mg/kg in *D. magna*, 1.4 mg/kg in *Euglena gracilis* and 1.2 mg/kg in mixed cultures of algae. In both papers there is clear evidence of bioaccumulation from very low ambient levels.

A stream polluted with waste water from a tannery was studied by Duval et al. (1980). In uncontaminated reaches the concentration of chromium in the sediment was 13 mg/kg dry weight but in the polluted zone concentrations rose to over 20 000 mg/kg, and other metals were present at elevated concentrations. The number of taxa was reduced from 34 to 12 over comparable zones, and the calculated diversity of the benthic community was much reduced in the polluted reaches. No data were given on concentrations of chromium in the water and it is difficult to assign a cause to the observed changes. Levels of dissolved oxygen in the polluted zones were very low in the summer and could have accounted for all the gross effects. Nevertheless, that the fauna were subject to the presence of chromium in an available state may be concluded from the heavy metal content of some of the organisms. For example the soft tissue of a mollusc, *Helisoma*, contained 440 mg/kg dry weight and another snail, *Physa*, contained 140 mg/kg.

The galvanizing of metals was considered to be the sole polluting factor in a study of a stream in the Pre-Alps by Ramusino, Pacchetti and Lucchese (1981). Of six species of Ephemeroptera, four disappeared downstream of the input of wastes from this industry, where the annual mean concentration of dissolved chromium was about 0.15 mg/l and concentrations in 5% of samples exceeded 0.36 mg/l. Two species of the genus Bactis were the most resistant but even they were severely reduced in number in the polluted zone. The water was very soft (total hardness 11-12 mg/l; alkalinity 9 mg/l, both as CaCO₃) with a pH value of 7.1, temperature at the time of sampling around 9°C and dissolved oxygen 11 mg/l.

The gastropods *Lymnaea* and *Viviparus*, collected from sites in Italy where the profundal water contained 0-0.004 mg/l, the sediments 6.4 (river) and 13.6-29.8 (lakes) mg/kg dry weight and the interstitial water 0-0.004 mg/l, did not accumulate chromium whereas oligochaetes did (Hall and Merlini, 1979 and 1979a). Oligochaetes of a number of species contained, on average, 3.7 mg/kg dry weight in the river and 9 mg/kg in the lake, perhaps reflecting the difference in concentrations in the sediments. On the other hand in a third site, the Lanca, where sediments contained 19.6 mg/kg, the oligochaetes contained
only 0.4 mg/kg. However, concentrations of chromium in the profundal water and pore water were 0.001 and 0.014 mg/l, a little lower than in the river and lake samples.

Hall and Merlini (1979b) were aware of the possibility that the oligochaetes were carrying chromium-laden sediment in their guts, which could have biased the results of the above survey (Hall and Merlini, 1979 and 1979a). They therefore carried out a number of procedures to define the influence of the gut content on the apparent whole-body concentrations. By starving worms for two days the concentrations of chromium were reduced from 5.36 to 0.48 mg/kg dry weight.

Tubificids in the Illinois River (Mathis and Cummings, 1973) contained the same order of concentrations of chromium as those in the Italian study. Unstarved worms were found to contain 4 to 21 (average 10) mg/kg in an area where concentrations of chromium in the water ranged from 0.005 to 0.038 mg/l and in the sediments from 2 to 87 (mean 17) mg/kg dry weight. Three species of clam from the same area contained average concentrations of 4.4 to 7.7 mg/kg wet weight (range 0.6-11.6 mg/kg). (Chromium concentrations in sediment from uncontaminated tributaries ranged from 3 to 7 mg/kg.)

Some species of tubificid worms appear to be insensitive to the toxic effects of chromium, and dipteran larvae also can be very tolerant. Surber (1959) found one species of midge (Cricotopus bicinctus) to occur in rivers carrying electroplating wastes, even though concentrations of chromium, copper and cyanide were sometimes as high as 25, 2.2 and 3.2 mg/l respectively. The wastes were treated with other sewage but when concentrated plating solutions were discharged in batches to the sewer the treatment work was unable to cope. Of 486 samples taken from the river over a 20-day period, 43 contained 0.5 to 3 mg/l hexavalent chromium. At another time, parts of the river contained 20 mg/l for four hours. Details were given of the decrease in concentration of chromium in the water as it passed downstream. Eighteen to twenty days after this latter incident, large numbers of larvae, pupae and adults of Cricotopus bicinctus were found in the river. It seems reasonable to suppose that they had been there during the pollution incident, although details of the life-cycle at relevant temperatures were not given. Surber (1959) did however list the other species present, chief of which was the tubificid Limnodrilus. In another river in Michigan Cricotopus was present, with other species including larvae of the caddis flies Hydropsyche and Cheumatopsyche, and the blackfly Simulium. Here the plating wastes contained chromium (0-4.7 mg/l), copper, zinc and cyanide but in the river chromium only occurred at 0-0.01 mg/l.

