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SCIENTIFIC OPINION

Scientific Opinion on the risks to public health related to the presence of chromium in food and drinking water[1]

EFSA Panel on Contaminants in the Food Chain (CONTAM)[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

This Scientific Opinion, published on 12 June 2014, replaces the earlier version published on 13 March 2014*.

ABSTRACT

EFSA received a request from the Hellenic Food Authority for a scientific opinion on estimation of the risk to human health from the presence of chromium (Cr) in food, particularly in vegetables, and Cr(VI) in bottled water. The CONTAM Panel derived a TDI of 0.3 mg/kg b.w. per day for Cr(III) from the lowest NOAEL identified in an NTP chronic oral toxicity study in rats. Under the assumption that all chromium in food is Cr(III), the mean and 95th percentile dietary exposure across all age groups were well below the TDI and therefore does not raise concerns for public health. In the case of drinking water, the Panel considered all chromium in water as Cr(VI). For non-neoplastic effects the lowest $BMDL_{10}$ for diffuse epithelial hyperplasia of duodenum in female mice and the lowest $BMDL_{05}$ for haematotoxicity in male rats in a 2-year NTP study were selected as reference points. The MOEs indicate that for non-neoplastic effects the current exposure levels to Cr(VI) via drinking water are of no concern for public health. For neoplastic effects, the CONTAM Panel selected a lowest $BMDL_{10}$ for combined adenomas and carcinomas of the mouse small intestine as the reference point. Overall, the calculated MOEs indicate low concern regarding Cr(VI) intake via drinking water (water intended for human consumption and natural mineral waters) for all age groups when considering the mean chronic exposure values with the exception of infants at the upper bound (UB) exposure estimates. MOEs below 10 000 were calculated at the UB 95th percentile exposure estimates, particularly for 'Infants', 'Toddlers' and 'Other children', which were highly influenced by the relatively high occurrence values under the UB assumption. To improve the risk assessment, there is a need for data on the content of Cr(III) and Cr(VI) in food and drinking water.

KEY WORDS

trivalent chromium, hexavalent chromium, chemistry, analysis, human dietary exposure, toxicity, risk assessment, benchmark dose, margin of exposure (MOE), tolerable daily intake (TDI)

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[1] On request from the Hellenic Food Authority, Question No EFSA-Q-2012-00379, adopted on 27 February 2014.

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SUMMARY

In March 2012, the European Food Safety Authority (EFSA) received a request from the Hellenic Food Authority (EFET) for a scientific opinion on estimation of the risk to human health from the presence of chromium (Cr) in food and Cr(VI) in bottled water.

Chromium is a metal widely distributed in the environment occurring in rocks, soil and volcanic dust and gases. Chromium can exist in a variety of oxidation states, with the trivalent (Cr(III)) and hexavalent (Cr(VI)) states being relatively stable and largely predominant. While Cr(III) is a natural dietary constituent present in a variety of foods and also in dietary supplements, Cr(VI) most commonly occurs in industrial processes and is present in drinking water usually as a consequence of anthropogenic contamination.

At human dietary exposure levels chromium absorption is relatively low (< 10 % of the ingested dose) and depends on its valence state and ligands. Most of the ingested Cr(VI) is considered to be reduced in the stomach to Cr(III), which is poorly bioavailable and presents low ability to enter cells. In contrast to Cr(III), Cr(VI) is able to cross cellular membranes. The interconversion of Cr(VI) to Cr(III) is of relevance for risk assessment since, in general, Cr(VI) compounds are much more toxic than Cr(III) compounds.

There are no maximum levels (MLs) for chromium in food. A parametric value of 50 µg Cr/L for total chromium in water intended for human consumption and a Maximum Limit of 50 µg Cr/L for total chromium in natural mineral waters are laid down in Council Directive 98/83/EC and in Commission Directive 2003/40/EC, respectively.

The International Agency for Research on Cancer (IARC) has classified Cr(VI) compounds as carcinogenic to humans (Group 1) with respect to the cancer of the lung and also cancer of the nose and nasal sinuses based on evidence from occupational studies.

Following a call for data on chromium (trivalent and hexavalent) levels in food and drinking water (water intended for human consumption and mineral waters), a total of 79 809 analytical results on chromium were available in the EFSA database by the end of February 2013. A total of 27 074 analytical results were reported for food and 52 735 for all types of drinking water (including e.g. tap water, bottled water and well water) covering the period from 2000 to 2012. Data were mainly from 1 Member State although 11 other European countries were represented. Information on oxidation state was not available for occurrence data in food, and for drinking water only 88 analytical results were received on Cr(VI), all in bottled water.

