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INTEGRATED CRITERIA DOCUMENT CHROMIUM: EFFECTS

J.A. Janus and E.I. Krajnc

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Advisers: P. van Beelen, J.H. Canton, C.A. van der Heijden, A.G.A.C. Knaap and G.R. Mohn

CONTENTS

INTRODUCTION

Data in the present Appendix are underlying those in the chapter on "effects" (chapter 5) of the "Integrated Criteria Document Chromium" (Slooff et al., 1989). The Criteria Document, prepared by the National Institute of Public Health and Environmental Protection in The Netherlands, comprises a systematical survey and a critical evaluation of the most important data on the "priority substance" chromium, as much as possible with regard to the specific situation in The Netherlands. The information in the Criteria Document will serve as a scientific basis for an "effect oriented policy" in The Netherlands, especially with regard to the general population and aquatic and terrestrial ecosystems.
The Criteria Document, including the present Appendix, has been written on behalf of the Ministry for Housing, Physical Planning and Environment, Directorate Substances and Risk-management. By order of this principal, in the present Appendix considerable reference has been made to previous reviews; these reviews are marked in the text by "R". However, in many cases data presented in reviews are too limited for evaluation. Therefore the original publications have also been studied whenever it appeared necessary; this applies especially with regard to data which are used in the risk assessments.
Extensive reviews on chemobiokinetics and metabolism in mammals and on effects on mammals have been published by the U.S. Environmental Protection Agency (U.S. EPA, 1984-R)) and, more recently, by the World Health Organization (WHO, 1988-R). Reviews on more specific items are mentioned in the sections in question. In some sections in the text the reader is referred to "R"; in these cases one is not referred to a specific review, but the section has been written on the basis of several reviews which are mentioned in the introduction of the section or chapter in question.

Chromium may have either beneficial or adverse effects on organisms, the ultimate effect depending on speciation (valency, chemical form), route of exposure and exposure level. Beneficial effects are ascribed to trivalent chromium, Cr(III), which is considered to be an essential trace element, in any case for mammals. Toxic effects, on the other hand, are mainly ascribed to hexavalent chromium, Cr(VI).
With respect to the general population, significant exposure to chromium can occur via oral intake from the diet or via inhalation of chromium in ambient air. The data which are considered to be necessary for a risk

assessment with regard to the impact of these routes of exposure to the general population, are described in chapter 1. Dermal exposure to chromium compounds other than metallic chromium is considered to be not relevant to the general population. Therefore, effects of chromium after dermal exposure have been left out of consideration in the present document.

[Dermal exposure to chromium compounds may result in ulcers and contact dermatitis due to the direct corrosive action, especially of Cr(VI) compounds. Repeated dermal exposure to chromium compounds may also lead to sensitization which ultimately results in allergic (eczematous) contact dermatitis. Again, Cr(VI) is believed to be more potent than Cr(III), due to the fact that Cr(VI) is capable of penetrating the skin, where it is subsequently reduced to the trivalent state which in turn binds to proteins or other macromolecules to form allergens. Chromium eczema is (one of) the most frequently occuring occupational dermatosis. For reviews on dermal exposure the reader is referred to Polak et al. (1973), Pederson (1982) and Langard and Norseth (1986).]

Data on the impact of chromium on aquatic and terrestrial organisms are described in chapter 2 and chapter 3, respectively. In chapter 4 data on agricultural crops and livestock are described. Chapter 5 contains the risk assessment for man and the environment.

An online literature search has been conducted in August 1988, especially in order to retrieve recent publications.

1 HUMAN TOXICITY

1.1 ESSENTIALITY AND REQUIREMENTS

On the basis of experiments with rats, Cr(III) is considered to be an essential dietary trace element for mammals, influencing chemobiokinetics and metabolism of glucose. The effect of chromium is insulin-dependent (Mertz and Schwarz, 1959; Schwarz and Mertz, 1959; Mertz et al., 1961). Furthermore, chromium is implicated in enzyme structures and activities (for example those of phosphoglucomutase and trypsin), in lipid metabolism and possibly in stabilizing RNA structures. An extensive publication on chromium occurence and function in organisms has been written by Mertz (1969-R).

In food (biological material) chromium is present in the form of a low molecular weight organic compound, the so-called "glucose-tolerance factor" (GTF), in which Cr(III) has been identified as the active component. GTF is composed of chromium, nicotinic acid and the amino acids glutamic acid, glycine and cysteine, but at present the precise structure is not yet known (Mertz. 1969-R; Van Schoor et al., 1988-R). GTF is also formed endogenously from dietary inorganic chromium (Riales and Albrink, 1981).

Low-chromium diets have resulted in impaired glucose tolerance in laboratory animals, characterized by delayed removal of (intravenously injected) glucose from the blood. In severe cases deficiency can lead to a diabetes-like syndrome, retarded growth and other adverse effects (Mertz, 1969-R).

In experiments with rats a single oral dose of 200 μg Cr(III).kg^{-1} bw (in the form of inorganic or organic complexes) was sufficient to overcome at least partially an impaired glucose tolerance at low-chromium state . In general there was an inverse relationship between the stability of these compounds and their influence on glucose tolerance. In the form of isolated GTF the effective dose was about 100 μg Cr.kg^{-1} bw (Schwarz and Mertz, 1959). The biological activity of chromium in human food samples (measured as metabolic rate of glucose *in vitro*) has been found to be more related to the ethanol-extractable fraction than to the total chromium content of these samples, indicating differences in biological availability. However, "relative biological values" for different food items were within a factor of 3, with exception of those for dried brewer's yeast and black pepper which were considerably higher (Toepfer et al., 1973).

In humans low-chromium state and chromium deficiency have been associated especially with malnourished children (low-protein diets), pregnant women (particularly multiparous women), aged people and insulin-requiring diabetics. Supplementary administrations of chromium (administered as yeast or inorganic chromium) have been shown to improve or normalize an impaired glucose tolerance in some but not all of these persons (Mertz, 1969-R; WHO, 1973-R; Van Schoor et al., 1988-R). An example of this contradiction is described in the study by Glinsmann and Mertz (1966), in which additional daily oral doses of 180 (3 times 60) µg Cr(III) (administered as chromic chloride in drinking water) resulted in an improved glucose tolerance in a diabetic exposed for 2.5 weeks, but not in another diabetic exposed for 19 weeks. In two studies using healthy volunteers, supplementary additions of inorganic chromium of 200 µg per day (5 and 7 days per week, respectively; administered as chromic(III)chloride) resulted in a trend of increasing glucose tolerance only or especially after 6 to 8 weeks; this effect appears to be related to serum glucose levels (Riales and Albrink, 1981; Anderson et al., 1983). In a number of studies supplementary additions of chromium influenced lipid metabolism, both in healthy persons and persons with an impaired glucose metabolism. In general supplementary additions of chromium, especially as brewer's yeast, result in a decrease of serum total-cholesterol levels (Van Schoor et al., 1988-R). In three studies using healthy persons the influence of chromium on different cholesterol fractions were studied. *In one study supplementary additions of 15 µg chromium per day (as brewer's yeast) resulted in a decrease in the LDL-/HDL-cholesterol ratio, which may have a protective effect against atherosclerosis (Riales, 1979, cited in Van Schoor et al., 1988-R).* In the studies by Riales and Albrink (1981) and by Anderson et al. (1983), see above, no decrease in this ratio was found at daily supplementary additions of 200 µg inorganic-Cr(III) per day (In the study by Riales and Albrink the HDL-cholesterol level was significantly increased after 12 weeks of exposure, but LDL- and total-cholesterol levels were unaffected. On the basis of these studies it appears that in yeast chromium is not the only causative agent involved in effects on lipid metabolism.

Two cases of chromium deficiency, resulting in glucose intolerance, weight loss and signs of neuropathy, have been reported after long-term total parenteral nutrition (TPN). In one case - a 45-year-old woman, given TPN for 5 months - a supplementary addition of 150 µg chromium chloride [50 µg Cr(III)] per day resulted in about normal glucose tolerance within a few days and in the disappearence of neuropathy and in some body weight gain after about one month (Freund et al., 1979). It is not mentioned what daily

amount of chromium was provided by the parenteral nutrition. In the second case - a 39-year-old woman, given TPN for 3.5 year - daily additions of 250 μg Cr(III), as chromium chloride, to the infusate for two weeks, abolished glucose intolerance. In the 1.5 year following this treatment a daily chromium addition of 20 μg to the infusate was sufficient for well-being. Without supplementary addition the protein hydrolysate in the nutrient solution provided about 5 μg Cr daily (Jeejeebhoy et al., 1977).
Based on a mean 24-hr urinary chromium excretion of 0.8 μg (range 0.4-1.8), the American "Food and Nutrition Board" has estimated the mean minimal daily requirement for absorbable chromium to be near 1 μg; at the lowest availability in humans (fractional absorption 0.5%, see 1.2) this requirement will be provided by a daily dietary intake of 200 μg. However, based on the absence of signs of chromium deficiency in the major part of the United States population, consuming on the average 60 μg chromium per day, a daily chromium intake of 50-200 μg has been recommended tentatively, for adults (NRC, 1980). Based on a mean fractional absorption value of 25% for chromium incorporated in food (see 1.2), 1 μg absorbable chromium corresponds with a daily dietary requirement of 4 μg (range 1.6-7.2 μg). Based on recent chromium levels measured in urine (0.3 - 1.0 $\mu g.l^{-1}$) and considering urine to be the major route of excretion of chromium actually absorbed (see 1.2) a daily dietary requirement of 2.4 to 8 μg can be estimated, on the basis of excretion of 2 l urine.
The safety of the upper limit recommended by NRC (1980) is based on a very limited study by Glinsmann and Mertz (1966) in which both diabetics (n = 6) and nondiabetics (n = 10) recieved daily oral additions of 150-3,000 μg Cr(III), for periods of 2-19 weeks. In this study physical examinations and measurements of blood and urine parameters revealed no signs of toxicity.

Summary and conclusions "essentiality and requirements"

Cr(III) -being present in biological material as an organic compound, the so-called "glucose-tolerance-factor"- is an essential trace element for mammals, influencing amongst others chemobiokinetics and metabolism of glucose. The minimal daily requirement for adults is estimated to be 0.5 to 2 μg absorbable Cr(III), corresponding with 0.01 to 0.03 $\mu g\ Cr(III).kg^{-1}\ bw.day^{-1}$. Assuming a fractional absorption value of 25% for Cr(III) incorporated in food, this amount of absorbable Cr(III) is provided by a daily dietary intake of 2 to 8 μg Cr(III), corresponding with 0.03 to 0.13 $\mu g\ Cr(III).kg^{-1}\ bw.day^{-1}$.

1.2 CHEMOBIOKINETICS AND METABOLISM

Data on this subject have been published in a number of reviews, for example Mertz (1969), Saner (1980), U.S. EPA (1984), Langard and Norseth (1986), Smith and Thorne (1986), Thorne et al. (1986) and Nieboer and Jusys (1988), the review by Thorne et al. (1986) being the most extensive one.

1.2.1 Absorption

Animals

After oral administration of inorganic chromium salts to experimental animals, fractional absorption values of < 0.5% to 6% have been estimated for chromium, as indicated by tissue distribution or urinary excretion (R). Since there is evidence that the intestines are also involved in chromium excretion (Visek et al., 1953; Hopkins, 1965), some studies may underestimate the amount actually absorbed. In contrast, more recent analyses of chromium in biological materials such as urine result in much lower values than those reported in the past (Van Schoor et al., 1988-R; see also "excretion"). On the basis of tissue distribution after both short- and long-term oral exposure of rats it is concluded that Cr(VI) is absorbed to a higher extent than Cr(III). Higher blood chromium levels and lower blood Cr(VI)/Cr(III) ratios after intragastric administration of Cr(VI) (as sodium chromate) compared with intraintestinal administration indicate that partial reduction of Cr(VI) to Cr(III) occurs in the stomach, which decreases the absorption (MacKenzie et al., 1958, 1959). These findings are in agreement with other data on rats exposed to trace amounts of chromium, showing considerably more urinary excretion of chromium administered as sodium chromate than of chromium administered as chromic chloride after intraintestinal administration (into the jejunum), but little or no difference after intragastric administration of these compounds. In the latter experiment a higher amount of chromium administered as Cr(VI) was excreted in the urine after intraintestinal than after intragastric administration, while the respective amounts of chromium administered as Cr(III) were similar (Donaldson and Barreras, 1966).

Quantitative data on absorption after inhalation are not available. At exposure of rats to an average concentration of 7.5 $mg.m^{-3}$ zinc chromate dust (zinc yellow pigment, particle size < 5 μm) for 6 hours per day for

four consecutive days, the concentration in the blood peaked after the third exposure; the average peak concentration of 0.46 $mg.l^{-1}$ was 65 times higher than the pre-exposure concentration of 0.007 $mg.l^{-1}$. After exposure for two months (6.5 hours per day, 5 days per week), the blood chromium concentrations were very similar (0.36-0.62 $mg.l^{-1}$ in individual animals), with an average concentration of 0.5 $mg.l^{-1}$. A pilot study showed increases in the blood chromium levels of individual rats up to 5-fold and 40-fold (compared with unexposed animals) after exposures for 100 and 250 minutes, respectively (Langard et al., 1978).
After intratracheal administration of soluble chromium compounds, Cr(III) was absorbed much more slowly than Cr(VI) (R).

Humans

After a single oral dose of a trace amount of chromium (6.5 ng) a mean 24-hr urinary excretion rate of 0.5% (range 0.1 - 1.2) was found after administration of Cr(III) (administered as $^{51}CrCl_3$) to volunteer patients who were hospitalized for dietary treatment of obesity or were recovering from surgery, while a mean value of 2.1% (range 0.2 - 4.4) was found after administration of Cr(VI) (administered as $Na_2{}^{51}CrO_4$). Intraduodenal administration of Cr(III) did not influence urinary and fecal excretion in comparison with oral administration, but that of Cr(VI) resulted in a mean urinary excretion rate of 11% and in a reduction of fecal excretion with about 50%, indicating that more chromium was absorbed. In jejunal perfusion studies the mean percentage of Cr(VI) absorbed was more than 15 times higher than that of Cr(III) in the same persons. After incubation of Cr(VI) with human gastric juice at pH 1.4 the jejunal absorption was strongly reduced; the resulting absorption was as low as that of Cr(III) (Donaldson and Barreras, 1966). These data are in agreement with animal data indicating intragastric reduction of Cr(VI). Donaldson and Barreras also showed the *in vitro* reduction of Cr(VI) to Cr(III) by acid human gastric juice (pH 1.3 - 1.4).
After oral administration of Cr(VI) more absorption (as indicated by an increased urinary excretion and a decreased fecal excretion) has been found in patients with pernicious anemia -which may be coupled with the absence of hydrochloric acid in the gastric juice- than in control patients; this is also in agreement with intragastric reduction of Cr(VI) in persons without gastrointestinal dysfunction (Donaldson and Barreras, 1966).

In studies reviewed in Thorne et al. (1986-R), gastrointestinal absorption of inorganic chromium compounds has been estimated to be up to 10%. On the basis of both animal and human data Thorne et al. have assigned a mean fractional absorption value of 5% for all inorganic chromium compounds, both Cr(III) and Cr(VI), in the diet.
Organic chromium in food ("biologically incorporated") is believed to be absorbted to a higher extent - up to 50% - than inorganic salts (R). Thorne et al. (1986-R) assigned a mean fractional absorption value of 25% for these biological forms of chromium in the diet.

In ambient air chromium aerosols are mainly in the small-size fraction. The majority of chromium particles has a mass median diameter (MMD) of about 1 μm (see Criteria Document). This means that a considerable part of chromium in air is respirable and is deposited in the tracheobronchial and pulmonary region of the respiratory tract. For general reviews on deposition, retention and clearence mechanisms of inhaled particles, the reader is referred to NRC (1979) and the Criteria Document on "Fine Particulate Matter" (Prins et al. 1985).
The increasing chromium level in human lungs from the age of 20 years and onwards shows that at least part of the chromium deposited in the respiratory tract is retained within that tissue.

1.2.2 Transport, distribution and metabolism

Animals

Once absorbed, the fate of chromium is dependent on the valence state. In the blood Cr(VI) readily penetrates through erythrocyte membranes, which on the other hand appear to be essentially impermeable to Cr(III) (R). For example, after gastrointestinal absorption of inorganic Cr(III), chromium in the blood of rats was found in the plasma (MacKenzie et al., 1959; Hopkins, Jr. and Schwarz, 1964). A small, physiological amount of Cr(III) was bound almost entirely to siderophilin, the iron-binding protein of the plasma. At higher concentrations binding also occured to other plasma proteins (Hopkins, Jr. and Schwarz, 1964). After gastrointestinal absorption of Cr(VI), chromium was found in both plasma and erythrocytes; the non-cellular fraction is believed to represent Cr(III) and the cellular fraction Cr(VI) (MacKenzie et al., 1959).

After entry in erythrocytes Cr(VI) is reduced to Cr(III); this reduction has also been found in studies with other cell types. Although the uptake of Cr(III) in cells other than erythrocytes has been found both *in vivo* and *in vitro*, biological membranes are considerd to be relatively impermeable to Cr(III) compared to Cr(VI). After penetration through membranes hexavalent chromium is assumed to be reduced rapidly to the trivalent state which binds subsequently to macromolecules (R).
Distribution studies using different routes of administration (intravenous, intraperitoneal, oral) and different inorganic Cr(III) and Cr(VI) compounds show that significant amounts of chromium may accumulate in liver, kidneys, spleen and bone marrow; some data also indicate significant accumulation in testes, brain, heart and lungs (R, MacKenzie et al., 1959; Hopkins, Jr., 1965); Tandon et al., 1979). The absolute amount which accumulates in different organs is dependent on both the valence state and the counter ion (Visek et al., 1953). In cats orally exposed to daily amounts of 50 to 1,000 mg Cr(III) salt [administered as chromic phosphate or chromic oxycarbonate; these amounts correspond with daily amounts of approximately 20 to 400 mg Cr(III)], only trace amounts were found in the tissues after 1 to 3 months of exposure. According to the authors the amount of chromium recovered in the tissues was at best 0.0004% of the total amount ingested (Akatsuka and Fairhall, 1934).
Clearance of Cr(III) from the blood occurs more rapidly than that of Cr(VI), whereas the latter is cleared more rapidly from other tissues (R).

After exposure of rats to chromite dust by inhalation for 28 days, the highest concentrations were found in kidneys followed by lung, spleen, liver and blood. Concentrations in blood and liver were similar and changed little between day 5 and day 28. Levels in lungs, kidney and spleen were increasing with time. After exposure of rats to 42 mg $CrCl_3.m^{-3}$ (14 mg $Cr(III).m^{-3}$), the concentration in the liver increased with time during the 5 day observation period following exposure. In experiments with rats inhaling zinc chromate dust it has been found that chromate may be taken up by the lungs in the hexavalent state and transferreed to the red blood cells before reduction to the trivalent state (R).
In two cats exposed to "fine" particles of chromic carbonate for 30 and 60 minutes per day, respectively (6 times per week, total exposure time about 3 months), chromium was retained completely (> 99% according to the investigators) into the lungs (Akatsuka and Fairhall, 1934).

After intratracheal administration of soluble chromium compounds, a higher percentage of the administered dose was found in the blood and other tissues (lung excluded) 10-minutes post-instillation in case of Cr(VI) than in case of Cr(III). In rats the highest concentrations were found in the lungs, followed by liver, kidneys and spleen, after intratracheal administration of chromate.
In the lungs water soluble chromium compounds can undergo conversion to insoluble compounds with long residence times (R).

Data on placental transfer of chromium are scarce. The data indicate that chromium is transferred to the fetus *in utero* to a limited extent. It appears that inorganic-Cr(VI) is transferred to a higher extent than inorganic-Cr(III) and that chromium in the form of GTF is transferred to a higher extent than inorganic chromium (R).