The work of Rodgers et al. (1977) confirms the general impression that concentrations of chromium in organisms from uncontaminated and polluted waters are intermediate between concentrations in the water and those in the sediment. They examined the invasion of the Asiatic clam Corbicula fluminea into the New River, Virginia and the influence of a coal-fired generating plant on the survival of the species through a harsh winter and on the uptake of heavy metals (see above).

In summary, few laboratory studies of accumulation of chromium in invertebrates have been made but data are available from the field. Some of these are from baseline studies of uncontaminated sites, others are from polluted waters. Although there are considerable variations between species, concentrations in organisms were intermediate between concentrations in the sediments and overlying water, although this should not be taken to imply strict correlation between environmental concentrations and those in animals.

As well as the data summarized above, the distribution of invertebrates in and near polluted waters have been described. Invertebrates seem to vary considerably in their sensitivity and certain oligochaetes and diptera are particularly tolerant.

5.11 Summary of Effects on Invertebrates

Many of the invertebrates studied proved more sensitive to the acute toxicity of chromium than fish, and the most sensitive were the species of Daphnia. For these the 24, 48 and 96-h LC50 values were 0.4-2.2, 0.26 and 0.05 mg/l respectively, although some studies indicated 21-d LC50 values of 2 mg/l and another freshwater crustacean, Gammarus, was much less sensitive (96-h LC50: 3.2 mg/l). Next in sensitivity were the Bryozoa.
(96-h LC50: 0.6-1.6 mg/l) and the oligochaete Tubifex (48-h LC50: 1.2-5.7 mg/l), although another worm, Nais, was more tolerant, having a 96-h LC50 of 9.3 mg/l.

Rotifers, snails and insects were the least sensitive species tested, with 96-h LC50 values in the ranges 3.1-15, 8.4-12.4 and 11-50 mg/l, respectively.

Data on the effects of other environmental variables on the toxicity of chromium to invertebrates were sparse, but the following relationships were suggested. The toxicity of chromium appears to increase with increases in temperature, but to decrease with increases in pH, alkalinity, hardness, salinity and the age of the organism. For example, as far as hardness was concerned, increasing hardness from 25 to 81 mg/l as CaCO₃ could increase the 96-h LC50 to the rotifer Philodina acuticornis from 3.1 to 15 mg/l. On the other hand an 8-fold increase in hardness from 34 to 261 mg/l only increased the 48-h LC50 to Tubifex tubifex by a factor of 3.

Among sub-lethal responses, reproduction of Daphnia could be inhibited by concentrations of chromium as low as 0.05 mg/l, or even 0.01 mg/l if the daphnid was exposed for the whole of its life. Crayfish were shown to be more sensitive to predation when exposed to chromium. Invertebrates in the field have been shown to accumulate chromium. Arthropods seemed to accumulate the least amounts of the metal (<2 mg/kg dry weight) and molluscs the most (140 and 440 mg/kg) with tubificid worms and other oligochaetes in between these extremes: <1-32 mg/kg. The importance of the gut contents as a major contributing source of chromium in worms from areas with contaminated sediments has been stressed; an accurate value for body burden must be obtained from starved animals. Generally, invertebrates seem to contain concentrations of chromium which are intermediate between those in the sediments and the overlying water.

Observations on field populations of invertebrates in polluted waters have demonstrated considerable differences in sensitivity between species. Certain insects seem particularly resistant to chromium, even in the presence of other pollutants, but not, it should be emphasized, in comparison with freshwater fish.

6. EFFECTS ON PLANTS AND MICRO-ORGANISMS

6.1 Algae and Macrophytes

The following data have been published concerning acutely lethal concentrations of chromium for algae and macrophytes. At an average concentration of 3.6 mg/l (Healey, 1973), chromium is toxic to algae. Hervey (1949) found 3.2 mg/l as hexavalent chromium to be inhibitory to Chlorella and 0.32 mg/l to Euglenoids. From more recent laboratory studies Meisch and Schmitt-Beckmann (1979) found that 2 mg/l inhibited growth of Chlorella pyrenoidosa and chlorophyll synthesis. Ryder (1978) from field studies found that Selenastrum capricornutum was inhibited by chromium-contaminated water containing more than 1 mg/l at a temperature of 24 ± 2°C, whereas Chlamydomonas sp was not and was therefore considered a more tolerant organism.