Almost 50 % of the results on food samples were left-censored. After data cleaning and validation and using different cut-offs based on the reported limits of quantification (LOQs), 24 629 analytical results for food were considered for this assessment. At FoodEx level 1 all the food groups were well represented, with a maximum of 4 647 samples in the food group 'Vegetables and vegetable products (including fungi)'. The five food groups of highest average chromium occurrence values were 'Products for special nutritional use', 'Herbs, spices and condiments', 'Sugar and confectionary', 'Vegetables and vegetable products (including fungi)', and 'Animal and vegetable fats and oils'.

There is a lack of data on the presence of Cr(VI) in food. The EFSA Panel on Contaminants in the Food Chain (CONTAM Panel) decided to consider all the reported analytical results in food as Cr(III). This assumption was based on the outcome of recent speciation work, the fact that food is by-and-large a reducing medium, and that oxidation of Cr(III) to Cr(VI) would not be favoured in such a medium.

However, the CONTAM Panel noted that if even a small proportion of total chromium in food was in the form of Cr(VI), it could contribute substantially to Cr(VI) exposure.

Chronic dietary exposure to Cr(III) was estimated combining the food mean occurrence data with the food consumption data at the individual level. Following the standard representation used for CONTAM opinions, lower bound (LB) and upper bound (UB) mean chronic dietary exposure values were calculated for Cr(III), across the different dietary surveys and age classes. Overall mean human chronic dietary exposure ranged from a minimum LB of 0.6 to a maximum UB of 5.9 µg/kg b.w. per

day. The 95th percentile dietary exposure values ranged from 1.1 (minimum LB) to 9.0 (maximum UB) µg/kg b.w. per day. Among the different age classes, 'Toddlers' showed the highest mean chronic dietary exposure to Cr(III) with minimum LB of 2.3 and maximum UB of 5.9 µg/kg b.w. per day. The adult populations ('Adult', 'Elderly' and 'Very elderly') showed lower exposure to Cr(III) than the younger populations. The mean chronic dietary exposure to Cr(III) varied between 0.6 µg/kg b.w. per day and 1.6 µg/kg b.w. per day (minimum LB and maximum UB, adults in both cases). The 95th percentile chronic dietary exposure ranged from 1.1 µg/kg b.w. per day (minimum LB, 'Elderly') and 2.6 µg/kg b.w. per day (maximum UB, adults).

In 'Infants' and 'Toddlers' the main contributors to the chronic exposure to Cr(III) were 'Foods for infants and small children', followed by 'Milk and dairy products' and 'Bread and rolls'. In the other age classes, the main contributors to the chronic exposure to Cr(III) were the food categories 'Milk and dairy products, 'Bread and rolls', 'Chocolate (cocoa) products' (except for 'Elderly' and 'Very elderly' population) and 'Non-alcoholic beverages'. The food group 'Vegetables and vegetable products (including fungi)' contributed to the exposure to Cr(III) with median values that ranged from 4 % in 'Adolescents' and 'Other children', to 8 % in the 'Elderly' population.

The assessment of the dietary exposure to Cr(III) in vegetarians was based on very limited data. The results indicated that virtually the same mean and 95th dietary exposure are likely in the vegetarian population as compared to the general population.

Overall, the Comprehensive Database contains limited information on the consumption of fortified foods, foodstuffs for particular nutritional use (PARNUTS) and food supplements. Based on previous EFSA opinions, the combined exposure from supplemental intake in adults (i.e. from fortified foods, PARNUTS and food supplements) would be between 910 µg/day for a typical intake and 1540 µg/day for upper intake (13 µg/kg b.w. per day and 22 µg/kg b.w. per day, respectively, for an adult of 70 kg b.w.).

In the FoodEx classification system, the different types of water are grouped under the generic name 'Drinking water'. Therefore, the generic term drinking water as used in this opinion includes both categories defined by the EU legislation, i.e. water intended for human consumption and natural mineral waters. Bottled water as used in this opinion includes natural mineral water, but also spring water and other bottled drinking waters, products that must comply with Council Directive 98/83/EC.

More than 90 % of the results for all types of drinking water were left-censored. Concerning the data on bottled water, 11 % of the samples analysed both for Cr(VI) and total chromium reported no quantified values for both parameters. After data cleaning and validation, and applying a cut-off value of 10 µg/L on the LOQs reported for total chromium, a total of 46 234 analytical results on water (including 88 results on Cr(VI)) were selected for exposure calculations. Tap water samples were the most reported (61 %) with LB and UB mean occurrence values of 0.2 µg/L and 1.9 µg/L, respectively. In bottled water, the mean occurrence values ranged between 0.3 µg/L for carbonated mineral water (LB) and 3.4 µg/L at the UB reported for unspecified bottled water.