Humans

Tissue chromium levels are depending on age. In most organs the highest levels are found in newborns; a survey of autopsy data in the United States shows concentrations ranging from 0.2 to 0.8 $mg.kg^{-1}$ on a wet weight basis. Levels are decreasing in the first months of life in some organs (e.g. spleen and heart) while that in other organs (liver, kidney) remain constant untill the age of 10 years, after which they start to decline. In contrast with other tissues, lung chromium levels decline until the age of 20 years, after which there is a steady increase throughout the remaining part of life. Chromium in lungs is found (partially) in the form of very insoluble compounds which indicates long residence times. The data on chromium levels in tissues in dependence on age indicate that levels in lungs and those in other organs are related to chromium inhaled and ingested, respectively. Cigarettes have been reported to contain chromium levels of 0.25 to 24 $mg.kg^{-1}$ (Criteria Document, chapter 4), but data on the relationship between smoking habits and lung chromium levels are not available.
Autopsy data on both occupationally and non-occupationally exposed humans show that chromium tissue levels are higher in the first group, but distribution pattern is similar. The highest concentrations in major organs are found in lungs, followed by spleen, liver, kidney and heart, which all contained approximately the same level in each individual person. Relatively (very) high concentrations have been reported in hilar lymph

nodes and in parts of the central nervous system, namely cerebral cortex, cerebellar cortex and nucleus caudatus.
After intravenous injection of Cr(III) in healthy persons and hemochromatotic patients, liver and spleen contained the highest levels; after 3 months half of the body burden was found in the liver (R).

1.2.3 Excretion

Data on both animals and humans indicate that after oral exposure to inorganic chromium, especially Cr(III) compounds, chromium is recovered almost entirely in the feces and that physiological amounts of chromium are reabsorbed almost completely in the renal tubuli (Visek et al., 1953; Donaldson and Barreras, 1966; R).

Animals

After dietary exposure of cats to chromic phosphate or chromic oxycarbonate (20 to 400 mg Cr(III) per day) for 1 to 3 months, no chromium was detected in the urine (Akatsuka and Fairhall, 1934).
The results of experiments in which rats were injected intravenously with Cr(III), as chromic chloride, indicate that the elimination process can be described as a three compartment model, with half-lives of hours, days and months, respectively. Clearance from the blood occurs more rapidly than that from tissues. Cr(VI) (as sodium chromate) was eliminated more rapidly than Cr(III) (as chromic chloride) after intubation or intravenous injection in rats; half-lives were 22 and 92 days, respectively. Excretion in the urine is reported to be the major route of elimination of chromium after parenteral administration (R). For example, 40% and 6% of an intravenously injected physiological amount (1 μg Cr(III).kg^{-1} bw, injected as $^{51}CrCl_3$) was excreted in urine and feces of rats, respectively, after 4 days of exposure (Hopkins, 1965). However, other authors reported that 15% and 20% of an intravenously injected dose of the same compound was excreted in urine and feces of rats, respectively, after 4 days of exposure. According to Hopkins (1965) the difference may be explained by the difference in amount administered. After i.v. injection of chromic chloride buffered by acetate or citrate, the amount excreted in urine was 4-7 times that excreted in feces; after injection of sodium chromate [Cr(VI)] the amount excreted in the urine was 2 times that excreted in the feces (Visek et al., 1953).

After exposure of rats by inhalation to respirable zinc chromate dust, the urine was found to be the major route of excretion (Langard et al., 1978). After intratracheal administration of chromium, a large part of the administered dose appears to have been cleared from the lungs to the gastrointestinal tract and to have been excreted subsequently into the feces (R).

Humans

In a 1-yr balance study in which two male subjects had a mean daily dietary intake of 200 and 290 μg Cr, a mean of 190 and 210 μg, respectively, was exceted daily. Of the total amount excreted, about 60% was recovered in the urine and 40% in the feces (Tipton and Stewart, 1970).
After oral administration of Cr(III) or Cr(VI) -administered as $^{51}CrCl_3$ and $Na_2{}^{51}CrO_4$, respectively- to six control patients, a mean of 99.6% (96.8-101.3) and 89.4% (84.7-96.7), respectively, of the dose was recovered in the feces; in the urine a mean of 0.5% and 2.1% was recovered, respectively (Donaldson and Barreras, 1966).
As in rats, also in humans a three compartment elimination process was found after i.v. injection of Cr(III), with half-lives of hours, days, and months. Highest clearance rates were found for adipose and muscle tissue and lowest ones for liver and spleen.
Data on urinary chromium levels are not consistent, due to the relatively low levels and the use of different analytical methods in the past and at present. Urine analyses from the years 1950 and 1963/1966 resulted in levels of 350 $\mu g.l^{-1}$ (maximum) and averages of 4-5 (range 2-11) $\mu g.l^{-1}$, respectively. More recent data indicate that urinary levels are as low as 0.3 to 1 $\mu g.l^{-1}$ (Mertz, 1969-R; Langard and Norseth, 1986-R; Van Schoor et al., 1988-R).

In humans occupationally exposed to chromium, for example to stainless steel welding fumes, correlations between exposure and urinary excretion have been found (Langard and Norseth, 1986-R).

Summary and conclusions "chemobiokinetics and metabolism"

The relatively low chromium levels in biological materials easily result in contamination problems at analysis. Due to the use of different analytical methods, data are not consistent: relatively high levels (for example in

urine) are reported in publications in the sixties and earlier, while more recent data result in lower levels. For this reason some data in this section may be open to question.

After oral exposure of experimental animals to inorganic chromium compounds, fractional absorption values < 0.5% to 6% have been reported; in human studies values up to 10% have been reported. On the basis of both animal and human studies a mean fractional absorption value of 5% is considered to be a good approximation for inorganic chromium, both Cr(VI) and Cr(III) compounds. For organic chromium [Cr(III)] in food a mean fractional absorption value of 25% has been assigned. In animals and humans Cr(VI) is absorbed to a higher extent than Cr(III) after oral exposure to inorganic chromium compounds. However, the absorption of Cr(VI) is decreased by reduction to Cr(III) in the stomach.

The absorption after inhalation depends on particle size and speciation of chromium in aerosols. The mass median diameter (MMD) of the majority of chromium particles in ambient air is approximately 1 μm. This means that a considerable part of chromium in air is deposited in the tracheobronchial and pulmonary region of the respiratory tract. Fractional absorption values are not available. A study with rats showed that chromium was quickly absorbed after inhalation of respirable zinc chromate dust.

Once absorbed, the fate of chromium is dependent on the valence state. Cr(VI) readily penetrates through cellular membranes, which are considered to be relatively impermeable to Cr(III). Due to this difference between Cr(VI) and Cr(III), chromium is found in both erythocytes and plasma of the blood after gastrointestinal absorption of Cr(VI), while chromium is found exclusively in the plasma after gastrointestinal absorption of Cr(III). Once transported through cell membranes, for example those of erythrocytes, Cr(VI) is rapidly reduced to Cr(III) which binds subsequently to macromolecules.

After oral or parenteral administration of different Cr(VI) and Cr(III) compounds to animals, chromium accumulates especially in liver, kidneys, spleen and bone marrow; some data also indicate a significant accumulation in testicles, brain, heart and lungs. The absolute amount which accumulates in different organs appears to be dependent on both the valence state and the counter ion. At exposure of animals by inhalation, the distribution also depends on the chromium speciation; the major organs in which accumulation is found are lungs, kidneys, spleen and liver. In inhalation experiments with rats it has been found that chromate may be taken up by

the lungs in the hexavalent form and transferred to the red blood cells before reduction to the trivalent state.
In humans, tissue chromium levels are depending on age. Levels start to decline during the first months of life in some organs and after about 10 years in other organs. Lung chromium levels decline untill the age of 20 years, after which there is a steady increase throughout the remaining part of life, indicating accumulation due to inhalation.
The distribution pattern in occupationally exposed humans on the one hand and non-occupationally exposed humans on the other hand, appear to be similar. The highest concentrations are found in hilar lymph nodes and lungs, followed by spleen, liver, kidneys and heart. Some parts of the central nervous system can (also) contain very high chromium concentrations.
In the lungs of both animals and humans, water soluble chromium compounds can undergo conversion to insoluble compounds with long residence times.

Data on placental transfer in animals indicate that chromium is transferred to the fetus *in utero* to a limited extent. Chromium tissue levels in newborns also point at placental transfer.

After oral exposure (animals, humans) to inorganic chromium compounds, especially Cr(III) compounds, chromium is recovered almost entirely in the feces. In animal studies urine was found to be the major route of elimination of chromium after parenteral administration, indicating that chromium that is actually absorbed into the body is excreted mainly in the urine. In a 1-yr balance study in which two humans had a mean daily dietary intake of 200 and 290 μg Cr, about 60% and 40% of the total amount excreted was recovered in the urine and feces, respectively.

1.3 TOXICITY

1.3.1 Short-term exposure (acute and subacute toxicity)

Animals

Oral LD50 values in rats for Cr(VI) compounds are ranging from 50 to 746 $mg.kg^{-1}$ body weight (bw), corresponding with 20 to 249 mg $Cr(VI).kg^{-1}$ bw, based on tests with sodium dichromate, potassium dichromate and calcium chromate. Oral LD50 values in rats for Cr(III) compounds are ranging from

1,410 to 3,250 mg.kg^{-1} bw, based on tests with chromic chloride, chromic chloride-hexahydrate and chromic nitrate-nonahydrate; on the basis of Cr(III) content these values are ranging from 183 to 617 mg Cr(III).kg^{-1} bw. Oral LD50 values in rats for Cr(II) compounds are 1,870 and 11,260 mg.kg^{-1} bw, corresponding with 790 and 3,110 mg Cr(II).kg^{-1} bw, based on tests with chromium(II)chloride and chromium(II)acetate hydrate, respectively (Smyth et al., 1969, Vernot et al., 1977, Lewis and Tatken, 1982-R; WHO, 1988-R).

After i.p. injection, 3-d LD50 values in mice for inorganic Cr(VI) salts were up to 3 times lower than that for inorganic Cr(III) salts, on the basis of Cr content; the mean values were 18 mg Cr(VI).kg^{-1} bw and 39 mg Cr(III).kg^{-1} bw, respectively. However, 10-d (asymptotic) LD50 values were similar for all compounds tested (Bryson and Goodall, 1983). Repeated i.p. injections of sodium chromate in rats (2 mg Cr(VI).kg^{-1} bw three times per week, treatment period 18 days) resulted in 100% mortality within the experimental period of 60 days. Using the same concentration of Cr(III), administered as chromium chloride, no mortality was found, while injections were given up to 60 days. In both cases adverse effects on liver and kidneys were found (Laborda et al., 1986).

In a very limited study using only one or two animals per dose and no controls, cats were exposed to Cr(III) salts in their feed (50 to 1,000 mg chromic phosphate or chromic oxycarbonate per day per cat, corresponding with approximately 20 to 400 mg Cr(III) per day) for a period of 1 to 3 months. At termination no signs of toxicity were noticed on the basis of appearance, growth, and macroscopic and microscopic examination (Akatsuka and Fairhall, 1934).

In 2-3 month experiments, one or more of the following parameters: general condition, growth and reproduction of young rats were affected at dietary levels of 0.125% zinc chromate (360 mg Cr(VI).kg^{-1} feed, corresponding with 36 mg Cr(VI).kg^{-1} bw.d^{-1} using a standard conversion factor of 10) and at 0.25% potassium chromate (675 mg Cr(VI).kg^{-1} feed, corresponding with 67 mg Cr(VI).kg^{-1} bw.d^{-1}). Dietary levels of 1% zinc chomate (2,900 mg Cr(VI).kg^{-1} feed) did not affect these parameters in half-grown rats and mature mice; this dose level corresponds with 290 and 415 mg.kg^{-1} bw.d^{-1} in rats (conversion factor: 10) and mice (conversion factor: 7), respectively. A concentration of 500 mg.l^{-1} potassium chromate (135 mg Cr(VI).l^{-1}) neither affected these parameters in mature mice and young rats. The numbers of animals used in these experiments are not given (Gross and Heller, 1946).

In preliminary experiments preceeding a lifetime experiment, "subchronic" exposure by inhalation (duration not specified), mice were exposed to 30 $mg.m^{-3}$ $CaCrO_4$ dust (10 mg $Cr(VI).m^{-3}$), 5 hours per day and 5 days per week. This exposure resulted in early death, rapid weight loss, fatty liver, and distended and atrophic intestines (Nettesheim et al., 1971).

Humans

Ingestion of chromates may result in a variety of effects, for example gastrointestinal disorders (cramps, bleedings), hepatotoxic and nephrotoxic effects (diffuse liver cell necrosis, renal tubular necrosis), effects on the vascular system (hemorrhagic diathesis) and convulsions. Doses which result in severe cases of poisoning are 1-5 g "chromate". Death may occur after a clinical picture of cardiovascular shock (Langard and Norseth, 1986-R).

In workers exposed to "chromate" (mist, particulate matter) ulcerations and perforations of the nasal septum have been reported, at indoor air concentrations of about 100 to 300 μg $Cr(VI).m^{-3}$. Asthmatic attacks, an allergic reaction after sensitization due to chromate(-containing) dust or chromic acid fumes have also been reported by many authors (Langard and Norseth, 1986-R).

Summary and conclusions "short-term exposure"

Acute oral LD50 values (rats) are ranging from 20 to 249 mg $Cr(VI).kg^{-1}$ bw and from 183 to 617 mg $Cr(III).kg^{-1}$ bw, based on tests with (di)chromates and chromic compounds, respectively. Based on these LD50 values and experiments in which chromium compounds were administered parenterally, Cr(VI) usually is more toxic than Cr(III). The two available subacute/semichronic oral studies are very limited with respect to the numbers of animals used, the parameters studied and/or the data reported.
"Subchronic" exposure of mice by inhalation to 30 $mg.m^{-3}$ $CaCrO_4$ dust (10 mg $Cr(VI).m^{-3}$, 5 hours per day and 5 days per week, resulted in early death.
In humans ingestion of 1-5 g "chromate" result in severe toxic effects. Signs and symptoms of acute poisoning are amongst others gastrointestinal disorders, hemorrhagic diathesis, hepatotoxic and nephrotoxic effects and convulsions; death may occur after a clinical picture of cardiovascular shock.

In workers exposed to "chromate" (mist, particulate matter) ulcerations and perforations of the nasal septum may occur at concentrations of 100 to 300 μg Cr(VI).m^{-3}. Asthmatic attacks, an allergic reaction due to sensitization to Cr(VI) compounds, are also common.

1.3.2 Developmental toxicity

In a 90-d feeding study with limited numbers of rats fed a diet containing 2% or 5% insoluble, non-hydrated chromic chloride pigment, females were paired with males from the same dosage group 60 days after the start of exposure. Neither effects on reproduction parameters (fertility, gestation period, litter sizes) nor malformations or other adverse effects in the young were found (Ivankovic and Preussmann, 1975; see also 1.3.3).

The effects of chromium on reproduction and teratogenicity were also studied in an inhalation test (rats) in which whole-body exposures to sodium dichromate aerosols (0.2 mg Cr.m^{-3}) were performed with an exposure time of 130 days per generation. Neither an increase in fetal chromium content nor reproduction and teratogenic effects were reported. However, effects on the immune system, hyperplasia in organs (especially in the lungs) and changes in haematological parameters were reported (Glaser et al., 1984 [not available]; cited in WHO, 1988-R).

Other studies in which developmental parameters were studied after oral exposure or exposure by inhalation are not available.

In specific teratogenicity studies with pregnant hamsters and mice, one or more of the following fetal effects were found after parenteral administration (intravenous, intraperitoneal, or subcutaneous) of chromium: increased fetal death, fetal growth retardation, external malformations (especially cleft palate) and internal malformations (especially hydrocephaly and skeletal defects). In these studies Cr(VI) and Cr(III) were administered as chromium trioxide and chromic chloride, respectively. In hamsters an increased incidence of cleft palate was seen in some strains, while no effects were reported in other strains (using the same test). In most studies these developmental effects were found to be associated with maternal toxicity (in hamsters: body weight loss and tubular necrosis of kidneys; in mice: body weight loss). However, maternal effects are not reported in all studies in which developmental effects were found. For an extensive review of the parenteral teratogenicity studies the reader is referred to U.S. EPA (1984) and WHO (1988).

Summary and conclusions "developmental toxicity"

The available data on oral exposure and exposure by inhalation are very limited and, therefore, do not allow an evaluation with respect to developmental effects after these routes of exposure.
After parenteral administration of either Cr(VI) or Cr(III) to pregnant hamsters and mice in specific teratogenicity studies, developmental effects (including malformations) have been reported. These effects appear to be associated with maternal toxicity, but definitive conclusions with respect to an association between maternal and fetal effects can not be made.

1.3.3. Long-term exposure (semichronic and chronic toxicity - noncarcinogenic and carcinogenic effects)

Animals

Oral exposure - Cr(III)

In a 90-d feeding study 3-months old inbred BD rats were fed a diet containing 0%, 2% or 5% insoluble, non-hydrated chromic oxide pigment (Cr_2O_3), 5 days per week. The number of animals per group was 12 to 19 (control: 6 males and 6 females; 2%: 14 males and 5 females; 5%: 5 males and 10 females). Parameters studied were survival, feed intake, body and organ weights, blood analyses, reproductive and developmental parameters (including teratogenicity), and macroscopic and histological changes of the major organs. According to the authors the only effect was a dose-related reduction of liver and spleen weights, found in both sexes (Ivankovic and Preussmann, 1975). However, because of 1) the small number of animals studied and the absence of statistical data, 2) the absence of pathological effects in these and other organs, and 3) the results of a 2-yr feeding study (using the same strain of rats and dose levels, see below) in which neither macroscopic nor histoligic signs of toxicity were found, the reduction of liver and spleen weights in the 90-d study is not considered to be relevant. On the basis of total intakes given by the investigators, the dose levels correspond with 560 and 1,260 mg Cr(III).kg^{-1} bw.d^{-1}, taking into account the number of males and females per group. Using a standard conversion factor of 20 (mg.kg^{-1} in feed : 20 = mg.kg^{-1} bw.d^{-1}), these levels correspond with 480 and 1,210 mg Cr(III).kg^{-1} bw.d^{-1}. The

highest dose is considered as the dose-without-effect (DWE). Urine analyses carried out during the study and at termination showed no significant differences between the treated and control animals, indicating that (very) little of the compound is actually absorbed.
In a life-time carcinogenicity study, groups of 60 male and female (3-month old inbred BD) rats were fed a diet containing 0%, 1%, 2%, or 5% insoluble, non-hydrated chromic oxide pigment (5 days per week; 600 treatment days). At the end of the treatment period, surviving animals were maintained on the control diet until they died or became moribund. Survival (average life expectancy) and body weight were not affected; data on toxicity parameters such as organ weights and blood analyses are not reported. Post-mortem findings showed neither macroscopic nor microscopic signs of treatment related toxicity in the major organs. The incidence of benign tumours (mammary fibroadenomas) was similar in all groups; no malignant tumours were found in the treatment groups (Ivankovic and Preussmann, 1975). On the basis of the total dose consumed given by the investigators, the highest dose corresponds with 1,450 mg Cr(III).kg^{-1}bw.d^{-1}. Using a standard conversion factor of 20, the highest dose (which is considered as DWE) corresponds with 1,210 mg Cr(III).kg^{-1} bw.d^{-1}.