Bringmann and Kühn (1980) found that inhibition of cellular multiplication in Scenedesmus quadricauda began to occur at 0.58 mg/l in an artificial medium at 27°C. The salt tested was sodium dichromate.

Chiaudani and Vighi (1978) found a 96-h EC50 on Selenastrum capricornutum in a normal Algal Assay Procedure medium of 0.031 mg/l, while in the same medium without the addition of EDTA, the 96-h EC50 was 0.004 mg/l.

Mangi et al. (1978) observed that with duckweeds (Lemma minor and Spirodela polyrhiza) grown in Bristol medium (Eyster, 1968) at a pH of 7.2 and 21°C, with 12 h of alternate light and dark, 10 mg/l proved inhibitory. Nasu and Kugimoto (1981) established that the same concentration reduced growth of the fronds of duckweed (Lemma paucicostata) to 20% of that of controls at pH values of 4.1 and 5.1 using M-medium.
Stanley (1974), in a study of Eurasian water-milfoil (Myriophyllum spicatum) grown under controlled greenhouse conditions, found a 50% reduction of (i) root weight at 1.9 mg/l as hexavalent chromium and 9.9 mg/l as trivalent chromium; (ii) root length at 8.0 mg/l as chromium (VI) and 24.4 mg/l as chromium (III); (iii) shoot weight at 2.6 mg/l as chromium (VI) and 14.6 mg/l as chromium (III); and (iv) shoot length at 9.5 mg/l as chromium (VI) and 26 mg/l as chromium (III).

As far as accumulation is concerned, Say, Harding and Whitton (1981) recorded concentrations of >200 mg/kg at pH values of 6.0 to 7.4 and total alkalinity of 1.5 meq/l (150 mg/l as CaCO₃) in tissues of the moss Rhynchostegium, in a river downstream of its confluence with a stream known to be polluted with chromium. Upstream, no chromium could be detected in the moss. Concentrations in the water were <0.01 mg/l throughout.

The alga Chlorella pyrenoidosa has been shown to absorb or adsorb trivalent chromium quickly but not hexavalent chromium, which, in any case, inhibited the growth of the alga (Schroll, 1978).

Cowgill (1973) investigated the sites of uptake and accumulation of chromium in plants. She recorded higher concentrations in the flowering and leaf structures than in the stem. Mangi et al. (1978) noted that absorption of chromium was apparent in both living and dead cells, and that chromium was adsorbed onto the outside of the cell walls (thus confirming the observations of Richards in 1936).

Mangi et al. (1978) also suggested that a 'sacrificial lamb' effect operated in such algal communities as the tufts of Oedogonium, mats of Hydridiotyon and globose clumps of Palmeta, whereby a portion of the outermost cells, their walls and capsules, removed and accumulated the chromium so effectively that the toxicity was considerably reduced, allowing the survival of a subsequent population. Rawlence and Whitton (1977) confirmed the idea of chromium absorption, finding that the metal had accumulated in senescent leaves.

Mangi et al. (1978) considered that at high levels of chromium the ratio of uptake by plants to the concentration in the medium was less than at lower concentrations of chromium.

### 6.2 Fungi and Micro-Organisms

At low concentrations of chromium fungal growth is stimulated, due to the inhibitory effect of chromium on other micro-organisms such as aquatic bacteria, algae and protozoa, which are all more sensitive than fungi to chromium (Trabalka, 1973; Draggan, 1977).

Draggan observed that fungal growth was stimulated at concentrations below 0.3 mg/l as hexavalent chromium (sodium chromate) and at 1.0 mg/l as trivalent chromium (chromium acetate and chromium chloride), confirming the earlier work of Trabalka.

However, both Trabalka and Draggan found that as the chromium concentration increased, fungal growth declined; with hexavalent chromium (sodium chromate) inhibition occurred above 0.3 mg/l and, with trivalent chromium between 3 and 15 mg/l; growth was significantly below that of control cultures. Inhibition of growth was complete at and above 20 mg/l for chromium acetate or 10 mg/l for chromium chloride.

Sudo and Aiba (1973) found the median inhibitory limit of chromium for the protozoa Vorticella microstoma, Colpidium campyllum and an Opercularia species to be 0.53 mg/l, 12.9 mg/l and 20.2 mg/l (21.2 mg/l in Conclusion section of their paper) respectively at a pH of 6.5, and that the bacterium Alcaligenes faecalis used to feed the protozoa was unaffected at such chromium concentrations.