The CONTAM Panel assumed that all chromium present in drinking water was Cr(VI) (worst case scenario) based on two reasons. First, the samples where both Cr(VI) and total chromium were quantified (71 out of 88 samples) showed an average ratio Cr(VI)/total chromium of 0.97. In addition the water intended for human consumption is usually treated with different oxidizing agents to make it potable, and this would promote the presence of Cr(VI) over that of Cr(III).

The CONTAM Panel estimated separately the exposure to Cr(VI) in all types of drinking water and in bottled water. The mean chronic exposure to Cr(VI) from consumption of all types of drinking water ranged from 0.7 (minimum LB) to 159.1 ng/kg b.w. per day (maximum UB). The 95th percentile exposure ranged from 2.8 (minimum LB) to 320.2 (maximum UB) ng/kg b.w. per day. The highest exposure to Cr(VI) through the consumption of all types of drinking water was estimated in the youngest populations ('Infants' and 'Toddlers'). No consumption of bottled water was reported in several dietary surveys. In those dietary surveys with reported data on consumption of bottled water, the highest exposure to Cr(VI) was also estimated in the youngest populations ('Infants' and 'Toddlers'), with a mean chronic exposure ranging from < 0.1 (minimum LB) to

149.8 ng/kg b.w. per day (maximum UB, infants). The 95th percentile exposure ranged from 0.0 (minimum LB) to 148.7 ng/kg b.w. per day (maximum UB, 'Toddlers').

An additional contribution to the exposure to Cr(VI) was considered from the water used to prepare certain foods (coffee, tea infusions, and infant dry and follow-on food mainly, but also some others such as instant soup, evaporated and dried milk, and dehydrated fruit juice). A worst-case scenario, with no reduction of the Cr(VI) present in water into Cr(III) when the foods are ingested immediately after their preparation, was assumed. This scenario led to an increase up to two-fold in the exposure levels to Cr(VI), in comparison to those estimated via the consumption of drinking water only.

The CONTAM Panel concluded that the exposure via the diet likely represents the most important contribution to the overall exposure to Cr in the general population. Inhalation of Cr compounds present in particular in cigarette smoke may contribute to the overall exposure levels but the currently available information does not allow quantification of its relative contribution.

Cr(III) compounds present low oral toxicity because they are poorly absorbed. Cr(III) compounds have the potential to react with DNA in acellular systems, however restricted cellular access limits or prevents genotoxicity. The CONTAM Panel decided to use the data from the chronic toxicity studies of the National Toxicology Programme (NTP) on chromium picolinate monohydrate to derive a health-based guidance value (HBGV) for the risk characterization of Cr(III). In the two year NTP chronic oral toxicity study in rats and mice, no carcinogenic or other adverse effects have been observed. The lowest no-observed-adverse-effect level (NOAEL) value derived from these studies amounted to 286 mg/kg b.w. per day in rats, which was the highest dose tested. Effects of Cr(III) on reproduction and developmental toxicity have been reported in some studies with the lowest lowest-observed-adverse-effect levels (LOAELs) in the order of 30 mg/kg b.w. per day, but the Panel noted that these studies had methodological limitations. In addition, no effects have been reported on reproductive organ weights, sperm parameters and oestrous cyclicity in subchronic dietary studies in rats or mice at the highest doses tested (506 mg/kg b.w. per day and 1090 mg/kg b.w. per day, respectively) (NTP studies). Taking these observations together, the Panel derived a Tolerable Daily Intake (TDI) of 300 µg Cr(III)/kg b.w. per day from the relevant NOAEL in the long-term rat NTP study of 286 mg/kg b.w. per day, applying a default uncertainty factor of 100 to account for species differences and human variability and an additional uncertainty factor of 10 to account for the absence of adequate data on reproductive and developmental toxicity.

Under the assumption that all chromium in food is Cr(III), the CONTAM Panel noted that the mean dietary exposure levels across all age groups (minimum LB of 0.6 µg/kg b.w. per day and maximum UB of 5.9 µg/kg b.w. per day) as well as the 95th percentile exposure (minimum LB of 1.1 µg/kg b.w. per day and maximum UB of 9.0 µg/kg b.w. per day) are well below the TDI of 300 µg Cr(III)/ kg b.w. per day.

Regarding the vegetarian population, although based on limited consumption data, the dietary exposure to Cr(III) seems to be similar to that estimated for the general population. Thus, also the dietary exposure of vegeterians is well below the TDI of 300 µg Cr(III)/ kg b.w. per day.