The effects of Cr(III) have also been studied in a number of experiments in which chromium compounds were added to the drinking water.
In a 1-yr study (9 male and 12 female Sprague-Dawley albino rats/group; 5 weeks old) a concentration of 25 mg Cr(III).l^{-1} -administered as chromic chloride, $CrCl_3$- did not result in effects on feed consumption and body weight gain. Neither gross changes in appearance nor microscopic changes in blood or other tissues were observed. The only effect found was some accumulation in different tissues. (MacKenzie et al., 1958). Data on water consumption and body weight are not reported. Therefore, in accordance with U.S. EPA (1984-R), a conversion factor of 10 is used to estimate the daily chromium intake on the basis of body weight; this factor is bases on a daily water intake of 0.035 l per rat and a body weight of 350 g. This results in a dose of 2.5 mg Cr(III).kg bw.d^{-1}, which is considered as DWE.
In life-time studies with mice and rats (54/sex/dose) Schroeder and co-workers investigated the effects of a (relatively low) concentration of 5 mg Cr(III).l^{-1} drinking water, administered as chromic acetate. In these studies the animals were fed a low chromium diet containing only 0.1 mg Cr.kg^{-1} wet weight and were kept in a low metal environment. No adverse effects on survival parameters and growth were found; the results of these studies point at the essentiality of Cr(III). Based on limited autopsy data

no indication for a carcinogenic activity was found (Schroeder et al., 1963a,b, 1964, 1965). Based on estimated water intakes during adult life (given by Schroeder et al.), exposure levels were 0.51, 0.45, 0.32 and 0.46 mg Cr(III).kg^{-1} bw.d^{-1} for male mice, female mice, male rats and female rats, respectively. In a 1-yr study preceeding these life-time studies, no effect of 5 mg Cr(III).l^{-1}, also administered as chromic acetate, was found on serum and hepatic cholesterol concentrations in rats (Schroeder et al., 1962).

Oral exposure - Cr(VI)

Using the same experimental procedure as described above (see experiments of Schroeder and co-workers with Cr(III) in drinking water), the effects of 5 mg Cr(VI).l^{-1} drinking water (added as potassium chromate) were studied. Survival parameters and body weight of the mice exposed were not affected during the 18-month exposure time. According to the authors there is suggestive evidence for a carcinogenic activity of Cr(VI) in this study (Schroeder and Mitchener, 1971). However, the very limited data reported do not allow an evaluation. Water intake is not given in this study; assuming a similar intake as mice in the Cr(III)-study (about 100 ml.kg^{-1} bw.d^{-1}), the exposure level is estimated to be 0.5 mg Cr(VI).kg^{-1} bw.d^{-1}.
Concentrations up to 11 mg Cr(VI).l^{-1} drinking water (administered as potassium chromate) resulted in a dose-related increase in chromium concentration in liver, spleen, kidneys and femur of Sprague-Dawley albino rats (8/sex/dose; 5-w old) after one year of exposure, but did not affect feed and water consumption, body weight, blood parameters and histology of organs. In an additional 1-yr study (9 male and 12 female rats/group) 25 mg Cr(VI).l^{-1} (administered as potassium chromate) did not result in effects on feed consumption and body weight gain. Neither gross changes in appearance nor pathological changes in blood and other organs were observed. Besides of accumulation of chromium in different tissues, the only effect found in the latter study was a 20% decrease in water intake (MacKenzie et al., 1958). Data on water consumption and body weight are not reported. Therefore, in accordance with U.S. EPA (1984-R), a conversion factor of 10 is used to estimate the daily chromium intake on the basis of body weight; this factor is bases on a daily water intake of 0.035 l per rat and a body weight of 350 g. This results in a dose of 2.5 mg Cr(VI).kg bw.d^{-1}, which is considered as DWE.
In a 29-month three generation test with NMRI mice, a drinking water concentration of 500 mg K_2CrO_4.l^{-1} (135 mg Cr(VI).l^{-1}, corresponding with 9

mg $Cr(VI).kg^{-1}$ $bw.d^{-1}$, on the basis of the water consumption measured in this study) had little or no effect on survival and growth. The study was negative with regard to carcinogenicity. No data on other parameters, for example reproduction, were reported (Borneff et al., 1968).

In a 4-yr study with different species of dogs (females, 2/dose) concentrations ranging from 0.45 to 11 mg $Cr(VI).l^{-1}$ drinking water, administered as potassium chromate, did not result in dose-related effects on feed intake, physical condition, growth rate, weights of liver, kidneys and spleen, urine and blood parameters or macroscopic appearance. Increased levels in (especially) liver and spleen were found, but these levels were largely independent of the doses (Anwar et al., 1961). Data on water consumption of the different species of dogs used in this study are not available. Therefore, a DWE on the basis of body weight can not be calculated.

Other routes of exposure - Cr(VI) and Cr(III)

Based on the occurence of increased mortality due to lung cancer -bronchogenic carcinomas- in workers exposed to chromium compounds (see below under "humans"), a variety of chromium compounds, especially Cr(VI) has been tested for carcinogenic activity in animal studies. An extensive review of these studies can be found in IARC (1980). Additional evaluations of the carcinogenicity (and mutagenicity) of chromium and chromium compound have been made by other scientific committees (NIOSH, 1975; U.S. EPA, 1984; WGD, 1985). In this section only the few studies in which animals were exposed by inhalation are discussed in detail; the results of the other studies are summarized briefly.

Exposure by inhalation

In a life-time inhalation study by Baetjer et al. (1959a), groups of mice (three different strains, having a high, moderately high and very low incidence of spontaneous lung tumors, respectively; the number of animals per group was ranging from 10 to 127) and one group of rats (about 100 animals) were exposed to a mixed chromium dust 4 hours per day, 5 days per week, untill they died or were killed. The mixed chromium dust (MMD 0.8 μm; 95% < 5 μm) contained about 12% chromium, of which at least half was Cr(VI), mostly water-soluble. Exposure concentrations were between 1 and 2 $mg.m^{-3}$ (expressed as CrO_3, corresponding with 0.5-1.0 mg $Cr(VI).m^{-3}$) for

mice and between 2 and 3 mg.m^{-3} (expressed as CrO_3, corresponding with 1-1.5 mg Cr(VI).m^{-3}) for rats. The chromium dust and chromium concentrations resembled those found in (old) chromate producing plants. Exposure times for mice ranged from 16 to 58 weeks. In mice exposure resulted in a significantly increased mortality in 3 out of 9 groups exposed and to a significantly higher incidence of non-carcinogenic abnormalities such as pneumonia. In rats survival and the incidence of non-carcinogenic abnormalities was not affected by treatment. The results on the whole with regard to carcinogenicity in mice and rats was negative: no differences were found with regard to tumour induction between treatment groups and their respective control groups (Baetjer et al., 1959a).

In an additional life-time inhalation study groups of rats, rabbits and guinea pigs were exposed -four to five hours per day, four days per week during their lifespan or untill signs of illness appeared- to the mixed chromium dust (rats) or to this dust in combination with aerosols of potassium dichromate, sodium chromate and "pulverized residue dust" of the mixed chromate dust (rabbits, guinea pigs). The average concentration in the air varied from 3 to 4 mg.m^{-3} (expressed as CrO_3, corresponding with 1.5-2.0 mg Cr(VI).m^{-3}). The result of this study was also negative with regard to carcinogenicity. Non-carcinogenic effects observed in one or more species (with a higher incidence in exposed animals) were perforation of the nasal septum, granulomata, multinucleated giant cells, abscesses and alveolar and interstitial inflammations (Steffee and Baetjer, 1965).

In two life-time inhalation studies with C57BL/6 mice (8-weeks old, 136/sex/group), the chronic effects of 13 mg.m^{-3} $CaCrO_4$ dust (particle size < 1 μm; 4.3 mg Cr(VI).m^{-3}) and 25 mg.m^{-3} Cr_2O_3 dust (average particle size 0.9 μm; 17 mg Cr(III).m^{-3}) were studied, respectively. The animals were exposed 5 and 5.5 hours per day, respectively, 5 days per week. Non-carcinogenic effects found at exposure to Cr(VI): higher mortality rate after 90 weeks, reduced growth, and histopathological changes in lungs and other organs. The tumour incidence of alveolar adenomas and adenocarcinomas after exposure to Cr(VI) was 6/136 (treated males), 3/136 (control males), 8/136 (treated females) and 2/136 (control females). A statistical analysis of these data is lacking. [Other groups of animals were pretreated with whole-body X radiation and/or influenza virus; addition of the tumours found in these groups to those found in the former groups results in a tumour incidence of 45/1,090 in Cr(VI) treated animals, which is significantly higher than the control incidence of 24/1,090, at $p < 0.05$]. (Nettesheim, 1971). In this study exposure to Cr(III) had no discernible effect on lung tumour incidence. Cr_2O_3 dust was very evenly distributed

throughout the lungs (conducting airways, alveoli). No fibrosis was seen (Nettesheim et al., 1970a,b).

Inhalation of calcium chromate by rats and hamsters resulted in one squamous-cell carcinoma of the lungs in either species; the number of animals is not reported (Laskin, 1972 [n.a.];cited in IARC, 1980-R).

Exposure by implantation and injection techniques

In a large number of experiments the carcinogenicity of chromium and chromium compounds has been investigated using injection and implantation techniques: 1) intratracheal injections or instillations 2) bronchial, intrapleural or intramuscular implantations, and 3) subcutaneous, intramuscular, intraperitoneal or intravenous injections. In these experiments local malignant tumours (especially sarcomas and carcinomas) were found after administration of a number of Cr(VI) compounds, especially calcium chromate which induced tumours in a variety of tests using different routes of administration. Incidentially, tumours were found after administration of chromium powder or Cr(III) compounds, but the tumour incidences usually were very low and in a number of these experiments the tumour incidence in the control group is not reported (IARC, 1980-R).

Humans

In a number of epidemiological studies an association has been found between exposure of workers to chromium compounds, especially in chromate producing plants, and mortality due to lung cancer (bronchogenic carcinomas). In workers exposed to [Cr(VI)] compounds also some clastogenic effects have been reported, see 1.3.4 (e.g. Baetjer et al., 1959a; Nettesheim et al., 1971; IARC, 1980-R). According to IARC the available epidemiological data do not permit a clear distinction between the relative carcinogenicity of chromium compounds of different oxidation states or solubilities, but it appears that exposure to a mixture of Cr(VI) compounds of different solubilities (as found in the chromate production industry) results in the highest risk to humans.

A number of epidemiological studies which include data on exposure levels, has been described and evaluated by two different scientific committees (U.S. EPA, 1984-R; WHO, 1987-R). On the basis of these studies these committees have calculated the carcinogenic potency of hexavalent-chromium. Calculations have been based on measured or estimated Cr(VI) levels in the air, because this valence state is considered as the most probable causative agent with regard to carcinogenicity (see also 1.3.4:

"genotoxicity"). The carcinogenic potency of Cr(VI) has been expressed as the "unit risk" (UR) which is defined as "the additional lifetime cancer risk occuring in a hypothetical population in which all individuals are exposed continuously from birth throughout their lifetimes to a concentration of 1 $\mu g.m^{-3}$ of the agent in the air they breathe (WHO, 1987). The results of these calculations are summarized in table 1.1.
On the basis of three different studies, the World Health Organization considered a UR of 40×10^{-3}, the geometric mean of the risk estimates of the three studies referred to, to be the "best estimate" UR (WHO, 1987).
One out of four studies reviewed, that by *Mancuso et al. (1975)* has been considered most suitable for estimating cancer risk by the Carcinogen Assessment Group of the U.S. Environmental Protection Agency, resulting in a "best estimate" UR of 12×10^{-3}. The other three studies reviewed by U.S. EPA were judged to have certain shortcomings (e.g. underestimation of ambient chromium concentrations, concurrent exposure to asbestos, ill-defined cohorts) which tend to overestimate the risk (U.S. EPA, 1984).

Summary and conclusions "long-term exposure"

Most long-term oral studies are considered to be inadequate for evaluation of toxicity, because of the parameters studied, the concentration(s) used, the numbers of animals used and/or the data reported.
In two oral studies with Cr(III), inbred BD rats were fed a diet containing up to 5% insoluble, non-hydrated chromic oxide pigment for 90-days and life-time, respectively. The highest dose, corresponding with 1,210 mg $Cr(III).kg^{-1}$ $bw.d^{-1}$ is considered to be the dose-without-effect (DWE). Other long-term feeding studies with Cr(III) are not available.
In a 1-year study in which rats were exposed to chromic chloride in their drinking water (25 mg $Cr(III).l^{-1}$), no adverse effects were found. Therefore this dose, corresponding with 2.5 mg $Cr(III).kg^{-1}$ $bw.d^{-1}$, is considered as DWE. Other studies using only one low Cr(III) concentration in drinking water (resulting in dose levels ranging from 0.3 to 0.5 mg $Cr(III).kg^{-1}$ $bw.d^{-1}$) are considered to be inadequate for establishing a DWE with respect to toxicity.
In the only carcinogenicity study available (life-time feeding study with rats, exposed to chromic oxide pigment, see above) no increased induction of tumours was found. The other oral studies with Cr(III) are considered to be inadequate with respect to carcinogenicity.

Long-term feeding studies with Cr(VI) compounds are not available. In a 1-year study in which rats were exposed to potassium chromate in their drinking water (25 mg Cr(VI).l^{-1}) no adverse effects were found. Therefore this dose, corresponding with 2.5 mg Cr(VI).kg^{-1} bw.d^{-1}, is considered as DWE. Other studies in which animals were exposed to Cr(VI) in drinking water are considered inadequate to establish a DWE with respect to toxicity.
In a 29-month 3-generation study in which mice were exposed to potassium chromate in their drinking water (500 mg K_2CrO_4.l^{-1}, corresponding with 9 mg Cr(VI).kg^{-1} bw.d^{-1}), no carcinogenic activity was found. The other oral studies with Cr(VI) are considered to be inadequate with respect to carcinogenicity.

The carcinogenicity of chromium, especially with regard to lung tumours, has been studied in a number of inhalation tests and in tests using inplantation or injection techniques. Although no bronchogenic carcinomas (the type of tumour that is found in humans) could be induced in inhalation studies using different animals species and chromium compounds, local malignant tumours in lungs and other tissues of experimental animals have been induced after implantation and injection techniques, especially in rats. In the IARC-evaluation (based on all animal studies) it is concluded, that there is sufficient evidence for the carcinogenicity of calcium chromate (which is a water-soluble compound) and of a number of other Cr(VI) compounds, namely sintered calcium chromate, lead chromate, strontium chromate, zinc chromate and sintered chromium trioxide (which are relatively insoluble), in rats. Furthermore it is concluded that there is limited evidence for the carcinogenicity of lead chromate oxide and cobalt-chromium alloy, in rats. Data on other compounds tested, both Cr(VI) and Cr(III) compounds, are considered inadequate for evaluation.
In epidemiological studies an association has been found between occupational exposure to chromium compounds and mortality due to lung cancer, especially in the chromate producing industry. On the basis of these studies the IARC concluded that there is sufficient evidence of respiratory carcinogenicity in humans occupationally exposed during chromate production. Data on lung cancer risk in other chromium-associated occupations and for cancer at other sides than the lungs are considered to be insufficient. According to the IARC the epidemiological data do not allow an evaluation of the relative contributions to carcinogenic risk of metallic chromium, Cr(III) and Cr(VI) or of soluble *versus* insoluble chromium compounds.

On the basis of the available data (amongst others the evaluation by IARC) it is concluded that Cr(VI) is carcinogenic to animals and humans.
In a number of epidemiological studies data are given on exposure levels. On the basis of these studies the WHO and U.S. EPA have calculated the "unit risk" (UR) which is the additional lifetime cancer risk due to exposure to 1 μg Cr(VI).m^{-3}. The two calculations result in UR values of 40 x 10^{-3} (WHO) and 12 x 10^{-3} (U.S. EPA), respectively.

1.3.4 Genotoxicity

The genotoxicity of both Cr(III) and Cr(VI) compounds has been studied extensively, using a variety of compounds and tests. For detailed information there are many reviews available, for example by IARC (1980), U.S. EPA (1984) and WHO (1988). The data in this section mainly originated from the IARC review.
The results clearly show that Cr(VI) compounds cause mutations and allied effects such as chromosomal aberrations in a very wide range of prokaryotic and eukaryotic systems, both *in vitro* and *in vivo*. In similar tests, there is good evidence that chromium (III) compounds are not active; the few positive results in assays for chromosomal aberrations were obtained only with extremely high doses (orders of magnitude higher than effective Cr(VI) concentrations) and could be explained by nonspecific cytotoxic effects". Furthermore, contamination with Cr(VI) and a partial oxidation of Cr(III) to Cr(VI) can not be ruled out.
The mutagenic activity of Cr(VI) is decreased or abolished by chemical and biological reducing agents, for example by rat liver microsomal fraction and human gastric juice (but not by the microsomal fractions of rat lung or muscle). Inactive Cr(III) compounds are converted to mutagens only by treatment with strong oxidizing agents and not by biological systems.
The difference in genotoxic action between Cr(III) and Cr(VI) is ascribed to differences in physicochemical properties. Cr(VI) readily penetrates through cellular membranes, while Cr(III) does not. Once transported into the cells, Cr(VI) is reduced to Cr(III) by tissue components. Cr(III), or intermediates such as Cr(V) which are formed during the reduction of Cr(VI), may subsequently form ligands with RNA and DNA, which results in physicochemical changes. So, Cr(III) and/or intermediates are believed to be the genetically active compounds. The validity of this mechanism is supported by the fact that Cr(VI) is active in intact cell systems but inactive in simplified systems such as isolated nuclei and purified DNA,

while Cr(III) is active in simplified systems but not in intact cell systems. For more details on the mechanism of chromium genotoxicity the reader is referred to Bianchi and Levis (1984).
In some studies increased incidences of chromosomal aberrations in peripheral blood cells, sister-chromatid exchange in lymphocytes or intermediate stage lesions in sputum (dystipical adenomatous proliferation, squamous- cell and basal-cell dysplasia) were found in workers exposed to [Cr(VI)] compounds.

Summary and conclusions "genotoxicity"

Cr(VI) compounds cause mutations and allied effects in a wide range of prokaryotic and eukaryotic systems, both *in vitro* and *in vivo*, while Cr(III) compounds are not active in similar tests, or only at extremely high (cytotoxic) concentrations. Therefore it is concluded that Cr(VI) is mutagenic and Cr(III) is not mutagenic. The mutagenic activity of Cr(VI) is decreased or abolished by reducing agents such as human gastric juice and rat liver microsomal fraction. The difference between the mutagenic action of Cr(VI) and Cr(III) can be explained by differences in physicochemical properties.

Table 1.1 Lifetime "unit risk" (UR) with regard to lung cancer mortality, at exposure to Cr(VI) in air

Average lifetime exposure ($\mu g\ Cr(VI).m^{-3}$)	Background lifetime risk	Relative risk	Unit risk	Concentration at which the additional lifetime cancer risk is 1×10^{-6} ($\mu g\ Cr(VI).m^{-3}$)	Reference	
E	Po	R	UR*	C**		
2.0 (cohort-1)	0.04	1.75	15×10^{-3}	67×10^{-6}	WHO, 1987	[1]
11.4 (cohort-2)	0.04	3.04	7×10^{-3}	143×10^{-6}	WHO, 1987	[1]
		Arithmetic mean:	**11×10^{-3}**	91×10^{-6}		
[A] 6.9	0.04	8.5	**43×10^{-3}**	23×10^{-6}	WHO, 1987	[2]
[B] 0.1 - 14.0	0.036	8.5	133×10^{-3}	8×10^{-6}	U.S. EPA, 1984	[2]
15.5	0.036	7.2	14×10^{-3}	71×10^{-6}	U.S. EPA, 1984	[3]
			$\underline{12 \times 10^{-3}}$	83×10^{-6}		
2.7	0.036	3.7	36×10^{-3}	27×10^{-6}	U.S. EPA, 1984	[4]
1.1 - 3.8	0.036	6.6	98×10^{-3}	10×10^{-6}	U.S EPA, 1984	[5]
11.0	0.04	38.0	**135×10^{-3}**	7×10^{-6}	WHO, 1987	[6]
WHO (1987)-"best estimate" UR:			40×10^{-3} -->	25×10^{-6}		[7]
U.S. EPA (1984)-"best estimate" UR:			12×10^{-3} -->	83×10^{-6}		[8]

* Calculations of UR made by WHO and U.S. EPA, based on E-, Po- and R-values given by these organizations. With exception of the value of $\underline{12 \times 10^{-3}}$, UR-values have been calculated using UR = Po (R-1) / E; the value of 12×10^{-3} has been calculated by U.S. EPA using a different method.