Poon and Bhayani (1971) noted that the species of fungus Geotrichum candidum overgrew bacteria present in activated sludge, again supporting the conclusion that fungi were less sensitive than bacteria to chromium.
6.3 **Summary of Effects on Plants and Micro-Organisms**

Growth rate has most often been used to indicate the toxicity of chromium to plants. For algae the lowest effective concentrations for a range of species lie between 0.3 and 3.6 mg/l. Macrophytes are a little more tolerant, equivalent concentrations being 1.9–10 mg/l.

Algae accumulate chromium and this can occur so readily that in algal colonies the outer cells may take up all the available metal, thus protecting the inner cells. Macrophytes accumulate chromium to a small extent but mosses are particularly effective, and therefore can be used to indicate the presence of transient pollution by chromium.

Fungi seem to be more tolerant of chromium than bacteria, algae or protozoa and, like plants, their growth is stimulated by low concentrations of the metal. Trivalent chromium is less toxic to fungi than hexavalent chromium.

Bacterial cells take up chromium from their surrounding media, and the rate of uptake is increased in the presence of copper or mercury, or in saline water.

Protozoa seem more sensitive than bacteria to chromium and this may have consequences in treatment of sewage containing chromium salts.

7. **SUMMARY AND CONCLUSIONS**

Chromium occurs widely in nature, often as trivalent salts (1.1). In fresh water the hexavalent forms predominate in the dissolved state as the component of anions rather than cations. Naturally occurring concentrations in water rarely seem to exceed 0.005 or 0.01 mg/l. Higher concentrations can occur as the result of Man's activities, particularly from the tanning and metal-plating industries and in cooling-water discharges (as a consequence of the use of chromium as an anti-corrosion agent).

According to thermodynamic principles (1.2) the readily adsorbed trivalent chromium should be converted in well-oxygenated waters to the more soluble hexavalent forms, which will tend to persist in fresh water unless conditions of low pH or relatively high concentrations of reducing agent are present.

Having examined all the relevant literature on the apparent toxicity of trivalent chromium it was decided that in view of the lack of definition of work on this transient species water quality criteria for the protection of freshwater fish could not be defined.

When defining water quality criteria for freshwater fish, laboratory data should be supported by evidence from field observations, particularly on relationships between the degree of success (e.g., diversity, productivity) of a fish community and ambient concentrations of the contaminant under consideration. In the case of chromium, unfortunately, there are no useful publications for this purpose, although other types of observation, discussed below, are available.

Hexavalent chromium can easily cross biological membranes and subsequently become reduced to the biologically more active trivalent state (2.1). Only high concentrations of dissolved chromium appear to be capable of causing histological damage although chromium seems to exert its toxic action in fish partly through impairment of respiratory and osmoregulatory function. It also seems that chromium has a specific toxic action on glucose metabolism and the related mechanisms involving proteases.

Chromium is not very toxic to fish, at least in the short term (2.2) and only in very soft water and at relatively low pH values were the most sensitive species (salmonids) affected by concentrations of about 3–4 mg/l (96-h LC50). The 7-d LC50 values of chromium to European non-salmonid fish (perch and roach) were 26 and >80 mg/l respectively.

Chromium can be less toxic in warmer water but more marked decreases in toxicity are found with increases in either pH or water hardness. (An increase in pH from 6.5 to 7.8 has roughly the same effect as an increase in hardness from 20 to 320 mg/l as CaCO3, of decreasing toxicity by a factor of 3 or 4, at least for rainbow trout.)
Changes in salinity have little if any effect on the toxicity of chromium to fish (although few data are available on which such a conclusion can be based), but chromium can affect the ability of fish to adapt to sea-water conditions and can make fish more prone to infection. Larger fish can be more tolerant of chromium than small fish of the same species.

In long-term exposure (2.3) concentrations as low as 0.2 mg/l have proved lethal to salmonid fish but non-salmonids can tolerate much higher concentrations. The data are conflicting, but in separate studies a concentration of 0.2 mg/l has neither killed nor retarded the growth of rainbow trout in exposure periods of up to 180 days, in hard and soft water. For perch and roach, the median asymptotic lethal concentrations are 26 and 32.5 mg/l respectively in hard water. Data on the effects of mixtures of chromium and nickel are conflicting for rainbow trout (2.4) but for roach only additive toxicity has been observed.

As far as developmental stages and studies of complete life cycles are concerned (2.5), for European species in hard water the lowest no-effect concentration appears to be about 0.2 mg/l but in waters of low pH the corresponding level is 0.02 mg/l.