A significant exposure to Cr(III) may occur via dietary supplement intake. Considering the exposure via dietary supplement intake (13 µg/kg b.w. per day and 22 µg/kg b.w. per day, for typical and upper intake from fortified foods, PARNUTS and food supplements, respectively, for an adult of 70 kg b.w.) and the maximum estimated contribution coming from the diet for adults (95th percentile of 2.6 µg/kg b.w. per day), the total exposure remains below the TDI of 300 µg Cr(III)/ kg b.w. per day.

After oral exposure, Cr(VI) has been shown to be carcinogenic in rats and mice of both sexes and genotoxic in some *in vivo* studies. The data available so far support that the reduction of Cr(VI) to Cr(III) along the gastrointestinal tract is efficient but it cannot be excluded that even at low dose levels a small percentage of Cr(VI) escapes gastrointestinal reduction to Cr(III). Once taken up in the cells, Cr(VI) is reduced to Cr(III) with formation of Cr-DNA adducts and production of oxidative stress (due to formation of reactive intermediates). Both modes of action can contribute to the genotoxicity and carcinogenicity of Cr(VI).

As recommended for substances which are both genotoxic and carcinogenic, the CONTAM Panel adopted a margin of exposure (MOE) approach for the risk characterisation of neoplastic effects of Cr(VI). To this end, lower 95 % confidence limit for a benchmark response of 10 % extra risk ($BMDL_{10}$) values were derived from the 2-year carcinogenicity study of the NTP investigating oral intake of Cr(VI) (as sodium dichromate dihydrate) via drinking water in male and female rats and mice. In this study increased incidence of tumours of the squamous epithelium of the oral cavity and of epithelial tissues of the small intestine was reported in male and female rats and mice, respectively. In a conservative approach, the CONTAM Panel selected a lowest $BMDL_{10}$ of 1.0 mg Cr(VI)/kg b.w. per day for combined adenomas and carcinomas of the small intestine in male and female mice as reference point (RP) for estimation of MOEs for neoplastic effects.

The EFSA Scientific Committee has concluded that for substances that are both genotoxic and carcinogenic, an MOE of 10 000 or higher, based on a $BMDL_{10}$ from an animal study, is of low concern from a public health point of view.

The MOEs calculated for all age groups on the basis of the mean chronic exposure to Cr(VI) via consumption of drinking water indicated a low concern (MOE values > 10 000) for all age groups with the exception of infants at UB exposure estimates (maximum UB - minimum LB, 6 300 - 71 000).

When considering the 95th percentile exposure, MOE values below 10 000 were found at UB exposure estimates, particularly for 'Infants' (maximum UB - minimum LB, 3 100 - 21 000), 'Toddlers' (maximum UB - minimum LB, 4 200 - 62 000), and 'Other children' (maximum UB - minimum LB, 6 600 - 360 000).

Similarly to the risk characterization carried out for all types of drinking water, in the case of exposure to Cr(VI) through the consumption of bottled water MOEs values below 10 000 were mainly found at UB estimates when considering the 95th percentile exposure in the youngest populations ('Infants', 'Toddlers' and 'Other children').

The CONTAM Panel noted that the MOE values calculated for exposure to Cr(VI) via consumption of all types of drinking water, as well as only bottled water were highly influenced by the high proportion of left-censored data. In addition, when interpreting the numerical values of the MOEs, it should be considered that they were calculated by using as RP the $BMDL_{10}$ for the combined incidence of adenomas and carcinomas in the mouse small intestine. Because of lack of *in vivo* data on the capacity and rate of reduction of Cr(VI) in the rodent and human gastrointestinal tract, there is a significant uncertainty associated with the use of tumour data in mice to estimate risk at doses of Cr(VI) relevant for human exposure.

Based on the MOE values for neoplastic effects, the CONTAM Panel concluded that the current levels of exposure to Cr(VI) via the consumption of all types of drinking water or of bottled water only are of low concern from a public health point of view for the average consumers but there might be a potential concern for high consumers particularly in 'Infants', 'Toddlers' and 'Other children'.

The inclusion of the water used in the preparation of specific foods (coffee, tea infusions, and infant dry and follow-on food) led to an increase up to two-fold of the exposure to Cr(VI). However, the CONTAM Panel was not able to consider this additional contribution to the exposure to Cr(VI) when deriving MOEs since no reliable data to quantify Cr(VI) in food exist.