** Theoretical concentration at which the additional lifetime cancer risk is 1×10^{-6}; this concentration has been calculated by the authors of the present Appendix using the formula: $\frac{(1 \times 10^{-6})}{UR} \times 1\ \mu g.m^{-3}$.

(1×10^{-6} is the additional lifetime cancer risk which is generally considered to be acceptable.

[1] Based on a study originally performed by Hayes et al. (1979); cumulative exposure data for two different cohorts by Braver et al., 1955.

[2] Based on a study by Langard et al. (1980).
[A]: Average lifetime exposure calculated on the basis of the geometric mean of the exposure conc.
[B]: On the basis of the lowest and highest exposure concentration, two different values for UR have been calculated; the geometric mean of these two UR-values (133×10^{-3}) is considered as the "best estimate" of the UR

[3] Based on a study by Mancuso et al. (1975); the UR value represents the "best estimate" of the carcinogenic potency of hexavalent chromium.

[4] Based on a study by Axelsson et al. (1980); the relative risk in this study was 2, which is not statistically significant; therefore, U.S. EPA used the 95% upper limit of the relative risk.

[5] Based on a study by Pokrovskaya et al. (1973); the UR is the geometric mean of the UR values, calculated on the basis of minimum and maximum exposure concentration, respectively.

[6] Based on a study by Langard & Norseth (1975) and Langard & Vigander (1983); the result of this study is based on a relatively small working population.

[7] The "best estimate" UR (40×10^{-3}) is calculated by WHO as the geometric mean of the 3 UR-values printed **bold: 11×10^{-3}, 43×10^{-3}** and **135×10^{-3}**.

[8] The "best estimate" UR (12×10^{-3}) is calculated by U.S. EPA on the basis of the Mancuso et al. (1975) study, see [3]

2 ECOTOXICITY - I: AQUATIC ORGANISMS

2.1 ACCUMULATION AND FOOD CHAIN TRANSFER

The chromium concentration in algae has been found to increase with increasing concentrations in the water. For example, at exposure of natural freshwater populations to concentrations ranging from 0.01 to 0.4 mg $Cr(VI).l^{-1}$ (test substance: potassium dichromate) for 2-4 weeks, concentrations in algae ranged from 100-120 to 900-3,350 $mg.kg^{-1}$ at the lowest and highest concentration, respectively. The resulting bioconcentration factors ($BCF = C_{organism} : C_{water}$) were ranging from 2,300 to 29,000; the BCFs appeared to decrease with increasing concentration in the water, but the results were not consistent (Patrick et al., 1975). In two long-term experiments (exposure time ± 1 month) using experimental freshwater microcosms consisting of diatoms, green algae and blue-green algae, BCFs of 10,000 and 29,000 were observed at 50 μg $Cr.l^{-1}$; chromium was added to the water as Cr(VI). In both experiments BCFs decreased with increasing concentration in the water (Patrick, 1978). It is not reported whether the concentrations in algae and the BCFs are based on wet weight or on dry weight (most probably these data are based on dry weight). At three locations in the river Rhine, chromium concentrations in mixed populations of algae were 0.49, 0.71 and 26 $mg.kg^{-1}$ dry weight at concentrations in the water of 0.0018, 0.035 and 0.12 $mg.l^{-1}$, respectively; the resulting BCFs were 270, 20 and 215, respectively, on the basis of dry weight (Vogt and Kittelberger, 1977). Studies by Stary and co-workers on the accumulation of chromium and other metals in freshwater algae suggest that the accumulation of metals is predominantly a physico-chemical process on the surface of the cells and that concentration factors for living and dead cells are similar (Stary and Kratzer, 1982).

According to the review by Moore and Ramamoorthy (1984), chromium is readily transferred through food chains to invertebrates. However, concentrations measured in invertebrates collected from polluted waters are in general not much higher (up to about 25 $mg.kg^{-1}$ <u>dry weight</u> than those measured in unpolluted waters (≤ 5 $mg.kg^{-1}$ <u>dry weight</u>). This range of concentrations has been reported in both freshwater organisms and in marine and estuarine organisms. Mean concentrations in tissues (muscle, soft parts) of both freshwater and marine invertebrates on the basis of <u>wet weight</u> have been reported to range from about 0.01 to 10 $mg.kg^{-1}$. In some accumulation studies with marine bivalve molluscs, BCFs in the range of 125 to 200 have been measured for both Cr(III) and Cr(VI); a similar BCF has

been found with a marine polychaete worm exposed to Cr(VI) (Moore and Ramamoorthy, 1984). In a laboratory study, exposure of two species of bivalve molluscs, *Mytilus edulis* and *Mya arenaria* to 1 mg Cr(III).l^{-1} in natural sea water resulted in whole-body (soft tissue) chromium concentrations of 430 and 765 mg.kg^{-1} dry weight, respectively, which are much higher than those above-mentioned (Capuzzo and Sasner, 1977).

In a 20-day field experiment on the fate of chromium (added to the inflowing water at high tides; the nominal chromium concentration in the water column ranged from 80 to 250 $\mu g.l^{-1}$) in an intertidal mesocosm, 0.02% of the chromium added to the system was fixed by the benthic fauna and 3.4% by the sediment. The remaining 96.6% was discharged into the environment with the outflowing water. The enclosure used in this study represents an open system with free exchange of sea water and seston, but with a very limited exchange of sediment and fauna (Schulz-Baldes et al., 1983).

Concentrations in both marine and freshwater species of fish collected from the environment generally are lower than those in invertebrates; mean concentrations in fish muscle have been reported to range from < 0.1 to about 1.5 mg.kg^{-1} wet weight, with maximum values up to about 5 mg.kg^{-1} wet weight. Because of muscle tissue makes up the bulk of the body burden of fish, whole-body concentrations are largely determined by concentrations in muscles. Levels in internal organs of fish may be higher than those in muscles, but are not consistently higher. Chromium levels in omnivorous and carnivorous species of fish were found to be similar, which indicates that chromium levels are not dependent on feeding habits (Mance et al., 1984-R; Moore and Ramamoorthy, 1984-R). Rainbow trout kept for two years in lake water polluted with chromates from cooling towers (concentration between 2 and 10 μg Cr(VI).l^{-1}) had whole-body chromium concentrations of 0.18 mg.kg^{-1}), corresponding with a BCF between 18 and 90 (Mance et al., 1984-R). It is not reported whether these data are based on wet weight or on dry weight.

2.2 TOXICITY

Introduction

In this section a distinction has been made between freshwater organisms and marine organisms (which included seawater and estuarine organisms) and between "short-term" and "long-term" exposure. In general, short-term exposure covers data on experiments with exposure times up to 96 hours; the

most relevant endpoint of "acute toxicity" studied in these experiments is mortality. Long-term exposure preferably covers experiments in which organisms are exposed lifetime or during a significant part of their lifetimes; the most relevant endpoints of "chronic toxicity" studied in these experiments are mortality and effects on reproduction and growth. Some organisms, for example algae, do have very short lifetimes; for this reason the section on long-term exposure includes experiments in which this kind of organisms is exposed for 2-4 days, because this exposure time covers one or more generations of these organisms.
The vast amount of data on chromium includes relatively many data on toxicity after long-term exposure, especially on toxicity of Cr(VI) in fresh water. Because these data -on chronic toxicity- are used preferably to establish an acceptable concentration ("limit value") in the water, this section has been focused on long-term toxicity. Data on toxicity after short-term exposure are summarized briefly on the basis of reviews. For more detailed information the reader is referred to these reviews, for example (U.S. EPA, 1980, 1983; 1986; EIFAC, 1983; Mance, 1984).
Unless otherwise noted, the concentrations reported herein are <u>total</u>-chromium concentrations ("dissolved"- plus "particulate"-chromium), expressed as chromium (μg $Cr.l^{-1}$ or mg $Cr.l^{-1}$), not as the chemical tested.

2.2.1 <u>Freshwater organisms</u>

<u>Short-term exposure</u>

Acute toxicity values (48-96 hr LC50- and EC50-values) for most invertebrates studied range from 2 to 60 $mg.l^{-1}$; these data are based on "single species" tests with both Cr(VI) and Cr(III) compounds. Many values for invertebrates are lower than those for fish species (see below). For some species of invertebrates, especially cladocerans such as *Daphnia magna* and other crustaceans, very low 48- to 96-hr L(E)C50-values, below 0.1 mg $Cr(VI).l^{-1}$ have been found in a number of tests, especially in relatively soft water ($\pm$ 50 $mg.l^{-1}$, as $CaCO_3$). The lowest and next lowest L(E)C50 reported for Cr(VI) are 0.007 and 0.024 mg $Cr(VI).l^{-1}$, respectively, based on tests in which *D. magna* was exposed to potassium dichromate. Tests with Cr(III), added as chromic sulphate and chromic nitrate, resulted in acute L(E)C50-values of 0.006 and 16.8-58.7 mg $Cr(III).l^{-1}$, respectively, for *D. magna* (Mance et al., 1984-R; U.S. EPA, 1983-R, 1986-R).

In a comparative study in which the acute toxicity of potassium dichromate to *D. magna* was investigated (a total of 129 tests, conducted in 47 laboratories), a mean 24-hr EC50 of 1.5 $mg.l^{-1}$ $K_2Cr_2O_7$ (0.5 mg $Cr(VI).l^{-1}$) was found; the range of values is not reported (Adema et al., 1981). A total of 40 tests (each test in duplicate) with potassium dichromate, conducted in two laboratories in The Netherlands, resulted in 48-hr LC50-values for *D. magna* ranging from 0.7 to 1.9 $mg.l^{-1}$ (0.24 to 0.66 mg $Cr(VI).l^{-1}$) (Kooijman, 1978).

In "single species" tests with hexavalent chromium, using 8 different species of fish, 96-hr LC50-values were found to range from 3.4 to 170 mg $Cr(VI).l^{-1}$; in tests with trivalent chromium, using about 15 different species of fish, 96-hr LC50 values ranged from 3.3 to 76 mg $Cr(III).l^{-1}$. The results were found to be dependent on both biotic factors (size) and abiotic factors (hardness, temperature, pH) (Mance et al., 1984-R). An example of the influence of the pH is reported by Hogendoorn-Roozemond et al. (1978): a 50- to 200-fold decrease in toxicity (48- to 120-hr LC50) of hexavalent chromium, added as sodium chromate, was observed in tests with rainbow trout *Salmo gairdneri* when the pH decreased from 7.8-8.0 (mean 7.9) to 6.4-7.2 (mean 6.8). LC50-values ranged from 28 to 58 mg $Cr(VI).l^{-1}$ at high pH and from 0.22 to 0.57 at low pH. In an ISO-test program in which the acute toxicity of potassium dichromate to the fish *Brachydanio rerio* was investigated in about 20 laboratories, 96-hr LC50-values were ranging from 51 to 174 mg $K_2Cr_2O_7.l^{-1}$ (18 to 61 mg $Cr(VI).l^{-1}$)(Adema et al., 1981).

In a comparative study the toxicity of two hexavalent chromium salts, namely potassium chromate and potassium dichromate, to six species including both freshwater (*Daphnia pulex, Pimephales promelas, Gasterosteus aculeatus, Lepomis macrochirus*) and marine organisms (*Mysidopsis bahia, Cyprinodon variegatus*) was investigated. For each single species the acute toxicity values (invertebrates: 48-hr L(E)C50; fish: 96-hr LC50) for both salts, expressed as chromium, were equal or very similar (within 35%) (Jop et al., 1987). In a number of tests to establish the suitability of hexavalent chromium (added as potassium chromate) as a reference toxicant, *D. magna* was found to be the most sensitive species: the mean 48-hr L(E)C50 of a total of 19 tests with this species was 0.13 mg $Cr(VI).l^{-1}$ (range 0.07 to 0.19). The next lowest 48-hr L(E)C50 (mean: 5.1 mg $Cr(VI).l^{-1}$; range 4.2 to 7.1; n = 8) was found for the other crustacean tested, *M. bahia*. The four species of fish were less sensitive, with mean 96-hr LC50--values ranging from 23.2 to 192 mg $Cr(VI).l^{-1}$ (Jop et al., 1986).

Long-term exposure

The results of long-term "single species" tests with freshwater organisms, mainly resulting in NOEC-values, are summarized in table 2.1 [tests with Cr(VI)] and table 2.2 [tests with Cr(III)].

Additional data long-term exposure

In a comparative study by Slooff and Canton (1983) the 7-hr NOEC (112 μg Cr (VI).1^{-1}) with regard to the specific growth rate of the bacterium *Pseudomonas fluorescens* was similar to long-term NOEC-values for relatively sensitive organisms. The results for the other species studied, have been published earlier (Adema et al., 1981; see table 2.1). Efficiency of bacterial denitrification in anaerobic "packed bed reactors" was reduced to 95% at concentrations in the influent ranging from 1 to 8 mg Cr (VI).1^{-1} (added as potassium dichromate), depending on carbon source; exposure times were ranging from 11-21 weeks. The efficiency deteriorated with increasing chromium concentrations (Lewandowski, 1985).

In an ISO-test program the toxicity (parameter: growth) of potassium dichromate to 6 different species of unicellular green algae was investigated by different laboratories which were allowed to use their common test method. The resulting EC50-values (using exposure times ranging from 4 to 18 days) ranged from 0.043 to 24 mg $K_2Cr_2O_7$ .1^{-1} (0.015 to 8.4 mg Cr.1^{-1}), showing highly variable results due to species differences and differences in methods. The highest variation in results for one species was found for *Selenastrum capricornutum:* EC50-values ranged from 0.05 to 24 mg $K_2Cr_2O_7$.1^{-1} (0.0175 to 8.4 mg Cr(VI).1^{-1} (Hanstveit, 1980). In a comparative study in The Netherlands using 8 species of unicellular algae (green algae, green flagellates, cyanobacteria and diatoms), 4- to 7-d EC50-values ranged from 0.003 to 6.8 mg $K_2Cr_2O_7$.1^{-1} (0.001 to 2.4 mg Cr(VI).1^{-1}. The lowest value was found for the diatom *Stephanodiscus hantzschii*, a planktonic species that is common in hard and eutrophic waters. The next lowest value was 230 times higher than the lowest one, so the difference in sensitivity between 7 out of 8 species was within a factor of 10 (Hanstveit et al., 1985).

In intermittent-flow experiments with exponentially growing *D. magna* populations consisting of cohorts of various ages, an EC50- and EC10-value of 640 and 500 μg Cr (VI).1^{-1} (actual concentrations) with respect to yield were found at exposure to potassium dichromate in lake water (hardness 225 mg.1^{-1}, as $CaCO_3$; pH 8.1). A no-observed-effect-concentration could not be established (Van Leeuwen et al., 1987).

The lowest Cr(VI) concentration reported to cause mortality in fish is 80 $\mu g.l^{-1}$, in studies using chinook salmon *Oncorhynchus ishawytscha* and rainbow trout *Salmo gairdneri* (Olson and Foster, 1956, 1957 and Olson, 1958; cited in Van der Putte et al., 1981). A concentration of 20 μg Cr(VI).l^{-1} has been reported to affect growth of early life stages (embryos through 4-month old juveniles) of these species. However, the difference with control fish was only 6% (Olson and Foster, 1956, cited in Benoit, 1976). According to Pickering (1980), reviewing the same study, growth was already affected at 13 μg Cr(VI).l^{-1}. Exposure of chinook salmon during 12 weeks to a concentration of 200 μg Cr(III).l^{-1} had no adverse effects on growth and survival, while exposure to 200 μg Cr(VI).l^{-1} caused 50% mortality and a reduction in weight of 40% (Olson, 1958; cited in Mance, 1984).

Exposure of adult fish *Barbus conchonius* to potassium dichromate (690 and 1,030 μg Cr (VI).l^{-1} for 30 and 60 days, respectively) resulted in hematological effects and in pathological effects in gills, kidneys and liver. Lower concentrations were not included in the test. Data on other parameters are not reported (Gill and Pant, 1987).

(Micro-)ecosystem studies

Exposure of natural phytoplankton populations (green and blue-green algae, diatoms, and other species of algae) for up to 23 days to potassium chromate (100 or 1,000 μg Cr(VI).l^{-1}) showed that growth of a number of species is stimulated by these concentrations (especially at 100 $\mu g.l^{-1}$), while that of other species is delayed or inhibited (especially at 1,000 $\mu g.l^{-1}$), resulting in changes in the community structure (Nosov et al., 1981).

Exposure of natural diatom-dominated algal communities to < 10 (control), 50, 100 or 400 μg Cr(VI).l^{-1} (added as potassium dichromate) in streamwater for 2-4 weeks resulted in a slight reduction of diatom abundance and diversity at 100 $\mu g.l^{-1}$, although the population was still dominated by these algae. At 400 $\mu g.l^{-1}$ the diatoms were almost completely replaced by other algae: blue-green algae and, to a lesser extent, green algae. This shift towards algal species which have less predator pressure may result in nuisance growths (Patrick et al., 1975; Patrick, 1978).

In a stream receiving waste waters from galvanic industries specialized in chromium plating, the structure of macrobenthic insect communities was clearly influenced at sites downstream the inflow site, resulting in a decreased diversity and decreased numbers of insects. Mean measured

dissolved Cr(VI) concentrations at the polluted sites were 350, 160 and 190 $\mu g.l^{-1}$ during the one year study period, with a range of 25 to 2,100. Total chromium concentrations were 15 to 100% higher than the Cr(VI) concentrations (Cotta Ramusino et al., 1983).

2.2.2 Marine organisms

Short-term exposure

For 18 different animal species, both invertebrates and vertebrates, 96-hr L(E)C50-values based on "single species" tests are ranging from 2 to 105 mg $Cr(VI).l^{-1}$. The lowest values (up to 7.5 mg $Cr(VI).l^{-1}$ are reported for polychaete worms and crustaceans. For Cr(III) only three 96-hr L(E)C50-values are available, namely 10, 31 and 56 mg $Cr(III).l^{-1}$. The value of 31 mg $Cr(III).l^{-1}$ for mummichog (fish) is similar to that of 91 mg $Cr(VI).l^{-1}$ for hexavalent chromium (U.S. EPA, 1983-R, 1984-R; Mance, 1984-R).

Long-term exposure

The results of long-term "single species" tests with marine organisms are summarized in table 2.3 [Cr(VI)] and table 2.4 [Cr(III)].

Additional data long-term exposure

Exposure of alga *Macrocystis pyrifera* to potassium dichromate for 4-5 days (1 and 5 mg $Cr(VI).l^{-1}$) resulted in inhibition of photosynthesis (U.S EPA, 1983-R). Exposure of two species of filter-feeding bivalve molluscs *Mytilus edulis* and *Mya arenaria* to 1 mg $Cr(III).l^{-1}$ natural sea water significantly reduced the filtration rate (Capuzzo and Sasner, 1977)

(Micro-)ecosystem studies

The influence of chromium (added as sodium dichromate) on growth of estuarine phytoplankton populations, collected from their natural environment and tested outdoor in water collected from the same location, is depending on salinity (which ranged from 0.03 o/oo to 32.5 o/oo) At "high" or "medium" salinities population growth was not affected at a chromium concentration of 100 $\mu g.l^{-1}$; at 1,000 $\mu g.l^{-1}$ an apparent lag was found. At "low" salinities 100 $\mu g.l^{-1}$ either completely eliminated growth or greatly reduced the rate of growth (Frey et al., 1983).