As indicated above, high concentrations of chromium can damage fish tissues and a number of examples of sub-lethal damage have been reported (3). Changes in haematocrit and in enzyme activity can occur on exposure to chromium, but their survival value is difficult to determine. Similarly locomotory activity and plasma cortisol levels have been changed, but the significance of these findings is not yet clear. More obviously damaging are the effects on gill tissue and gill mucus, the latter being observed after 6 months exposure of rainbow trout to 0.2 mg/l. Glucose uptake by cells seems to be inhibited by the presence of chromium albeit at relatively high levels (2.5 mg/l). However, in a 6-month experiment, significant reductions in liver glucidic content have been reported at a concentration of 0.2 mg/l.

Exposure of fish to dissolved chromium can lead to accumulation of the metal in various fish tissues (4). Release can be rapid from most tissues, but not from gill, liver (and gall bladder and bile) or kidney (4.1). These two rates of turnover could be due to the rapid elimination of hexavalent chromium and the slower elimination of the trivalent metal. Bioconcentration factors of about 10 were not uncommon, and, for rainbow trout, asymptotic concentrations in kidney were about 6-10 mg/kg dry weight, in liver 0.1-1.7 mg/kg and in muscle 0.01-0.3 mg/kg. Chromium certainly seemed to occur in the soluble fraction of gill, liver and kidney but results on the significance of the nuclear fraction seem to conflict.

In the field (4.2), the chromium contents of fish in contaminated and uncontaminated waters often do not differ, although fish have been shown to accumulate chromium as they age. Among European species the chromium content of muscle was commonly in the range of 0.1 to 0.5 mg/kg. No biomagnification is apparent from prey to carnivorous fish. There is evidence that fish possess regulatory mechanisms for handling absorbed chromium.

Many species of invertebrate have been shown to be less tolerant of chromium poisoning than fish (5). In order of increasing tolerance they can be listed as Daphnia, Bryozoa, Gammarus and Tubifex. Other oligochaetes, rotifers, snails and insects are similar to fish in their sensitivity to chromium.

Unlike the situation with fish, toxicity to invertebrates increased with increasing temperature. As with fish, however, toxicity decreased with increases in pH, alkalinity and hardness. It also decreased as organisms aged and as salinity increased.

Reproduction of Daphnia proved sensitive to chromium. Exposure to 0.01 mg/l from the moment of release from the brood pouch until that individual itself reproduced resulted in impaired reproduction.

Chromium was accumulated by snails (to 140-440 mg/kg), tubificid and other worms accumulated less and arthropods least (<2 mg/kg dry weight). From studies of invertebrate communities affected by chromium discharges it appears that certain insects are particularly tolerant of chromium, but not, it should be emphasized, in comparison with the tolerant shown by many freshwater fish.
Chromium is toxic to algae, inhibitory concentrations being reported in the range of 0.3–3.6 mg/l (6.1). Some larger plants were more resistant. Algae and macrophytes can absorb chromium and among algae this can happen to such an extent at the periphery of a colony that the rest of the cells may show no effect of the chromium at all. (Dead cells as well as living ones take up chromium from the water.)

Fungal growth can be promoted at low concentrations of chromium but inhibited above 0.3 mg/l (6.2). Even so, fungi are more tolerant of chromium than some bacteria, but certainly not all. For protozoa however median inhibitory concentrations of 0.5 to 20 mg/l have been reported. Bacteria can take up the metal from their environment, and uptake is stimulated by the presence of certain other metals, and under saline conditions.

8. TENTATIVE WATER-QUALITY CRITERIA

Tentative standards for the protection of freshwater fish may be derived in a number of ways. For example, Stephan et al. (1980) calculated a 'final chronic value' by dividing a 'final acute value' by an 'acute-chronic ratio'. In the present report another approach has been adopted. Attention has been focussed on the individual results of long-term tests, where such data are available, and also on the lowest concentrations producing adverse effects, e.g., on growth and reproduction, as well as on survival.

8.1 Salmonid Fish

The mean aqueous concentration of 'soluble' chromium should not exceed 0.025 mg/l and the 95 percentile should not exceed 0.1 mg/l. This standard is widely applicable to most natural waters. However, more stringent values may be necessary in very soft, acid waters and less stringent values may be satisfactory in alkaline waters.

8.2 Non-Salmonid Fish

The few data available would suggest that the mean concentration of chromium should not exceed 0.1 mg/l and the 95 percentile should not exceed 0.4 mg/l. High concentrations should not occur during the breeding season. However, this standard would not protect the most sensitive species of invertebrates. Therefore, in the absence of any field data indicating the presence of a flourishing population of invertebrates and plants in waters containing these concentrations of chromium, it is proposed that the salmonid standard should also be applied to non-salmonid waters. Nevertheless, the standards could be relaxed if it could be shown than an acceptable diversity of invertebrates can flourish under such conditions.

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