After repeated oral administration of Cr(VI), in addition to the cancer effects, several toxic effects were identified in rats and mice including microcytic, hypochromic anaemia, and non-neoplastic lesions of the liver, duodenum, mesenteric and pancreatic lymph nodes and pancreas. BMD analysis was performed on the suitable dose-response data for non-neoplastic effects. The $BMDL_{10}$ values of 0.27, 0.11 and 0.011 mg Cr(VI)/kg b.w. per day were calculated for non-neoplastic lesions in pancreas (acinus, cytoplasmic alteration), duodenum (diffuse epithelial hyperplasia) and liver (histiocytic infiltration), respectively. The Panel noted that the biological significance and cause of histiocytic cellular infiltration are unknown and therefore it cannot be considered a critical adverse effect. The $BMDL_{10}$ value of 0.11 mg Cr(VI)/kg b.w. per day for diffuse epithelial hyperplasia of the duodenum in male mice was selected as the RP for the estimation of the MOE for non-neoplastic lesions in the intestine. In the case of haematological effects a $BMDL_{05}$ of 0.2 mg Cr(VI)/kg b.w. per day was

calculated for decrease of haematocrit in male rats. The CONTAM Panel selected this value to be used as the RP for MOE estimation of haematotoxic effects of Cr(VI). The comparison of these RPs with estimated daily intakes of Cr(VI) via drinking water ranging up to 159.1 and 320.2 ng/kg b.w. per day (maximum UB for mean and 95th percentile exposure) for the different age groups resulted in MOEs of 690 and 340 for non-neoplastic lesions, and MOEs of 1300 and 630 for hematotoxic effects, respectively. The CONTAM Panel considered that for the critical thresholded effects, MOEs larger than 100 would indicate no concern for human health and therefore concluded that for non-neoplastic lesions and haematological effects the current exposure levels to Cr(VI) via drinking water are of no concern from a public health point of view.

The Panel recommended the generation of data using sensitive analytical methodologies which specifically measure the content of Cr(III) and Cr(VI) in food and drinking water in different EU Member States. In addition the CONTAM Panel recommended that further data for the characterisation of Cr(VI) reduction in the GI tract at doses relevant for human exposure and at the doses used in the rodent bioassays should be generated.

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BACKGROUND AS PROVIDED BY THE HELLENIC FOOD AUTHORITY (EFET)

Chromium is a steely-gray, hard metal that occurs naturally everywhere in the environment. It can exist in a number of different oxidation states, ranging from - 2 to + 6 but the most stable forms are elemental chromium, trivalent chromium (chromium III) and hexavalent chromium (chromium VI). Chromium is released into the environment by natural processes (mainly dust from rocks and volcanic activity) and, to a greater extent, by human activities (metal industries, burning of oil and coal, waste incineration etc). Due to its strong resistance to corrosion, chromium is commonly used in the production of stainless steel and for surface coating through electroplating. Other uses of chromium include dyes and colour pigments, tanning of leather, wood preservatives and catalysts.

The International Agency for Research on Cancer (IARC) has classified chromium VI as carcinogenic to humans (Group 1) while metallic chromium and chromium III compounds were not classifiable as to their carcinogenicity to humans (Group 3) (IARC, 1990)[4]. The occurrence of hexavalent chromium compounds is rare and nearly always man-made. Chromium III is considered to be an essential element both in animal and human nutrition[5].

Exposure to chromium for the general population occurs primarily via food and drinking water, but also through inhalation of ambient air. Cigarette smoking is another important source of chromium exposure.

There is presently no EU regulation regarding maximum levels of chromium in food. For water intended for human consumption, a quality standard of 50 µg/L for total chromium is laid down in Council Directive 98/83/EC[6], but no level is available specifically for chromium VI.

In 2011 the Hellenic Food Authority (EFET) monitored the presence of total chromium in food crops and bottled water. In food crops concentrations of up to 0.96 mg/kg total chromium were measured. All the tested samples of bottled water contained total chromium at concentrations lower than the drinking water quality standard of 50 µg/L. However, there is evidence from the surveys carried out in Greece that the concentrations of chromium VI can reach up to 36 µg/L in bottled water.

TERMS OF REFERENCE AS PROVIDED BY THE HELLENIC FOOD AUTHORITY (EFET)

In accordance with Art 29 (1) of Regulation (EC) No 178/2002, the Hellenic Food Authority asks the European Food Safety Authority to provide a scientific opinion on the risk to human health related to the presence of chromium in food addressing particularly the presence of chromium in vegetables and hexavalent chromium (chromium VI) in bottled water.

The scientific opinion should:

- Consider any relevant information on toxicity of chromium III and chromium VI, considering all relevant toxicological endpoints;
- Assess the contribution of different foodstuffs to human exposure to total chromium. This should particularly include the contribution of chromium in vegetables and chromium VI in bottled water. An indication of non-dietary sources of exposure (e.g. air, cigarette smoke) should be given.
- Contain a dietary exposure assessment of chromium taking into account the recent analytical results on the occurrence on chromium III and chromium VI in food and bottled water, and the consumption patterns of specific (vulnerable) groups of the population (e.g. high consumers, children, people following a specific diet, etc).