2.2.3 Water-sediment systems

Reviews on the bioavailability of trace metals, including chromium, from sediments have been written by de Kock and Marquenie (1982) and by Luoma (1983). The data in these reviews show that the bioavailability of trace metals to aquatic organisms is dependent on hydrodynamical, geochemical and biological mechanisms. For more details the reader is referred to these reviews. The results of an additional study are reported below.

The accumulation and toxicity of chromium present in natural sediments (polluted *in situ* by tannery wastes) and in artificial sediments (either bentonite or kaolinite, treated with a $CrCl_3$ solution) was determined in long-term studies with two species of filter-feeding bivalve molluscs, either the blue mussel *Mytilus edulis* or the soft shell clam *Mya arenaria*. The animals were exposed to suspensions of these sediments in aquaria by means of a continuous flow of filtered seawater and air, supplied to the bottom of the aquaria. After exposure to natural sediments for 24 weeks (*M. edulis*), the filtration rate was significantly reduced in sediments containing 150 and 990 mg $Cr.kg^{-1}$ clay; exposure to a "control" sediment (10 mg $Cr.kg^{-1}$ clay) suspension did not result in a reduced rate (The clay content of these sediments is not reported). Exposure to the artificial sediments (bentonite containing 1,000 mg $Cr(III).kg^{-1}$ clay or kaolinite containing 1,200 mg $Cr(III).kg^{-1}$ clay) for 4-6 weeks also resulted in significantly reduced filtration rates, with exception of that of *M. arenaria* exposed to bentonite. In *M. edulis*, bentonite (relatively high cation exchange capacity) had more effect than kaolite (relatively low cation exchange capacity), although whole-body soft tissue chromium levels were lower at exposure to bentonite. In *M. arenaria* the greatest effect on both filtration rate and whole-body chromium levels were found at exposure to kaolite. Exposure to chromium in solution (1 mg $Cr(III).l^{-1}$ sea water) resulted in whole-body chromium levels that were 10-30 (*M. edulis*) and 35-110 (*M. arenaria*) times higher than those after exposure to the artificial sediment suspensions (Capuzzo and Sasner, 1977).

Summary and conclusions "aquatic organisms"

Accumulation and food chain transfer

Chromium is accumulated to a limited extend by aquatic animals. The concentrations in aquatic organisms decrease with increasing trophic level. In general, concentrations up to 10 and 5 $mg.kg^{-1}$ fresh weight have been

reported in invertebrates and vertebrates (fish), respectively. These concentrations are based on both freshwater and seawater organisms. Based on these data it is concluded that biomagnification (the occurence of a substance at successive higher concentrations with increasing trophic levels in food chains) of chromium is of no account.

Toxicity to freshwater organisms

Short-term "single species" tests with most species of invertebrates have resulted in L(E)C50-values of 2,000 to 60,000 $\mu g.l^{-1}$, based on tests with both Cr(III) and Cr(VI). Crustaceans, especially cladocerans *(Daphnia sp.)* are more sensitive, with L(E)C50-values of 10 to 500 $\mu g.l^{-1}$. In general, fish are less sensitive than invertebrates: LC50-values for fish are 3,000 to 190,000 $\mu g.l^{-1}$. The toxicity of Cr(VI) increases with decreasing pH and hardness of the water.

Long-term "single species" tests with Cr(VI) have resulted in NOEC-values of 0.35 to 6,650 $\mu g.l^{-1}$. Relatively low values have been reported for organisms of different, important taxonomic groups, namely algae, crustaceans and fish. In general, fish are less sensitive than invertebrates. Long-term tests with Cr(III) have resulted in NOEC-values of 48 to 330 $\mu g.l^{-1}$.

In experimental micro-ecosystems (algae) a concentration of 100 μg $Cr(VI).l^{-1}$ has resulted in a reduction of diatom abundance and diversity.

Toxicity to marine organisms

Short-term "single species" tests with Cr(VI) have resulted in L(E)C50-values of 2,000 to 105,000 μg $Cr(VI).l^{-1}$; these values are based on tests with both invertebrates and vertebrates. The lowest values have been reported for crustaceans and polychaete worms. L(E)C50-values based on tests with Cr(III) are 10,000 to 56,000 μg $Cr(III).l^{-1}$.

Long-term "single species" tests with Cr(VI) have resulted in NOEC-values of 13 to 770 μg $Cr(VI).l^{-1}$. The only available NOEC resulting from a test with Cr(III) is 50,400 μg $Cr(III).l^{-1}$; in this test only 20 μg $Cr(III).l^{-1}$ was found to be dissolved (< 0.1 μm).

Based on these NOEC-values and additional data it is concluded that Cr(III) is less toxic than Cr(VI) in sea water.

Table 2.1 Long-term "single species" toxicity tests with Cr(VI) compounds - freshwater organisms

Organism	A	Test-type	Test-subst.	Test-water.	pH	Hardness	Exp.-time	Crite-rion	Result µg Cr(VI).l^{-1}		Reference
Algae											
Chlorella pyrenoidosa	+	S	$K_2Cr_2O_7$	n.m.	7.7	54	4-d	$NOEC_{g[n]}$	**63**	[16]	Adema et al.,'81
Chlorella pyrenoidosa 211-8b	-	-	$K_2Cr_2O_7$	n.m.	-	--	4-d	$NOEC_{g[n,W,c]}$	100	[9]	Meisch & Schmitt-Beckmann,'79
Chlorella vulgaris 211-11b	-	"F"	$K_2Cr_2O_7$	n.m.	-	--	4-d	$EC50_{g[n]}$	160	[15]	Jouany et al.'83
Chlorella vulgaris 211-11b	-	S	$K_2Cr_2O_7$	n.m.	-	--	2-d	$EC50_{g[n]}$	264	[11]	Jouany et al.,'82
Chlorella WR 1 (wild strain)	-	-	$K_2Cr_2O_7$	n.m.	-	--	4-d	$NOEC_{g[n,W,c]}$	**100**	[9]	Meisch & Schmitt-Beckmann,'79
Euglena gracilis	+	S	$K_2Cr_2O_7$	n.m.	7.7	54	4-d	$NOEC_{g[n]}$	**35**	[16]	Adema et al.,'81
Gomphonema parvulum	+	S	$K_2Cr_2O_7$	n.m.	7.5	8	4/7-d*	$NOEC_{g[n]}$	**35**	[16]	Hanstveit et al.,'85
Microcystus aeruginosa	+	S	$K_2Cr_2O_7$	n.m.	7.8	24-54	4/8-d*	$NOEC_{g[n]}$	**196**	[16]	Adema et al.,'81
Microcystus aeroginosa	+	S	$K_2Cr_2O_7$	n.m.	7.8	24	4-d	$NOEC_{g[n]}$	112	[16]	Hanstveit et al.,'85
Microcystus aeroginosa	-	S	$K_2Cr_2O_7$	n.m.	7.7	54	4-d	$NOEC_g$	350	[18]	Slooff & Canton, '83
Oscilltoria agardhii	+	S	$K_2Cr_2O_7$	n.m.	7.8	24	4/7-d	$NOEC_{g[n]}$	**35**	[16]	Hanstveit et al.,'85
Scenedesmus pannonicus	+	S	$K_2Cr_2O_7$	n.m.	7.7	54	4/14-d*	$NOEC_{g[n]}$	**112**	[16]	Adema et al.,'81
Selenastrum capricornutum	+	S	$K_2Cr_2O_7$	n.m.	7.7	54	4-d	$NOEC_{g[n]}$	**196**	[16]	Adema et al.,'81
Stephanodiscus hantzschii	+	S	$K_2Cr_2O_7$	n.m.	7.5	8	4/7-d	$NOEC_{g[n]}$	**0.35**	[16],[20]	Adema et al., '81; Canton & Mathijsen-Spiekman,'84
Macrophytes											
Lemna gibba	+	S	Na_2CrO_4	n.m.	7-8	--	1-w	$NOEC_g$	**100**	[5]	Staves & Knaus,'85
Lemna minor "M 19"	+	S	$K_2Cr_2O_7$	n.m.	8.0	248	1-w	$NOEC_g$	**11**		Adema & De Zwart,'84
Lemna minor "M 19"	-	S	$K_2Cr_2O_7$	n.m.	-	102	1-w	$NOEC_{g[W]}$	112		Slooff & Canton,'83
Lemna paucicostata "6746"	-	S	$K_2Cr_2O_7$	n.m.	4/5	--	1-w	$NOEC_g$	500/1,000	[6a]	Nasu & Kugimoto,'81
				n.m.	6/7	--	1-w	$NOEC_g$	< 500	[6b]	
Spirodela punctata	+	S	Na_2CrO_4	n.m.	7-8	--	1-w	$NOEC_g$	**100**	[5]	Staves & Knaus,'85
Spirodela polyrhiza	+	S	Na_2CrO_4	n.m.	7-8	--	1-w	$NOEC_g$	**100**	[5]	Staves & Knaus,'85
Protozoa											
Colpidium campylum	-	S	$K_2Cr_2O_7$	art.	6.5	--	2-d	$NOEC_{g[n]}$	**3,200**	[14]	Sudo & Aiba,'73
Opercularia sp.	-	S	$K_2Cr_2O_7$	art.	6.5	--	2-d	$NOEC_{g[n]}$	**6,400**	[14]	Sudo & Aiba,'73
Vorticella microstoma	-	S	$K_2Cr_2O_7$	art.	6.5	--	2-d	$NOEC_{g[n]}$	**100**	[14]	Sudo & Aiba,'73

(to be continued)

Table 2.1 Long-term "single species" toxicity tests with Cr(VI) compounds - freshwater organisms (continued)

Organism	A	Test-type	Test-subst.	Test-water.	pH	Hardness	Exp.-time	Criterion	Result µg Cr(VI).l^{-1}		Reference
Coelenterata											
Hydra oligactis	-	R	$K_2Cr_2O_7$	DSW	8.2	208	3-w	$NOEC_{g,s}$	**1,120**		Slooff & Canton,'83
budless											
Molluscs											
Lymnaea stagnalis	-	R	$K_2Cr_2O_7$	DSW	8.2	208					Slooff & Canton,'83
5-m old							6-w	$NOEC_{r,s}$	**112**		
eggs							1-w	$NOEC_{h}$	350		
Crustaceans											
Atlanto-astacus pallipes p.	+	F	$--Cr_2O_7$	well	6.9	--	20-w	$NOEC_{h,g,s}$	**9**	α	Chaisemartin,'78
P (egg-bearing females) --> F (juveniles)											
Daphnia magna	+	R	$K_2Cr_2O_7$	DSW	8.2	208	3-w	$NOEC_{r,s}$	**35**		Adema et al.,'81
P < 1-d --> F [lc]											
Daphnia magna	-	R	$K_2Cr_2O_7$	lake	8.1	225	3-w	LC50	500		Van Leeuwen et al.'87
P < 1-d --> F [lc]							3-w	$NOEC_{r,s}$	350	[8]	
Daphnia magna	+	R	Na_2CrO_4	spring	8.3	--	4-w	$EC_{r,s}$	10	[10]	Trabalka & Gehrs,'77
P < 1-d --> F [lc]											
Insects											
Culex pipiens	-	R	$K_2Cr_2O_7$	DSW	8.2	208	± 4-w	$NOEC_{d,s}$	**1,120**		Slooff & Canton, '83
first instar larvae											
Fish											
Brachydanio rerio	+	R	$K_2Cr_2O_7$	DSW	8.2	208	≥ 4-w	$NOEC_{e\text{-}d,g,s}$	**3,500**		Canton et al.,'84
eggs --> post-hatching [els]											
Catostomus commersoni	+	F	$Na_2Cr_2O_7$	well	7.0	32-46	10-w	$NOEC_{h,g,s}$	**290**	α	Sauter et al.,'76
eggs --> 60-d post-hatching [els]											
Channa punctatus	-	-	$K_2Cr_2O_7$	tap	7.1	160	17-w	NOLC	2,600	[19]	Sastry & Sunita,'83
20 cm											
Gasterosteus aculeatus	+	R	$K_2Cr_2O_7$	DSW	8.2	208	5-w	$NOEC_{e\text{-}d,g,beh,s}$	**6,650**	α	Van den Dikkenberg et al.,'89
eggs < 6-hr --> 4-w post-hatching [els]											

(to be continued)

Table 2.1 Long-term "single species" toxicity tests with Cr(VI) compounds - freshwater organisms (continued)

Organism	A	Test-type	Test-subst.	Test-water.	pH	Hardness	Exp.-time	Crite-rion	Result $\mu g\ Cr(VI).l^{-1}$		Reference
Fish (continued)											
Ictalurus punctatus	+	F	$Na_2Cr_2O_7$	well	7.2	35-38	10-w	$NOEC_{h,g,s}$	**150**	α	Sauter et al.,'76
eggs --> 60-d post-hatching [els]											
Jordanella floridae	+	R	$K_2Cr_2O_7$	DSW	8.2	208	± 6-w	$NOEC_{e-d,g,s}$	**1,120**		Adema et al.,'81
eggs < 36-h --> 4-w post-hatching [els]											
Lepomis macrochirus	+	F	$Na_2Cr_2O_7$	well	6.9	33-45	9-w	$NOEC_{h,g,s}$	**522**	α	Sauter et al.,'76
eggs --> 60-d post-hatching [els]											
Nuria denricus	-	S	$K_2Cr_2O_7$	well	6.2	5	3-w	LC50	1,720		Abbasi & Soni,'84
adults											
Oryzias latipes	+	R	$K_2Cr_2O_7$	DSW	8.2	208	± 6-w	$NOEC_{e-d,g,s}$	**3,500**		Adema et al.,'81
eggs < 36-h --> 4-w post-hatching [els]											
Pimephales promelas	+	F	$K_2Cr_2O_7$	pond	7.7	209	16-m	$NOEC_{h,g,r,s}$	**1,000**	α [4]	Pickering,'80
P (juveniles 4-w) --> F_1 (60-d post-spawning) [lc]											
Pimephales promelas	-	F	$Na_2Cr_2O_7$	well	7.8	220	4-w	$NOEC_{g,s}$	400	[17]	Broderius & Smith,'79
1-d old larvae											
Poecilia reticulata	+	R	$K_2Cr_2O_7$	DSW	8.2	208	4-w	$NOEC_{g,s}$	**3,500**		Adema et al.,'81
3-4 w											
Rutilus rutilus	-	R	$K_2Cr_2O_7$	tap	7-8	25	4-w	$NOEC_{b,beh,s}$	**1,000**	[7]	Strik et al.,'75
Salmo gairdneri	+	F	$Na_2Cr_2O_7$	well	6.8	30-42	13-w	$NOEC_{h,g,s}$	51	α	Sauter et al.,'76
eggs --> 60-d post-hatching [els]											
Salmo gairdneri	+	F	Na_2CrO_4	tap		80					Van der Putte,'82
eggs (late eyed stage) --> juveniles [els]					7.8		32-w	$NOEC_{h,g,s}$	200	(α) [1]	
					6.5		32-w	$NOEC_{h,g,s}$	20	(α) [1]	
alevins (5 w) --> juveniles					6.5/7.8		32-w	$NOEC_{g,s}$	200	(α) [1]	
yearlings (14 m)					7.8		12-w	$NOEC_{b,g,hs,s}$	2,000	(α) [1]	
					6.5		12-w	$NOEC_{b,g,hs,s}$	200	(α) [1]	
Salmo gairdneri	+	F	$Na_2Cr_2O_7$	lake	7-8	45	8-m	$NOEC_{g,s}$	**100**	α [2]	Benoit,'76
alevins (1 w) --> juveniles											
Salmo gairdneri	-	F	$K_2Cr_2O_7$		7.4	320	6-m	NOLC	≥ 200		Arillo et al.,'82
adults								$NOEC_b$	< 200		
Salvelinus fontinalis	+	F	$Na_2Cr_2O_7$	lake	7-8	45	8-m	$NOEC_{g,h,s}$	**100**	α [2]	Benoit,'76
eyed eggs --> juveniles [els]											

(to be continued)

Table 2.1 Long-term "single species" toxicity tests with Cr(VI) compounds - freshwater organisms (continued)

Organism	A	Test-type	Test-subst.	Test-water.	pH	Hardness	Exp.-time	Criterion	Result $\mu g\ Cr(VI).l^{-1}$		Reference
Fish (continued)											
Salvelinus fontinalus alevins (1 w) --> adults --> 3-m juveniles [lc]	+	F	$Na_2Cr_2O_7$	lake	7-8	45	22-m	$NOEC_{g,(r),s}$	< 350	α	[3] Benoit,'76
Salvelinus namaycush eggs --> 60-d post-hatching [els]	+	F	$Na_2Cr_2O_7$	well	6.9	31-42	16-w	$NOEC_{h,g,s}$	**105**	α	Sauter et al.,'76
Amphibians											
Xenopus laevis < 2-d	-	R	$K_2Cr_2O_7$	DSW	8.2	208	14-w	$NOEC_{d,g,s}$	**350**		Slooff & Canton,'83

b = biochemistry; beh = behaviour; d = development; e-d = egg-development; g = growth [n: number of cells; w: weight of cells; c: chlorophyll content of cells]; h = hatchability; hs = histology; r = reproduction; s = survival

lc = life cycle test; els: early life stage test (egg-larval test)

* "x"/"y": different tests; parameter either "x" or "y"

art. = artificial (reconstituted); n.m. = nutrient medium; DSW = Dutch Standard Water

[1] Actual concentrations were within 5% of nominal ones which were 20, 200 and 2,000 $\mu g.l^{-1}$.

[2] At the next highest concentration of 200 $\mu g.l^{-1}$, 8-m old rainbow and brook trout weighted 30% and 20% less than controls, respectively (no statistics reported).

[3] Temporary effect on growth during the first year of exposure; after 12-22 months of exposure, weights varied only 10%-12% from controls (no statistics reported).

[4] At all concentrations tested (range 18 to 1,000 $\mu g.l^{-1}$, weight of the first generation was reduced significantly. However, final lenghts and weights of adult fish were not different from controls at any concentration tested. Growth of the second generation was not affected at any concentration.

[5] Next lowest test concentration: 1,000 $\mu g.l^{-1}$. Nutrient medium: 10-20 g fresh manure per litre water.

[6a], [6b] Different nutrient media used.

[7] Test concentrations 100, 1,000 and 10,000 $\mu g.l^{-1}$.

[8] Mean carapace length of surviving adults sign. ($p < 0.01$) reduced at 110 $\mu g.l^{-1}$; $NOEC_g$ = 60 $\mu g.l^{-1}$.

[9] Tests were conducted either in continuous light or in synchronous light (photoperiod 16:8 light/dark).

(to be continued)

Footnotes Table 2.1 (continued)

[10] Measured concentrations were within 10% of the nominal concentration indicated. A significant ($p < 0.01$) dose-related reduction of life span was found at the lowest test concentration of 10 $\mu g.l^{-1}$; fecundity (total no. of young; no. of young/animal) was reducted also at this concentration.

[11] Photoperiod 16:8; 72-hr and 96-hr EC50 values were 346 and 442 $\mu g.l^{-1}$, respectively.

[12] Result independent of photoperiod used (either continuous or synchronous). At 500 $\mu g.l^{-1}$ all parameters were reduced ≥ 35%.

[13] Alkalinity 43 $mg.l^{-1}$, as $CaCO_3$; lowest effect concentration: 38 $\mu g.l^{-1}$.

[14] At the lowest effect concentration: reduction of specific growth rate with ≥ 20%; at times of exposure longer longer than 2 days, growth of the protozoa was no longer linear with log Concentration.

[15] Photoperiod 16:8; "F" = pseudodynamic: periodic addition of test solution without removal. In a static test the 4-d EC50 was 590 $\mu g.l^{-1}$.