[4] IARC Monograph on the Evaluation of Carcinogenic Risks to Humans (1990). Chromium, Nickel and welding. Volume 49. Available at: http://monographs.iarc.fr/ENG/Monographs/vol49/mono49.pdf.
[5] Opinion of the Scientific Committee on Food on the Tolerable Upper Intake Level of Trivalent Chromium (expressed in 4 April 2003). Available at http://ec.europa.eu/food/fs/sc/scf/out197_en.pdf
[6] Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption, OJ L 330, 5.12.98, p. 32-54.

- Available biomonitoring data should be taken into account and the results be compared with the calculated exposure levels.

ASSESSMENT

1. Introduction

Chromium (Cr) was discovered in the second half of the 18th century: its elemental state and compounds have been the subject of extensive research, also due to their diverse industrial applications. It occurs in a variety of valence states with trivalent (Cr(III)) and hexavalent (Cr(VI)) being the most stable and biologically relevant oxidation states. For the general population, food is the major source of exposure to chromium (> 90 % of the total intake). Drinking water may also be a substantial source of exposure if chromium levels are exceptionally high. Cr(III) is a natural dietary constituent present in a variety of foods and also in dietary supplements. Conversely, Cr(VI) seems to be absent in food and its presence in drinking water is usually a consequence of anthropogenic activity. Over the last years there have been several reports of naturally occurring Cr(VI) in groundwater. Although in most cases the Cr(VI) concentrations found appear to be in the order of a few µg/L or some tens of µg/L, values of a few hundreds of µg/L are not unusual.

Chromium absorption after dietary exposure in humans is relatively low (< 10 % of the ingested dose) and is affected by the valence state and the nature of its ligands. Cr(VI) is reduced in the stomach to Cr(III), which lowers the absorbed dose from ingested Cr(VI). The interconversion of the two species is of relevance for risk assessment since, in general, Cr(VI) compounds are more toxic than Cr(III) compounds. This is mostly due to the more effective cellular uptake of Cr(VI) as compared to Cr(III). Cr(III) presents a low oral toxicity due to poor bioavailability. Oral exposure to Cr(VI) compounds is associated with gastrointestinal system cancers in experimental animals. In humans, Cr(VI) is a known carcinogen by the inhalation route of exposure and Cr(VI) compounds are classified by the International Agency for Research on Cancer (IARC) as carcinogenic to humans (Group 1).

There are no maximum levels (MLs) set for chromium in food. A parametric value of 50 µg Cr/L for total chromium in water intended for human consumption and a Maximum Limit of 50 µg Cr/L for total chromium in natural mineral waters are laid down in Council Directive 98/83/EC and in Commission Directive 2003/40/EC, respectively.

In March 2012, the European Food Safety Authority (EFSA) received a mandate from the Hellenic Food Authority (EFET) for a scientific opinion on estimation of the risk to human health from the presence total Cr in food and Cr(VI) in bottled water. This scientific opinion addresses the risks for public health related to the presence of total Cr in food and Cr(VI) in water intended for human consumption and natural mineral waters.

1.1. Chemistry and physico-chemical properties

In this Section, a summary of the current knowledge on a number of physico-chemical, environment-related properties of chromium is given. Due to the very large number of scientific publications, technical reports and reviews, and educational and press releases available on these topics, no references are provided in the text unless specifically required. For additional detailed information, a number of general scientific references are available (e.g. Papp and Lipin, 2001; WHO, 2003; OEHHA, 2011; Saha et al., 2011; Zhitkovich, 2011; ATSDR, 2012; McNeill et al., 2012a, b).

1.1.1. General aspects

Chromium (Cr; CAS registry No. 7440-47-3) is widely distributed in the earth's crust, almost always in the trivalent chromic state (Cr^{3+} or Cr(III); CAS registry 16065-83-1); its concentration is in the order of few tens of mg/kg in most soils. The metal is produced in large quantities for industrial purposes, its principal ore being ferrochromite ($FeCr_2O_4$ or $FeOCr_2O_3$, in short chromite), in which the element is present as Cr(III) and iron as Fe^{2+} (Fe(II), ferrous state). For incorporation in iron alloys, chromite is simply reduced with carbon in an electric arc furnace where ferrochrome - also known as ferrochromium, an alloy of iron and approximately 50-70 % chromium - is concurrently generated:

- $FeCr_2O_4 + 4\,C \rightarrow Fe + 2\,Cr + 4\,CO$

Ferrochrome is commonly used as a raw material to produce stainless steel, a popular corrosion-proof alloy usually formed by adding chromium to iron in concentrations above 11 %. To obtain pure chromium different methods exist. For instance, chromite can be treated with oxygen in molten alkalis to oxidize Cr(III) to the hexavalent oxidation state (Cr^{6+} or Cr(VI); CAS registry 18540-29-9). The latter (chromate) is dissolved in water and eventually precipitated as sodium dichromate: this is reduced to Cr(III) oxide which, in turn, is reduced with aluminium to the pure metal (aluminothermic method):

- $Na_2Cr_2O_7 + 2\,C \rightarrow Cr_2O_3 + Na_2CO_3 + CO$
- $Cr_2O_3 + 2\,Al \rightarrow 2\,Cr + Al_2O_3$

Chromite ore and chromite concentrates are produced mainly by South Africa and, to a lesser but similar extent, India and Kazakhstan: these countries together accounted for some 70 % of total world production (approximately 24×10^6 metric tonnes) in 2008. Other important producers are Albania, Brazil, Finland, and Turkey. In the same year, over 90 % of the global chromite production was converted to ferrochrome for metallurgical applications (Korinek and Kim, 2010).

1.1.2. Uses and applications

Most chromium produced today is used in alloys, including stainless steel, a metal with wide applications. Chromium is also used to cover the surface of other metals by electroplating (specifically, chrome-plating) to protect the base metal from corrosion and give the surface a lustrous appearance. Some chromium is also used to make refractory bricks, a material that can withstand very high temperatures such as those of high-temperature ovens. Chromium and its salts are used in the leather tanning industry, the manufacture of catalysts, pigments, paints, and fungicides/pesticides, the ceramic and glass industry, the production of synthetic ruby and recording tapes, photography, and as laboratory reagents. Cr(III) organic complexes, such as Cr(III) nicotinates and picolinate (Figures 1a and b), are used as nutritional supplements for human use (EFSA, 2008a; EFSA ANS Panel, 2010a). A selection of chromium compounds is presented in Table 1.

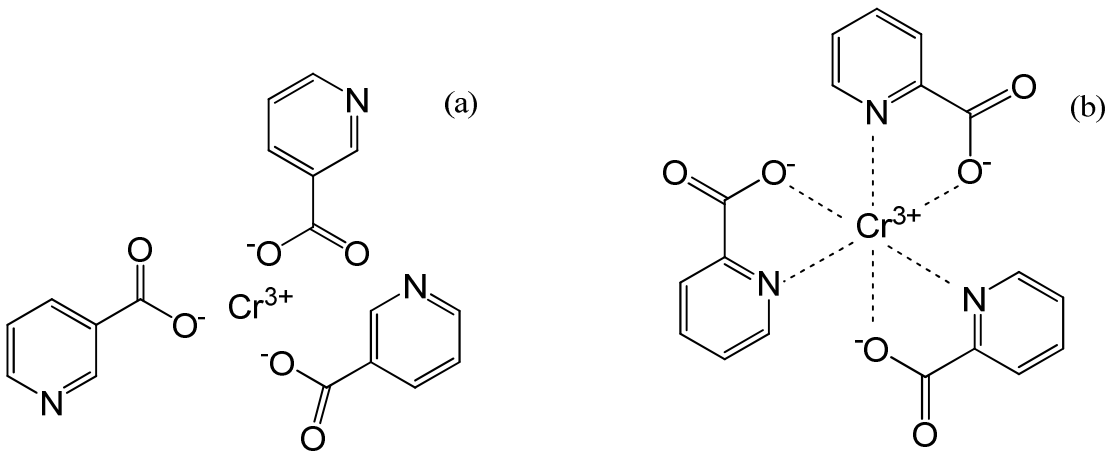

Figure 1: Chemical structures of chromium trinicotinate (a) and chromium picolinate (b).

Table 1: Some relevant chromium compounds and their key properties[(a)].