[16] Continuous light; according to NEN 6506; NOEC-values did not differ more than 10% from control values.

[17] Mortality 40%, both in control and treatment groups (concentrations up to 3,000 $\mu g.l^{-1}$). No dose-related effect on growth (as mean individual dry weight) higher than 20% was found up to 400 $\mu g.l^{-1}$; growth at 800 $\mu g.l^{-1}$ and upwards was reduced ≥ 60%.

[18] Continuous light.

[19] Biochemical changes were found in several tissues, especially in liver, kidneys and gills.

[20] The diatom Stephanodiscus hantzschii is common in hard and eutrophic waters in The Netherlands.

Tests conducted by Adema et al.,'81:

Actual concentrations were within 30% of the nominal ones, both before and after renewal.

Table 2.2 Long-term "single species" toxicity tests with Cr (III) compounds - freshwater organisms

Organism	A	Test-type	Test-subst.	Test-water.	pH	Hardness	Exp.-time	Crite-rion	Result µg Cr(III).l^{-1}		Reference
Algae											
Chlorella pyrenoidosa 211-8b	-	-	Cr-glycine	n.m.	-	-	4-d	$NOEC_{g[n,w,c]}$	**100**	[1]	Meisch & Schmitt-Beckmann,'79
Chlorella WR 1 (wild strain)	-	-	Cr-glycine	n.m.	-	-	4-d	$NOEC_{g[n,w,c]}$	**100**	[1]	Meisch & Schmitt-Beckmann,'79
Crustaceans											
Daphnia magna	-	R	$CrCl_3$	lake	7.7	44-53	3-w	$NOEC_{r,g,b,s}$	≤ **330**	[2]	Biesinger & Christensen, '72
P ≤ 1-d --> F [lc]											
Daphnia magna	-	-	$Cr(NO_3)_3$	-	-		-				U.S. EPA, '83 ~
P --> F [lc]						50		NOEC	47		
P --> F [lc]						100		NOEC	129		
P --> F [lc]						200		NOEC	< 44		
Fish											
Oncorhynchus kisutch	-	-	-	-	-	-	12-w	$NOEC_{g,s}$	200	[4]	Mance, '84 ~
Pimephales promelas	-	-	$KCr(SO_4)_2$	-	-	200	-	NOEC	750		U.S. EPA, '83 ~
[lc]											
Salmo gairdneri	+	F	$Cr(NO_3)_3$	-	7.3	20-30					Stevens &
newly fertilized eggs --> 30-d post-swimup [els]								$NOEC_{g,h,s}$	**48** α	[3]	Chapman,'84
eyed eggs --> 30-d post-swimup [els]								$NOEC_{g,h,s}$	157 α	[3]	
Salmo gairdneri	-	-	$Cr(NO_3)_3$	-	-	25	-	NOEC	19		U.S. EPA, '83 ~
[els]											

b = biochemistry; g = growth [n: number of cells; w: weight of cells; c: chlorophyll content]; h = hatchability; r = reproduction; s = survival

lc = life cycle test; els: early life stage test (egg-larval test)

n.m. = nutrient medium

[1] Tests were conducted either in continuous light or in synchronous light (photoperiod 16:8 light/dark).
[2] Reproductive impairment 16% at 330 µg.l^{-1}; this represents the minimal reproducible value below which the variability in reproduction could not be detected from controls.
[3] Mean measured total chromium concentrations: control, < 5, 9, 13, 19, 30, 48, 89, 157, 271 and 495 µg.l^{-1}; dissolved (0.45 µm) chromium concentrations were ≥ 85% of total concentrations.
[4] A concentration of 200 µg.l^{-1} hexavalent chromium resultes in 50% mortality ansd a reduction of weight of 40% using the same experimental procedure.

Table 2.3 Long-term "single species" toxicity tests with Cr (VI) compounds - marine organisms

Organism	A	Test-type	Test-subst.	Test-water	Salinity o/oo	Exp.-time	Criterion	Result µg Cr(VI).l^{-1}		Reference
Algae										
Thalassiosira pseudo-	+	S	$Na_2Cr_2O_7$	n.s.w.					[4]	Frey et al.,'83
nana clone 3H					0.03	1-w	EC_g	10-100	[5]	
					0.3	1-w	$NOEC_g$	20		
					≥ 2.1	1-w	$NOEC_g$	200		
Annelida										
Capitella capitata	-	S	$K_2Cr_2O_7$	s.w.	35	5-m	**$NOEC_{r,l\text{-}d,s}$**	**50**		Reish,'75
P (trochophore larvae) --> F [lc]										
Capitella capitata	-	S	CrO_3	s.w.	--	3-w	LC50	280		Reish et al.,'76
adults										
Ctenodrilus serratus	-	S	CrO_3	s.w.	--	3-w	$NOEC_{r,s}$	< 50		Reish & Carr,'78
specimens lacking reproductive buds										
Ophrytrocha diadema	-	S	CrO_3	s.w.	--	3-w	**$NOEC_{r,s}$**	**500**		Reish & Carr,'78
mature										
Neanthes virens	-	-	--	--	--	3-w	LC50	1,000		Reish,'75 ~
(= Nereis virens)										
Neanthes arenaceodentata	-	-	--	--	--	8-w	LC50	200		Reish,'75 ~
						---	$EC50_r$	< 12		
Neanthes arenaceodentata	+	R	$K_2Cr_2O_7$	n.s.w.	33	10-m	$NOEC_r$	17	[1]	Oshida,'77
P (young) --> F_2 [2 generation test]										
Neanthes arenaceodentata	+	R	$K_2Cr_2O_7$	n.s.w.	33	14-m			[2]	Oshida et al.,'81
P (juveniles) --> F_3 [3 generation test]										
							$NOEC_r$	< 13 α		[P --> F_1]
							$NOEC_r$	**13** α		[F_1 --> F_2]
							$NOEC_r$	25 α		[F_2 --> F_3]
						2-m	NOLC	100 α		
Neanthes arenaceodentata			--				$NOEC_r$	25		Oshida,'77 ~
P --> F_3 [3 generation test]										
Neanthes arenaceodentata	-	S	CrO_3	s.w.	--	3-w	LC50	550	[9]	Reish et al.,'76
Nereis sp.	+	S	Na_2CrO_4	s.w.	--	3-w	LC50	± 1,000	[6]	Raymont & Shields,'63
						5-w	NOLC	500		

(to be continued)

Table 2.3 Long-term "single species" toxicity tests with Cr (VI) compounds - marine organisms (continued)

Organism	A	Test-type	Test-subst.	Test-water	Salinity o/oo	Exp.-time	Criterion	Result $\mu g\ Cr(VI).l^{-1}$	Reference
Crustaceans									
Callinectes sapidus	+	R	Na_2CrO_4	s.w.	30	6-w	$NOEC_{l-d,s}$	770 α	Bookhout et al.,'84
larvae --> zoeal stages --> megalops --> first crab									
Carcinus maenas	-	S	Na_2CrO_4	s.w.	--	2-w	NOLC	40,000 [7]	Raymont & Shields,'63
Leander squilla	-	S	Na_2CrO_4	s.w.	--	1-w	NOLC	5,000	Raymont & Shields,'63
"small"									
Mysidopsis bahia	-	-	$K_2Cr_2O_7$	-	--	-	NOEC	88	U.S. EPA, '83
[lc]									
Rhithropanopeus harrisii	+	R	Na_2CrO_4	s.w.	20	3-w	$NOEC_{l-d,s}$	360 α [3]	Bookhout et al.,'84
larvae --> zoeal stages --> megalops --> first crab									
Tisbe holothuriae	-	S	Na_2CrO_4	s.w.	--	± 7-w	$NOEC_{r,s}$	< 500	Verriopoulos &
P (ovigerous females) --> F_1 [lc]									Moraïtou-Apostopoulou
Fish									
Citharichthys stigmaeus	+	F	$K_2Cr_2O_7$	n.s.w.	33	3-w	LC50	5,000	Mearns et al.,'76
1.5-17 g									

l-d = larval development; g = growth; r = reproduction; s = survival

s.w.: sea water, no further data given; n.s.w.: natural sea water; a.s.w.: artificial sea water

[1] The mean number of offspring per brood of the P generation was significantly ($p \leq 0.05$) increased at this concentration, that of the F_1 generation was similar to that of the control. The next higher concentration (38 $\mu g.l^{-1}$) resulted in a reduced number of offspring per brood of the F_1 generation.
Mean percentage of chromium lost between renewals was 20%.

[2] At 100 $\mu g.l^{-1}$ no spawning occurred; no significant shifts from Cr (VI) to Cr (III) could be detected in 3-w periods.

[3] Lowest effect concentration: 2,300 $\mu g.l^{-1}$.

[4] Growth measured as relative fluorescence and chlorophyll a content; salinity of the bay water ranged from 0.03 to 32.5 o/oo; 2 months after addition of a nominal concentration of 100 μg Cr (VI).l^{-1}, 85% and 95% was still recovered as Cr (VI) in fresh and sea water, respectively.

[5] Concentrations of 10 and 100 $\mu g.l^{-1}$ were "moderately" and "severely" inhibitory, respectively; quantitative data are not reported.

(to be continued)

Footnotes Table 2.3 (continued)

[6] LC50 not calculated; approximately 50% mortality at 1,000 $\mu g.l^{-1}$.

[7] At 60,000 $\mu g.l^{-1}$ mortality was 42% (5/12); control mortality was 17% (2/12).

[8] "Significant reduction in the number of offspring above a level that was between 25 and 50 $\mu g.l^{-1}$".

[9] The 3-w LC50-values for adults and juveniles were 550 and 700 $\mu g.l^{-1}$, respectively.

Table 2.4 Long-term "single species" toxicity tests with Cr (III) compounds - marine organisms

Organism	A	Test-type	Test-subst.	Remark on medium	Salinity o/oo	Exp.-time	Criterion	Result $\mu g\ Cr(III).l^{-1}$	Reference
Annelida									
Neanthes arenaceodentata	+	R	$CrCl_3$	n.s.w.	33	10-m			[1] Oshida et al.,'81
P (juveniles) --> F_2 [2 generation test]									
							$NOEC_{r,s}$	≥ 50,400 α	total concentration
							$NOEC_{r,s}$	≥ 20 α	dissolved (0.1 μm)
Neanthes arenaceodentata	-	-	--	--	--	3-w	NOLC	≥ 12,500	Reish, '75 ~
Fish									
Citharichthys stigmaeus	-	F	$ClCl_3$	n.s.w	33	3-w	NOLC	≥ 10,000	[2] Mearns et al.,'76

r = reproduction; s = survival

[1] Precipitation of chromium, resulting in both internal and external exposure to undissolved chromium.

[2] Precipitation of chromium (hydroxide).

List of abbreviations tables 2.1 to 2.4

A	+: Test substance analysed in test solution; -: Test substance not analysed in test solution, or: no data.
α	Value based on actual (measured) concentrations in test solutions, as mentioned explicitly in the literature source. Values not indicated by "α" are considered to be nominal concentrations. The concentrations are expressed as chromium, not as the compound tested, and represent total-chromium ("dissolved"-plus "particulate"-chromium).
~	Secundary literature source; primary source not available.
> and ≥	Value indicated is highest concentration used in the test.
< and ≤	Value indicated is lowest concentration used in the test.
Test type	S: static; R: renewal; F: flow-through (continuous flow).
Test time	hr: hour(s); w: week(s); m: month(s).
Criterion	LC50: Lethal concentration for 50% of the organisms exposed. EC50: Effect concentration for 50% of the organisms exposed. NOLC: No-observed-lethal-concentration. NOEC: No-observed-effect-concentration.

3 ECOTOXICITY-II: TERRESTRIAL ORGANISMS

Data in the present chapter are on soil micro-organisms and invertebrates. For data on agricultural crops and livestock the reader is referred to the next chapter.
Most data refer to Cr(III), the form of chromium which is stable in most soils (Williams, 1988-R; see also the Criteria Document).

3.1 ACCUMULATION

All data available on accumulation in terrestrial animals are on earthworms.
In adult earthworms of the species *Eisenia andrei*, exposed for three weeks to concentrations up to 1,000 mg Cr(III).kg^{-1} dry weight (dw) -added to the soil as chromium(III)nitrate, in solution-, a dose-related increase in chromium content was found. Bioconcentration factors (BCF = $C_{organism}$: C_{soil}) on the basis of dry weights were 0.03-0.05 at the lowest concentrations and 0.02 at the highest concentrations. The soil used was an artificial soil (8% clay, 8% organic matter, pH 6.0 ± 0.5). After transfer of the worms to untreated soil a rapid elimination of chromium occurred: at all concentrations with exception of the highest level tested the chromium content decreased to the level of the control worms. At the highest level tested the content in worms still was slightly increased compared to the controls: 1.1 *versus* 0.7 mg.kg^{-1} dw (van Gestel et al., 1989).
Adult earthworms of the species *Lumbricus rubellus*, collected from a untreated pasture on a sandy soil (pH-KCl 5.9; 5% organic matter), contained 3-7 (average: 4) mg Cr.kg^{-1} dry weight (dw); chromium levels in the top layer (0-15 cm) of the soil were 13-21 mg.kg^{-1} dw. After treatment of the pasture with different amounts of contaminated sludge for several years, all but one worm samples contained 7-17 (average: 11) mg.kg^{-1} dw, at soil chromium levels in the top layer (0-15 cm) of 27-399 mg.kg^{-1} dw. Although soil chromium levels were increasing considerably with increasing amounts of sludge, levels in the worms appeared to be independent of the amount of sludge applied (Ma, 1983). In another field study conducted in The Netherlands, both the chromium concentration in adult earthworms (*Allolobophora caliginosa;* collected from six different agricultural soils treated with municipal waste compost) and the bioconcentration factor were found to be independent on the cation exchange capacity (CEC), pH and organic matter content (% OM), on the basis of linear regression analysis.

The soils studied were three loamy soils and three sandy soils. In the different soils CEC, pH and % OM ranged from 5-29 meq.100 g^{-1}, 4.7-7.1, and 3%-14%, respectively. Maximum concentrations in the worms were 8-10 $mg.kg^{-1}$ dw; maximum concentrations in soils were 110-125 $mg.kg^{-1}$ dw. Although there was a trend of increasing chromium levels in the worms with increasing chromium soil concentrations due to treatment with compost, the concentration in the soil could only explain 27% of the variation in the levels in the worms. Levels in adult and subadult worms collected from the same plots were found to be similar (Ma, 1982, 1983). In these two studies the earthworms were analysed with guts voided, to prevent overestimation of the amount actually accumulated in the tissues.

In a field study the chromium concentration in earthworm *Eisenia foetida*, collected during an interval ranging from 2 to 28 weeks from sludge containing 200-650 mg $Cr.kg^{-1}$, ranged from 1 to 13 $mg.kg^{-1}$ (Hartenstein, 1980a). Although it is not reported, these levels are most probably expressed as $mg.kg^{-1}$ dry weight. In a study in which one or more species of earthworms were collected from 20 soils, both "natural" and "contaminiated" soils, and analyzed per species, a correlation coefficient of -0.2 (statistically not significant) was found between the concentration in the worms and that in soils. This may be explained by species differences and different soil characteristics. The concentration in the worms (guts voided) ranged from $\leq$ 0.25 to 53 $mg.kg^{-1}$ dw and that in soils from 5 to 70 $mg.kg^{-1}$ dw (Beyer and Cromartie, 1987).

With the exception of a few locations in the studies reported, worm samples contained (much) less chromium than soil samples, on the basis of dry weights. Therefore, concentration factors are below unity.

Similar results have been reported in other studies (Hartenstein et al., 1980b; Pietz et al., 1984; Diercxsens et al., 1985) in which earthworms were collected from sludge or from sludge-amended soils.

3.2 TOXICITY

3.2.1 Microbes and microbe-mediated processes

Reviews on the effects of chromium -and other metals- on microbial numbers and species diversity, microbe-mediated processes (such as respiration, ammonification and nitrification), and on physicochemical soil characteristics influencing toxicity, have been published by Doelman and Haanstra (1983), Doelman (1985), Babich and Stotzky (1985) and Williams

(1988). Most data reviewed are on short-term tests in which effects were measured after exposure times ranging from a few hours to a few weeks. It is assumed that soil metal concentrations reported in these reviews are expressed on the basis of soil dry weights.

Data reviewed by Doelman (1985) show that chromium concentrations without effect on respiration, ammonification and nitrification are about 150-200 $mg.kg^{-1}$ (dw). At concentrations of 150-350 $mg.kg^{-1}$ (dw) data are not consistent: in some studies inhibition of these processes is found, while in other studies no inhibition is reported. The differences are ascribed to different test conditions and different soils used. Concentrations $\geq$ 400 $mg.kg^{-1}$ (dw) have been found to inhibit these microbe-mediated processes in all cases reported in this review. Data on chromium valency and duration of exposure are not reported in the figure from which these boundaries have been derived.

Data reviewed by Doelman and Haanstra (1983) and Babich and Stotzky (1985) show that concentrations up to about 200 $mg.kg^{-1}$ (dw, expressed as Cr(III) or "chromium") usually do not result in irreversible effects after long-term exposure. In a study by Chang and Broadbent (1981, 1982) the no-effect-concentration with regard to respiration and N-transformation was reported to be below 50 and 100 mg $Cr(III).kg^{-1}$ dw, respectively, after 3 months of exposure (table 3.1). Examples of relatively low effect-concentrations (26 to 100 mg $Cr(III).kg^{-1}$ dw) after short-term exposure have been reported by Tabatabai (1977) and Ross et al.(1981).

Decreased numbers of selected classes of bacteria have been reported at 10 and especially 100 mg $Cr(VI).kg^{-1}$ dw after 2-6 days of exposure. The lowest concentration tested, 1 mg $Cr(VI).kg^{-1}$ dw significantly reduced respiration (cumulative amount of CO_2 evolved) after 13 days of exposure. After 24 days of exposure the no-effect-concentration with regard to respiration was 10 mg $Cr(VI).kg^{-1}$ dw; at 100 mg $Cr(VI).kg^{-1}$ dw respiration still was reduced (Drucker et al., 1979).

In some comparative studies the toxicity of both Cr(III) and Cr(VI) on microbe-mediated processes have been investigated. In a study on the effect on nitrification, Cr(VI) was found to be a more effective inhibitor than Cr(III). A concentration of 240 mg $Cr(VI).kg^{-1}$ still inhibited nitrification after 3 months of exposure; concentrations of 60 and 120 mg $Cr(VI).kg^{-1}$ resulted in a temporary inhibition. Concentrations up to 800 mg $Cr(III).kg^{-1}$ did not result in an inhibition (Fenke, 1977, cited in Williams, 1988). In a 3-w study on the effect on respiration in two different soils, concentrations of 10 and 100 mg $Cr(VI).kg^{-1}$ dw and 100 mg

Cr(III).kg^{-1} dw resulted in decreased respiration, with little or no differences between the different treatments (Ross et al., 1981).

In a laboratory study in The Netherlands the effects of Cr(III) on soil respiration, enzyme activities and microbial populations were studied in 5 different, representative Dutch soils (two sandy soils, a sandy loam soil, a clay soil and a sandy peat soil, containing background chromium concentrations of 2-4, 34, 76 and 11 mg.kg^{-1} dw, respectively). Cr(III) was added to the soils as chromic chloride; the test concentrations used were 55, 150, 400, 1,000, 3,000 and 8,000 mg Cr(III).kg^{-1} dw. Some effects were measured both shortly after treatment of the soil samples and 1-1.5 year after treatment, to study both short- and long-term effects. Only the results after long-term exposure will be discussed here.
Soil respiration was significantly ($p < 0.05$) reduced in one soil at 150 mg.kg^{-1} dw (the sandy peat soil) and in four soils at 400 mg.kg^{-1} dw. Higher concentrations (1,000, 3,000 and 8,000 mg.kg^{-1} dw) were mostly inhibitory, but in some cases a significant stimulation of respiration was found, even in soils in which lower concentrations were inhibitory. For example, 8,000 mg.kg^{-1} dw stimulated respiration in one of the sandy soils, while 400 and 3,000 mg.kg^{-1} dw were inhibitory in this soil. The three lowest EC10-values with regard to respiration were 8 mg.kg^{-1} dw in one of the sandy soils, 54 mg.kg^{-1} dw in the sandy loam soil and 71 mg.kg^{-1} dw in the sandy peat soil; in the remaining two soils EC10-values were much higher (the exact values were not reported). EC50-values with regard to this parameter were higher than 5,000 mg.kg^{-1} dw in all soils or could not be calculated because of the highly variable results. The influence on enzyme activities was also highly depending on soil characteristics; extracellular enzymes (arylsulphatase, urease) were more sensitive than intracellular enzymes (β-glucosidase, protease). For arylsulphatase EC50-values were 10, 180, 410 and 570 mg.kg^{-1} dw. The lowest and next lowest value were found in the sandy soils; in the sandy peat soil the EC50 was much higher (exact value not reported). The lowest and next lowest EC50 for urease were 410 and 630 mg.kg^{-1} dw in the clay soil and one of the sandy soils, respectively. EC50-values for the other enzymes tested, including phosphatase, were (much) higher. With regard to the total numbers of micro-organisms or of "specific" (groups) of organisms responsible for a specific proces (for example the nitrification of NH_4^+ --> NO_2^-), concentrations up to 400 mg.kg^{-1} dw were without effect, while at $\geq$ 1,000 mg.kg^{-1} dw decreased numbers were found. However, the results were not consistent with regard to dose-effect relationships. The two lowest EC50-values with regard to the

mineralization of glutamic acid were 600 and 800 mg.kg^{-1} dw, in one of the sandy soils and the sandy peat soil, respectively.
The results of this study indicate that the impact of chromium (and other metals) is highly depending on the parameter studied and on soil characteristics. For example, the relative toxicity of chromium in the series of the metals tested in the present study (chromium, cadmium, lead, copper, nickel and zinc) with regard to soil respiration ranged from lowest in the one soil tested to highest in one of the other soils tested (Doelman and Haanstra, 1983).

3.2.2 Invertebrates

Data on effects on invertebrates are scarce. The only data available are on earthworms; these data are summarized in table 3.2.
In a 3-w test with the earthworm *Eisenia andrei*, the highest concentration tested (1,000 mg Cr(III).kg^{-1} dw) resulted in adverse effects on growth and reproduction; the resulting no-observed-effect-concentration was 300 mg Cr(III).kg^{-1} dw (measured concentration). In this test Cr(III) was added to the artificial soil (8% clay, 8% OM, pH 6.0 ± 0.5) as chromium(III)nitrate, in solution (van Gestel et al., 1989). Growth and mortality of *Eisenia foetida* hatchlings were not affected at exposure to a silt loam covered with contaminated sludge containing up to 6,300 and 45,000 mg Cr(III).kg^{-1} on the basis of wet weight and dry weight, respectively. This concentration corresponds with approximately 2,600 and 5,500 mg Cr(III).kg^{-1} substrate (soil plus sludge) on the basis of wet weight and dry weight, respectively. The chromium was added to the sludge as insoluble, solid chromic oxide (Hartenstein et al., 1981).
Exposure of two species of earthworms to Cr(VI) -added to the soil as potassium dichromate- resulted in increased mortality after 8 weeks of exposure to the lowest concentrations tested: 2 and 10 mg Cr(VI).kg^{-1} substrate, respectively. In both tests the earthworms were transferred to freshly contaminated soils once a week; the test substance was added to the soils in solution (Soni and Abbasi, 1981; Abbasi and Soni, 1983). It is not reported whether these concentrations are expressed on a wet weight or on a dry weight basis.

Summary and conclusions "terrestrial organisms"

Accumulation

In most studies on the accumulation of chromium in earthworms, concentrations were reported to be ≤ 10 mg Cr.kg^{-1} dry weight; the highest concentration reported is about 50 mg Cr.kg^{-1} dry weight. Concentrations in earthworms may increase with increasing concentration in soil, but bioconcentration factors are small (mostly < 0.1). Therefore, chromium is accumulated but not concentrated from the soil by these organisms.
Data on other terrestrial organisms are not available.

Toxicity

In some short-term experiments effects on microbes (numbers, diversity) and microbe-mediated processes (respiration, ammonification, nitrification, enzym-activities) have been reported at concentrations of 25 to 100 mg.kg^{-1} dry weight [Cr(III) or "chromium"] and 1 to 10 mg Cr(VI).kg^{-1}. However, in most short-term experiments and in long-term experiments no effects have been reported at concentrations up to 200 mg.kg^{-1} (dry weight) [Cr(III) or "chromium"].
In a test with the earthworm *Eisenia andrei*, a concentration of 1,000 mg Cr(III).kg^{-1} dry weight resulted in adverse effects on growth and reproduction. The resulting NOEC is 300 mg Cr(III).kg^{-1} dry weight.
In two tests in which other earthworm species were exposed to Cr(VI), concentrations of 2 and 10 mg Cr(VI).kg^{-1}, respectively, resulted in increased mortality.
Relevant data on other terrestrial organisms are not available.

Table 3.1 Effects of relatively low Cr(III) concentrations on microbe-mediated processes after long-term exposure

Soil & soil characteristics	Test-subst.	Exposure mg $Cr(III).kg^{-1}$ dw substrate	Exp.-time	Criterion & Result	Reference
Silt loam (2.2% OM, CEC 27 meq/100 g), amended with 1% dry sewage sludge and 1% ground alfalfa; background soil chromium level 2.6 mg/kg	$CrCl_3$	0-50-100-200-300-400 (added in solution)	3-m	Respiration NOEC < 50 [1] 50 < EC50 < 100	Chang & Broadbent, '81
Silt loam (see above)	$CrCL_3$	0-100-200-400 (added in solution)	3-m	N-Transformation NOEC < 100 [2] 100 < EC50 < 200	Chang & Broadbent, '82

[1] Incubation at 25 ^{o}C. The lowest concentration added, 50 mg/kg, resulted in a 45% decrease in cumulative CO_2 evolution.

[2] Incubation at 25 ^{o}C. The lowest concentration added, 100 mg/kg, resulted in a 40% decrease in nitrate-N concentration.

Table 3.2 Toxicity of chromium to earthworms

Species	Test-type	Test-subst.	Substrate	Concentration in the substrate mg Cr.kg^{-1} dry weight	Reference
Cr(III)					
Eisenia andrei clitellated adults	S	$Cr(NO_3)_3.9H_2O$	artificial soil, 8% clay, 8% OM, moist. 55% w/w pH 6.0 ± 0.5	0-15-40-100-300-1,000 (added in solution)	van Gestel et al. '89 [1]
Eisenia foetida hatchlings	R 1-time	Cr_2O_3	silt loam, covered with treated sludge; no data on soil characteristics	0-55-550-2,750-5,500 (added as solid)	Hartenstein et al. '81 [2]
Cr (VI)					
Octochaetus pattoni	R weekly	$K_2Cr_2O_7$	soil mixed with animal dung; no data on soil characteristics	0-2-10-20 (added in solution)	Abbasi & Soni, '83 [3]
Pheretima posthuma adults	R weekly	$K_2Cr_2O_7$	paddy-field soil; no data on soil characteristics	0-10-20-40-60-80-100 (added in solution)	Soni & Abbasi, '81 [4]

Result: Growth (relative weight gain), cocoon production and percentage of fertile cocoons significantly ($p < 0.05$) reduced at 1,000 mg.kg^{-1} after the 3-w exposure period. During the 3-w recovery period growth of the worms exposed to this concentration was significantly increased, and cocoon production did recover partially. The resulting no-observed-effect-concentration (NOEC) in this study was 300 mg.kg^{-1}.

Result: After 8 weeks of exposure there was no effect on mortality and growth.

Result: After 8 weeks of exposure, 25% mortality was found in the lowest two dose groups and 70% in the highest dose group; there was no mortality in the controls. 8-week LD50: 15 mg.kg^{-1}. The number of juveniles was not affected. In the treatment groups the number of cocoons and bits appear to be decreased and increased, respectively.

Result: At relatively short-term exposure (4 weeks) mortality data between 3 experiments were highly variable, but increased mortality was found in any dose group. After 8 weeks of exposure mortality in all dose groups ranged from 50% to 100%, while that in controls was 0% to 2.5%. Complete mortality in all dose groups was found after 16 weeks of exposure.

Test-type: S = single application of test substance; R = renewal of test medium

[1] Soil (prepared according to OECD Guidelines) was treated with chromic nitrate 1 week before the addition of the worms. The worms were pre-incubated in untreated soil for 1 week, followed by a 3-w exposure period in treated soil and a 3-w recovery period in untreated soil. Concentrations indicated are measured concentrations (nominal: 10-32-100-320-1000).

(to be continued)

[2] Highest concentration in sludge: 6,300 mg Cr(III).kg^{-1} wet weight, corresponding with 45,000 mg Cr(III).kg^{-1} dry weight.
The highest concentration in sludge corresponds with 2,600 mg Cr(III).kg^{-1} wet weight in the substrate (50 g dry soil + 30 ml water; covered with 50 g wet sludge); this corresponds with 5,500 mg Cr(III).kg^{-1} dry weight.
After 4 weeks of exposure old sludge material was removed and a fresh supply of Cr-treated sludge was added.

[3] For data on treatment the reader is referred to Soni & Abbasi (1981), so it is assumed that also in this experiment substrates were renewed weekly. It is not reported whether the exposure concentrations are expressed on the basis of wet weight or on the basis of dry weight.

[4] It is not reported whether the exposure concentrations are expressed on the basis of wet weight or on the basis of dry weight.

4 TOXICITY TO AGRICULTURAL CROPS AND LIVESTOCK

4.1 AGRICULTURAL CROPS

In a number of cases additions to soil of Cr(III), but not Cr(VI), has resulted in effects such as increased growth and development of seedlings and increased yields of different crops, which indicates that there are chromium-deficient soils (Mertz, 1969-R). However, according to the review by Williams (1988-R), conclusive evidence that Cr(III) is essential to plants is not available at present.

Most data in the present subchapter refer to Cr(III), because Cr(VI) usually is not stable in soil and sludge. Although in some soils Cr(III) may be oxidized to Cr(VI) by manganese oxides, it is assumed that Cr(III) prevails in most soils and sludges (Williams, 1988-R; see also the Criteria Document).

4.1.1 Accumulation

Chromium is regularly present in plants. Levels in vegetables have been found to range widely; for example, a range of 0.01 to 1.0 mg $Cr.kg^{-1}$ dry weight (dw) was found in vegetables from 25 plant families. The variation probably depend on species, soil characteristics and season during which the plants are harvested (Mertz, 1969-R). Levels in shoots of crops grown on uncontaminated soils usually do not exceed 0.5 $mg.kg^{-1}$ dw. Levels $\geq$ 3 $mg.kg^{-1}$ dw probably indicate an increased accumulation, but contamination of plant samples with soil may obscure actual uptake. This contamination problem and the immobility of chromium within plants are considered to be two major reasons for the poor relationships that have been found between levels in plants and soils (Williams, 1988-R).

Chromium levels in soil solutions and extractability of soil chromium with mild extractants such as ammonium acetate, acetic acid or EDTA are very low (Williams, 1988-R). For example, in a silt loam amended with 1% sewage sludge and 1% ground alfalfa, H_2O- and 1 M KNO_3- extractable chromium concentrations were 0.002-0.04 and 0.09-0.9 $mg.kg^{-1}$ soil after additions of 50-400 mg $Cr(III).kg^{-1}$ dw (as $CrCl_3$); using 1 M HNO_3 only 10%-14% was extractable (Chang and Broadbent, 1981). These data indicate a low bioavailability of chromium in soil, but according to Williams the

significance of the use of extractants for assessing availability to plants is doubtful.

From both soils and nutrient solutions, chromium is accumulated in the roots rather than in the shoots. Root/shoot concentration-ratios are roughly in the range from 5 to 250 and increase strongly at relatively high soil chromium concentrations, indicating little transformation from roots to shoots (Smilde, 1976-R; Williams, 1988-R).

In a pot experiment the uptake of chromium from soil treated with inorganic Cr(III) -added as chromic acetate- and that from soil mixed with sewage sludge treated with chromic acetate were compared. Three crops (maize, rye and maize, in that order) were grown successively in the control and treated substrates for 6 weeks each and were harvested at the soil surface. In soil treated with inorganic Cr(III), additions of 350 and 700 mg Cr(III).kg^{-1} dw resulted in shoot chromium concentrations of 15-46, 26-58 and 4-7 mg.kg^{-1} dw in the first (maize), second (rye) and third crop (maize), respectively; significantly higher than control levels which were below the limit of detection (3 mg.kg^{-1} dw). In soil amended with treated sludge -resulting in similar Cr(III) concentrations in the substrate- shoot chromium concentrations were 5-8, 5-10 and $\leq$ 3 mg.kg^{-1} dw in the first, second and third crop, respectively. These data show that in the presence of sludge significantly less chromium was accumulated in the crops and that in the second harvest of maize much less chromium was accumulated compared with the first harvest of the same crop (Cunningham et al., 1975). In a pot experiment conducted in The Netherlands the accumulation in oats of inorganic Cr(III) -added as chromic acetate; added concentrations up to 800 mg Cr(III).kg^{-1} dw- was studied in three loamy clay soils (< 16 μm clay content: 12%, 40% and 58%; organic matter content 1.6%-3.2%; CEC 15, 21 and 33 meq.100 g^{-1} dw) and three sandy soils (organic matter content 3.4%, 6.8% and 19.4%; clay content 4%-5%; CEC 9, 19 and 47 meq.100 g^{-1} dw). All soils were slightly acid, with pH-KCl values of 4.6 to 5.6. Concentrations in the green crop (< 150 mg.kg^{-1} dw in the clay soils and the sandy soil with the highest % OM, and 500-800 mg.kg^{-1} dw in the other two sandy soils) were much higher than those in the straw (< 50 and 25-300 mg.kg^{-1} dw in clay soils and sandy soils, respectively). It is not reported whether whole plants or only shoots were analyzed. The concentration in grain was below detection limit (0.1 mg.kg^{-1} dw) in all samples. Transfer coefficients (increase in Cr concentration in crop : increase in Cr concentration in soil) for both green crop and straw were not significantly related to soil CEC (r-values -0.47 and -0.31, respectively) (De Haan et al., 1985).

In barley, grown on a sandy loam soil (pH 5.6, 6.4 and 7.8) with additions of 200 mg Cr(III).kg^{-1} or 200 mg Cr(VI).kg^{-1}, concentrations in leaves (2.2-3.2 mg.kg^{-1}) were only slightly increased compared with controls (0.5-0.8 mg.kg^{-1}). The concentrations in roots were much higher: 46-146 mg.kg^{-1} and 115-168 mg.kg^{-1} after treatment with Cr(III) and Cr(VI), respectively; control levels were 1.9-2.6 mg.kg^{-1}. A trend of increasing accumulation with increasing pH was found (Patterson, 1971; cited in Williams, 1988-R).
In a series of pot experiments the accumulation of chromium in butterhead lettuce, radish, spring wheat and red fescue grass was studied; the crops were grown on 5 different fluvial clay soils (reclaimed or dredged sediments) with similar soil texture (5-10% OM, 27-31% clay) and chromium concentrations ranging from 100 to 600 mg.kg^{-1} dw. Concentrations in radish tubers increased with increasing soil chromium concentrations, but the highest concentration of 6.6 mg.kg^{-1} dw was only four times higher than the lowest concentration of 1.6 mg.kg^{-1} dw. The concentrations in radish leaves and in the other crops were below 1.5 mg.kg^{-1} dw; these concentrations were not related to soil chromium concentrations (Van Driel et al., 1985). The concentrations in potato tubers grown on three sandy soils and three clay soils treated for 15 years with municipal waste compost (40 ton.yr^{-1}, the highest application rate) were not increased compared to the concentrations in tubers grown on the soils without treatment. All concentrations were in the range from 0.2 to 0.4 mg.kg^{-1} dw, (0.05 to 0.1 mg.kg^{-1} fresh weight). Average soil chromium concentrations were 18 and 85 mg.kg^{-1} dw in untreated sandy soils and clay soils, respectively, and 31 and 101 mg.kg^{-1} dw in treated sandy soils and clay soils, respectively. In experiments in which potatoes were grown on either uncontaminated soils or on contaminated substrates (sludges, harbour sediments), chromium concentrations in the tubers were very similar: $\leq$ 0.3 mg.kg^{-1} dw (0.075 mg.kg^{-1} fresh weight). Based on these results and reviewed data, concentrations of 0.3 and 1.0 mg.kg^{-1} dw are considered to be "normal" and "(too) high", respectively, by de Haan and Lubbers; these concentrations correspond with 0.075 and 0.25 mg.kg^{-1} fresh weight, respectively (De Haan and Lubbers, 1983).
Additional data, based on both pot experiments and field experiments show that chromium tissue levels of vegetable crops (leaves) are not or only little affected when plants are grown in soils treated with chromium-containing sludge, resulting in substate concentrations up to about 400 mg Cr.kg^{-1}; effects on tissue concentration have been reported in some cases for cereals, but sample contamination with the substrate was suspected (Smilde, 1976-R; Williams, 1988-R).

4.1.2 Toxicity

In a pot experiment conducted in The Netherlands the toxicity of inorganic Cr(III) -added as chromic acetate at concentrations of 50, 100, 200, 400 and 800 mg Cr(III).kg^{-1} dw- to oats was studied in three loamy clay soils (< 16 μm clay content: 12%, 40% and 58%; organic matter content 1.6%-3.2%; CEC 15, 21 and 33 meq.100 g^{-1}dw; background Cr level 40-88 mg.kg^{-1} dw) and three sandy soils (organic matter content 3.4%, 6.8% and 19.4%; clay content 4%-5%; CEC 9, 19 and 47 meq.100 g^{-1} dw; background Cr levels 14-20 mg.kg^{-1} dw). All soils were slightly acid, with pH-KCl values of 4.6 to 5.6. In all soils studied, added concentrations up to 200 mg Cr(III).kg^{-1} dw did not result in yield depression of either grain or straw after 5 months of exposure. The relationship between maximum chromium applications without yield depression and the CEC of the soils (r-value -0.56) was not statistically significant (De Haan et al., 1985).

In a series of pot experiments in fluvial clay soils (reclaimed or dredged sediments) with similar soil texture (5-10% OM, 27-31% clay) and chromium concentrations ranging from 60 to 600 mg.kg^{-1} dw, the yield and dry-matter content of butterhead lettuce, radish, spring weat and red fescue grass were not related to chromium concentrations (Van Driel et al., 1985).

In sludge-amended soil the effect on yield of four different crops grown in pots was very variable, depending on crop, origin of sludge (bovine hide tannery sludge, sheepskin tannery sludge, dried sewage sludge), and time after application (different effects on successive crops). Concentrations of 200 and 500 mg Cr.kg^{-1} substrate resulted in both growth reductions and stimulations; however, most effects were not statistically significant at $p \leq 0.05$. At 200 mg.kg^{-1} only crop yield of grass after addition of sewage sludge was significantly reduced. At 500 mg.kg^{-1}, yields of beans (source: sheepskin tannery sludge) and grass (source: sewage sludge) were significantly reduced. Yields of lettuce and radish were not significantly reduced at any treatment (Cunningham et al., 1975b; cited in Williams, 1988-R).

In a 4-yr trial in general no adverse effect on successive crop yields of red beet, lettuce and celery was found after one single or four annual additions of "unpolluted"- "low"- or "high"-Cr sludge to 2 different soils (soil 1: sandy loam, pH 5.8-6.4, CEC 11 meq.100 g^{-1}, OM 1,8%; crops: red beet and lettuce # soil 2: silty clay loam, pH 6.3-6.6; CEC 22 meq.100 g^{-1}, OM 4.9; crops: red beet and celery). Total chromium concentrations (aqua regia soluble) after a single dressing of "high"-Cr sludge were 350 and 440 mg.kg^{-1} in soil 1 and soil 2, respectively, measured at the end of the

trial. Build-up due to four annual dressings of "high"-Cr sludge resulted in measured concentrations of 320 $mg.kg^{-1}$ in soil 1. Control soils contained 30 to 40 $mg.kg^{-1}$. Significant ($p \leq 0.05$) reductions in yield only occured in beet after a single dressing of "high"-Cr sludge, 1 and 2 years after application in soil 2 (440 $mg.kg^{-1}$) and soil 1 (350 $mg.kg^{-1}$), respectively; concentrations of 185 $mg.kg^{-1}$ (soil 1) and 230 $mg.kg^{-1}$ (soil 2) were without effect (MAFF, UK Field Experiments 1968-1972; cited in Williams. 1988-R).

Additional data show that Cr(III) concentrations (added as inorganic compounds) up to 200 $mg.kg^{-1}$ soil do not result in adverse effects on crops. Additions of inorganic Cr(VI) compounds has resulted in effect-concentrations ranging from 10 to 80 mg $Cr(VI).kg^{-1}$, using different crops and different soils. In some experiments the effect of Cr(III) and Cr(VI) has been compared; two examples of these experiments are summarized in table 4.1. In these experiments Cr(VI) was found to be consistently more toxic than Cr(III). The toxicity of Cr(VI) was higher at higher pH-values (Smilde, 1976-R; Williams, 1988-R).

4.2 LIVESTOCK

On the basis of data on experimental animals (see chapter 1), chromium is considered to be "probable essential" to ruminants (Hansard et al.,1983). Specific data with regard to essentiality to livestock are not available.

4.2.1 Accumulation

Quantitative data on fractional absorption of chromium from the gastro-intestinal tract are not available. On the basis of data on chemobio-kinetics, a mean fractional absorption value of 25% is estimated for dietary chromium (chapter 1).
Chromium levels of 30 $mg.kg^{-1}$ in liver and 4 $mg.l^{-1}$ in blood are suggested as indicative of chromium poisoning (NRC, 1980b-R).

4.2.2 Toxicity

A single oral dose of 30-40 mg $Cr(VI).kg^{-1}$ bw resulted in acute poisoning in young calves; a single oral dose of approximately 700 mg $Cr(VI).kg^{-1}$ bw has been reported as the acute lethal dose for mature cattle.
Chromic oxide has been used as a fecal marker in cattle and sheep for periods of several weeks at levels of 3,000 $mg.kg^{-1}$ feed without evidence of adverse effects. In chicks fed chromic chloride 2,000 $mg.kg^{-1}$ feed resulted in reduced growth; 1,000 $mg.kg^{-1}$ feed was without effect. On the basis of these data "maximum tolerable dietary levels" for domestic animals are set at 3,000 and 1,000 $mg.kg^{-1}$ feed for chromium oxide and chromium chloride, respectively, by the American National Research Council. These levels correspond with 2,000 and 330 mg $Cr(III).kg^{-1}$ feed, respectively.
Potassium chromate and sodium chromate have been fed to chicks at levels of 100 $mg.kg^{-1}$ feed without adverse effects; this level corresponds with 27 and 32 mg $Cr(VI).kg^{-1}$ feed, respectively (NRC, 1980b-R).
According to Williams (1988) applications of chromium containing sludge on grassland are very unlikely to result in toxicity to grazing animals.

Summary and conclusions "agricultural crops and livestock"

Agricultural crops

Cr(III) may be essential to plants, but conclusive evidence is not available.

Accumulation

The bioavalability of chromium to plants appears to be low. "Normal" concentrations in shoots usually are ≤ 0.5 $mg.kg^{-1}$ dry weight; concentrations ≥ 3 $mg.kg^{-1}$ dry weight probably indicate an increased accumulation. Chromium is accumulated especially in the roots, with little translocation to shoots. After addition to soils of inorganic Cr(III) concentrations in crops are higher than after addition as sludge. After addition of sludge, soil chromium concentrations up to 400 $mg.kg^{-1}$ (dry weight) do not result in significantly increased accumulation in crops.

Toxicity

Additions to soils up to 200 mg $Cr(III).kg^{-1}$ (dry weight) generally do not result in yield depression. When crops are grown on substrates with relatively high chromium concentrations (sediments, soils treated with sludge), chromium concentrations up to 350-500 $mg.kg^{-1}$ (dry weight) generally do not result in yield depression.

Cr(VI) has been found to be consistently more toxic than Cr(III): effect-concentrations range from 10 to 80 mg $Cr(VI).kg^{-1}$.

Livestock

Data on livestock are vere limited. On the basis of data on experimental animals chromium is expected to be essential to livestock. A single oral dose of 30-40 and 700 mg $Cr(VI).kg^{-1}$ bw has been reported as the acute lethal dose for young calves and mature cattle, respectively. Feed chromium concentrations of 330-2,000 mg $Cr(III).kg^{-1}$ and approximately 50 mg $Cr(VI).kg^{-1}$ did not result in adverse effects. Applications of chromium containing sludge on grasslands are not expected to result in toxicity to grazing animals.

Table 4.1 Comparative studies of the toxicity of Cr(III) and Cr(VI) to agricultural crops

Crop	Soil	Test subst.	Substr. Cr conc. (mg Cr.kg^{-1})	Exp.-time	Criterion	Result (mg Cr.kg^{-1})		Reference
Barley	Sandy loam pH 5.6, 6.4 and 7.8		0, 50 and 200	4-w ?				[A]
		$K_2Cr_2O_7$			EC_g Cr(VI)	50	at pH 7.8	
		idem			$NOEC_g$ Cr(VI)	50	at pH 5.6 and 6.4	
		$Cr_2(SO_4)_3$			$NOEC_g$ Cr(III)	200	independing on pH	
Maize	Sandy loam pH 5.5 and 7.0		0, 80 and 320					[B]
		$Na_2Cr_2O_7$			EC_y Cr(VI)	80	[1]	
		$Cr_2(SO_4)_3$			EC_y Cr(III)	320		
		idem			$NOEC_y$ Cr(III)	80		
	idem	sludge	0, 70, 340 and 1,360		$NOEC_y$	1,360		

Parameters: g = growth; y = yield

[1] Yield depression was greater at pH 7.0 compared to pH 5.5 (parent pH).

[A] Patterson, 1971; cited in Smilde, 1976 and Williams, 1988.

[B] Mortvedt & Giordano, 1975; cited in Smilde, 1976 and Williams, 1988).

5 RISK ASSESSMENT

5.1 RISK ASSESSMENT FOR MAN

Cr(III) -being present in biological material as an organic compound, the so-called "glucose tolerance factor"- is an essential trace element for mammals. It is estimated that the minimal requirement for adults is provided by a daily dietary intake of 2 to 8 μg Cr(III), corresponding with 0.03 to 0.13 μg Cr(III).kg bw.day^{-1}.
There is conclusive evidence that Cr(VI) is carcinogenic to mammals, including man. Because of the mutagenic activity, Cr(VI) is considered to be a genotoxic carcinogen. Therefore, a dose-without-effect with regard to carcinogenicity can not be established.
After parenteral administration of either Cr(VI) or Cr(III) to pregnant hamsters and mice in specific teratogenicity studies, developmental effects (including malformations) have been reported. However, developmental toxicity tests in which animals were exposed orally or by inhalation are very limited and, therefore, do not allow an evaluation of the risk to humans which are exposed only orally or by inhalation.

5.1.1 Oral exposure

The oral studies available with regard to Cr(III) are considered to be too limited to establish an acceptable daily intake. Based on feeding studies with insoluble chromic oxide pigment (Cr_2O_3), a dose-without-effect (DWE) of 1,210 mg Cr(III).kg^{-1} bw.day^{-1} was established; assuming a fractional absorption of 0.5%, this DWE corresponds with 6 mg absorbed-Cr(III).kg^{-1} bw.day^{-1}. Based on a study in which animals were exposed to relatively soluble chromic chloride ($CrCl_3$) in drinking water, a DWE of 2.5 mg Cr(III).kg^{-1} bw.day^{-1} was established; assuming a fractional absorption of 5%, this DWE corresponds with 0.125 mg absorbed-Cr(III).kg^{-1} bw.day^{-1}.
Data on the daily dietary intake of chromium in The Netherlands are not available. Based on foreign data ("total diet studies") summarized in the Criteria Document, the average daily dietary intake is estimated to be 100 μg total-Cr.day^{-1}, with a range of 50 to 200 μg total-Cr.day^{-1}. Data on the speciation of chromium in the diet are not available. Based on the speciation of chromium in biological material [organic Cr(III)] and the small contribution of drinking water [containing 0.1-0.8 μg total-Cr.l^{-1} in The Netherlands] to the chromium level in the total diet, it is assumed

that by far most chromium in the diet is present as Cr(III). Therefore, the upper limit of 200 μg total-Cr.day^{-1} is corresponding with 0.83 μg absorbed-Cr(III).kg^{-1} bw.day^{-1} (based on a fractional absorption of 25% and a body weight of 60 kg). The two above-mentioned DWE-values (125 and 6,000 μg absorbed-Cr(III).kg^{-1} bw.day^{-1}, respectively) based on long-term animal experiments are 150 times and 7000 times, respectively, higher than the upper limit of human intake (0.83 μg absorbed-Cr(III).kg^{-1} bw.day^{-1}). Based on these "margins of safety" adverse effects are not expected to occur at the current exposure levels.
Because of data on the speciation of chromium in surface water in The Netherlands, it must be noticed that it can not be excluded that chromium in drinking water may be present partially as Cr(VI). If so, Cr(VI) will be converted partially to Cr(III) due to intragastric reduction; quantitative data on this reduction are not available. The available studies in which animals were orally exposed to Cr(VI) are too limited for a risk assessment for man. Because Cr(VI) is considered to be a genotoxic carcinogen, exposure to Cr(VI) should be as low as possible.

5.1.2 Exposure by inhalation

Exposure to Cr(III) by inhalation results in a daily intake which is < 0.1% of the dietary intake, assuming an airborne concentration of 5 ng.m^{-3} and a respiration volume of 12 m^3 per day. Because exposure to Cr(III) by inhalation is not expected to result in different effects than oral exposure, exposure to Cr(III) by inhalation is considered to be not relevant. Therefore, this risk assessment is limited to Cr(VI).
Although there appear to be differences in the carcinogenic potency of different Cr(VI) compounds, it is not possible to quantify these differences. Moreover, data on the speciation of Cr(VI) in air are not available.
The "unit risk" of 40 x 10^{-3}, calculated by the World Health Organization (WHO, 1987), is considered to be the best estimate of the carcinogenic potency of Cr(VI). The "unit risk" is defined as "the additional lifetime cancer risk occuring in a hypothetical population in which all individuals are exposed continuously from birth throughout their lifetimes to a concentration of 1 μg.m^{-3} of the agent in the air they breathe". Assuming an acceptable risk of 1 x 10^{-6} (one extra case of lung cancer per million persons exposed lifetime), this risk corresponds with an airborne concentration of 25 x 10^{-6} μg Cr(VI).m^{-3} [0.025 ng Cr(VI).m^{-3}].

5.2 RISK ASSESSMENT FOR THE ENVIRONMENT

5.2.1 Aquatic organisms

Introduction

At present there are no generally accepted methods for extrapolation of the results of laboratory "single species" toxicity studies to natural ecosystems. Therefore different theoretical methods (see below) are used provisionally to calculate acceptable concentrations in fresh and sea water, at long-term exposure. In this risk assessment the use of these methods is in accordance with a proposal of a Dutch advisory board (Gezondheidsraad, 1988).

In the method according to Slooff et al. (1986), calculated concentrations are based on one toxicity value, either an L(E)C50 from a short-term test or an NOE(L)C from a long-term test. In both cases the value used in the calculation is the lowest value that is considered reliable. Using an L(E)C50 two different concentrations can be calculated, namely an NOEC for single species ($NOEC_{ss}$) and an NOEC for ecosystems ($NOEC_{eco}$). Using an NOEC, only an $NOEC_{eco}$ can be calculated. The values calculated must be divided bij an "uncertainty factor" which depends on the formula used. The final results are considered to be "safe" concentrations.

In the method according to Kooijman (1987) also different toxicity values can be used, namely short-term L(E)C50-values, long-term L(E)C50-values or long-term NOEC-values. In this method all available data of a kind are used, for example all long-term NOEC-values. Using short-term L(E)C50-values or long-term L(E)C50-values, a HCS ("hazardous concentration for sensitive species") is calculated; at these concentrations there is a probability (for example 10%, arbitrary) that up to 50% of individuals of the most sensitive species will die at short-term and long-term exposure, respectively. At the HCS calculated from long-term NOEC-values, there is a probability that adverse non-lethal effects will occur in up to 50% of individuals of the most sensitive species.

The method according to van Straalen (1987) is similar to that according to Kooijman. In the method of van Straalen all long-term NOEC-values are used, resulting in an HC5 ("hazardous concentration for 5% of the species"). At the HC5 there is a probability of 5% that adverse non-lethal effects will occur in up to 5% of the species exposed. The values of 5% also are arbitrary chosen. The HC5 is considered to be a "threshold" value ("limit" value).

Because of the high number of long-term NOEC-values available for chromium, only these values have been used in the present risk assessment. The results of the methods of extrapolation in which NOEC-values can be used, are summarized in table 5.1. By far most NOEC-values are from tests with Cr(VI) in fresh water; the distribution of these values is shown in figure 5.1.
All NOEC-values used in the methods according to van Straalen (1987) and Kooijman (1987) are printed **bold** in the tables 2.1, 2.2 and 2.3 in chapter 2 of the present document. In cases of the presence of two or more NOEC-values for one single species, only one value has been used. The value used has been selected on the basis of both test procedure (reliability, test medium, exposure time, effect-parameters) and representative value for the species involved. Therefore, in all cases only values from primary literature sources have been used in the methods of extrapolation.

Fresh water

Based on both L(E)C50- and NOEC-values it is concluded that there is no or no relevant difference in toxicity between Cr(III) and Cr(VI), the valence states in which chromium is found. Therefore one "limit" value for chromium is proposed. In accordance with the proposal of the "Gezondheidsraad" (1988), the "limit" value is based on the method according to van Straalen (1987). Because by far most NOEC-values are from tests with Cr(VI), the result of this method using these NOEC-values: 7 $\mu g.l^{-1}$ is considered to be the most reliable "limit" value for chromium in fresh water. This value is based on "total"-chromium concentrations ("dissolved"- plus "particulate"-chromium) in the test waters used. However, because of the differences between test waters used and surface waters (especially with regard to the difference in particulate matter content), it is assumed that in most tests chromium was present as "dissolved"-chromium. For this reason the concentration of 7 $\mu g.l^{-1}$ is considered to be "dissolved"-chromium (< 0.45 μm). Based on data in the Criteria Document on the concentrations "dissolved"-chromium *versus* "particulate"-chromium in the major surface waters in The Netherlands, the concentration of 7 $\mu g.l^{-1}$ corresponds with approximately 50 $\mu g.l^{-1}$ "total"-chromium. In conclusion, concentrations of 7 $\mu g.l^{-1}$ and 50 $\mu g.l^{-1}$ are provisionally recommended as "limit" values for "dissolved"-chromium and "total-chromium, respectively.

Sea water

The concentration calculated according to the method of van Straalen (1987) -on the basis of tests with Cr(VI)- is 1.3 $\mu g.l^{-1}$. This value is based on a very limited number of NOEC-values and of species of different taxonomic groups. The lowest relevant NOEC in sea water is very similar to that in fresh water. Therefore, and because of the low solubility of Cr(III) in sea water, the concentration of 7 $\mu g.l^{-1}$ (calculated on the basis of NOEC-values of Cr(VI) in fresh water) is provisionally recommended as "limit" value for "dissolved"-chromium, Cr(III) plus Cr(VI), in sea water.

5.2.2 Terrestrial organisms

Most probably Cr(III) is essential to terrestrial organisms such as plants and livestock. On the basis of the data available, a minimal required concentration in soils can not be established.
Because of physicochemical properties of Cr(III) and Cr(VI) it is assumed that Cr(III) is the stable form of chromium in soil and sludge. Therefore the present risk assessment has been based on data on Cr(III).
Based on data with regard to 1) effects on microbes (numbers, diversity) and microbe-mediated processes such as respiration, ammonification, nitrification and enzym-activities, 2) accumulation in, and toxicity to earthworms, and 3) accumulation in, and toxicity to crops, it is concluded that soil "total"-chromium concentrations up to 200 $mg.kg^{-1}$ dry weight do not result in irreversible effects. Therefore this concentration is provisionally recommended as "limit" value in soils.

Table 5.1 Calculated concentrations (μg Cr/l) in fresh water and sea water, based on the methods of extrapolation according to Slooff et al. (1986), Kooijman (1987) and van Straalen (1987)

			Fresh water		Sea water*
			Cr(VI)	Cr(III)	Cr(VI)
Lowest relevant NOEC (long-term tests)			10**	48	13
Slooff et al.					
$NOEC_{eco}$: UF	--->		30,2 : 33,5 = **0,9**	114,5 : 33,5 = **3,4**	37,7 : 33,5 = **1,1**
NOEC-values (long-term tests)			m = 35	m = 4	m = 5
Kooijman [1]	--->	HC	**0,005**	**0,3**	**0,0004**
Van Straalen [2]	--->	HC5:	**7,0**	**11,2**	**1,3**

* A reliable NOEC with respect to Cr(III) in sea water is not available.

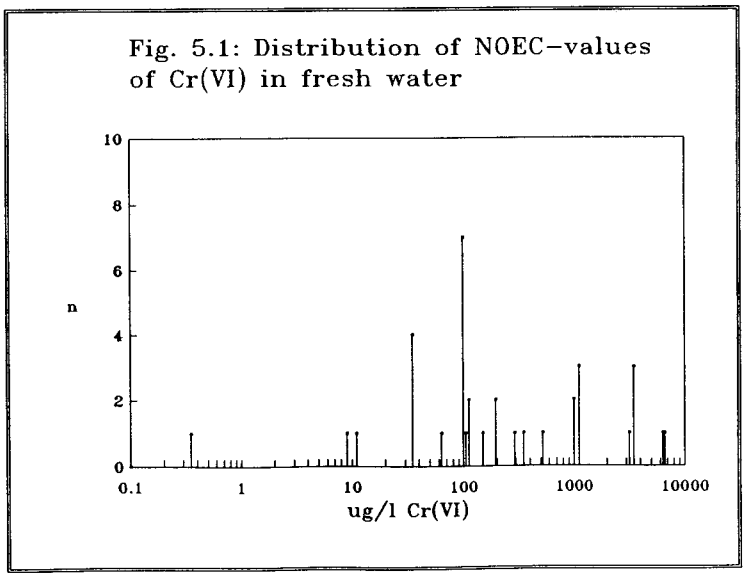
** The value of 10 μg/l is based on two NOEC-values, namely 9 μg/l ((Atlanto-astacus pallipes) and 11 μg/l Lemna minor). The exceptionally low NOEC (0.35 μg/l) for Stephanodiscus hantzschii has been left out of consideration.

m Number of NOEC-values used, for m different species.

UF "Uncertainty factor".

[1] Dm from table 1 in Kooijman (1987) at d = 0.1; theorethical number of species in the ecosystem ("n") is 1000.

[2] Dm from table 1 in kooijman (1987) at d = 0.05.



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