Compound	Formula	CAS Registry	MW (amu)	Water solubility[(b)]	MP (°C)	BP (°C)
		Chromium(0) compounds				
Hexacarbonyl[(c)]	$Cr(CO)_6$	13007-92-6	220.06	■	90	130[(d)]
		Chromium(III) compounds				
Acetate [(e)]	$Cr(CH_3COO)_3 \cdot H_2O$	25013-82-5	247.14	■■	Solid[(l)]	—
Boride [(f)]	CrB	12006-79-0	62.81	■	2760	—
Chloride	$CrCl_3$	10025-73-7	158.35	■	≈ 1150	1300[(d)]
Chloride [(g)]	$CrCl_3 \cdot 6H_2O$	10060-12-5	266.45	■■■	83	—
Fluoride [(f)]	CrF_3	7788-97-8	108.99	■	> 1000	—
Dinicotinate [(f,h)]	$Cr(C_6H_4NO_2)_2^+ \cdot X^-$	—	370.26	—	Solid[(l)]	—
Trinicotinate [(f)]	$Cr(C_6H_4NO_2)_3$	64452-96-6	418.30	■	Solid[(l)]	—
Nitrate [(i)]	$Cr(NO_3)_3 \cdot 9H_2O$	7789-02-8	400.15	■■	60	100[(d)]
Oxide	Cr_2O_3	1308-38-9	151.99	■	2435	3000
Picolinate	$Cr(C_6H_4NO_2)_3$	14639-25-9	418.30	■	Solid[(l)]	—
Potassium sulphate [(f,j)]	$CrK(SO_4)_2 \cdot 12H_2O$	7788-99-0	499.40	■■■	89	330
Sulphate	$Cr_2(SO_4)_3$	10101-53-8	392.18	■	Solid[(l)]	—
		Chromium(IV) compounds				
Dioxide	CrO_2	12018-01-8	83.99	■	Solid[(l)]	—
		Chromium(VI) compounds				
Chromic acid	H_2CrO_4	7738-94-5	118.01	■■■	196	—[(d)]
Lead chromate	$PbCrO_4$	7758-97-6	323.19	■	844	—[(d)]
Potassium chromate	K_2CrO_4	7789-00-6	194.19	■■■	975	—
Potassium dichromate	$K_2Cr_2O_7$	7778-50-9	294.18	■■	398	500[(d)]
Sodium chromate	Na_2CrO_4	7775-11-3	161.97	■■■	792	—
Sodium dichromate	$Na_2Cr_2O_7$	10588-01-9	261.97	■■■	357	400[(d)]
Sodium dichromate [(k)]	$Na_2Cr_2O_7 \cdot 2H_2O$	7789-12-0	298.00	■■■	357	400[(d)]
Trioxide	CrO_3	1333-82-0	99.99	■■■	197	—[(d)]

MW: molecular weight; MP: melting point; BP: boiling point.

(a): Most data derived from ATSDR (2012). All data shown in the table are cross-checked with diverse literature and Internet sources.

(b): Generally reported at, or near, room temperature ■: insoluble or slightly soluble; ■■: fairly soluble; ■■■: very or freely soluble.

(c): Data from Patnaik (2003, 2007).

(d): Decomposition.

(e): Monohydrate.

(f): Data from Internet sources.

(g): Hexahydrate.

(h): X^-, glycinate anion ($H_2N\text{-}CH_2\text{-}COO^-$).

(i): Nonahydrate.

(j): Dodecahydrate.

(k): Dehydrates at 100 °C.

(l): In the absence of a reliable melting point estimate, the term merely indicates the physical state under standard conditions.

1.1.3. Physico-chemical properties

Elemental chromium is a silvery, shiny, hard, and brittle metal with the following key physico-chemical properties (Table 2).

Table 2: Some physico-chemical properties of elemental chromium

Atomic number: 24	Boiling point: 2672 °C
Atomic mass: 51.9961 amu	Vapor pressure: 990 Pa (1857 °C)
Chemical family: Group 6, transition metals	Density: 7.19 g/cm^3 (20 °C)
Electron shell configuration: [Ar], $3d^5$, $4s^1$	Solubility in water: insoluble
Electronegativity (Pauling scale): 1.66	Resistant to ordinary corrosive agents
Melting point: 1857 (± 20) °C	Dissolves fairly readily in non-oxidizing mineral acids (e.g. hydrochloric acid), but not in oxidizing acid media (e.g. nitric acid) due to passivation

The solubility of chromium compounds depends in part on the oxidation state. What follows refers to observations at or around room temperature. The monohydrate acetate, hexahydrate chloride, hydroxide sulphate, and nitrate salts of Cr(III) are soluble in water and possibly in common polar organic solvents; however, Cr(III) chloride, (dichromium) iron tetraoxide, oxide, phosphate, sulphate, and picolinate exhibit a scant or no solubility in water (chromium picolinate is more soluble in polar organic solvents). Jelly-like $Cr(OH)_3$ (chromium(III) trihydroxide) has an amphoteric behaviour, the pH value having a strong influence on its solubility and the type of hydroxo-species that are formed following interaction with the aqueous media (Rai et al., 1987, 2004): a minimum solubility is observed between pH 7 and 10. Cr(IV) dioxide (CrO_2) is insoluble in water. As to Cr(VI) compounds, zinc and lead chromates are practically insoluble in water, whereas the chromates of alkaline earth metals are only slightly soluble; CrO_3 (chromium trioxide or chromic acid) and its ammonium and alkali metal salts are in general readily or quite soluble in water. Some Cr(VI) compounds also show a solubility in polar organic solvents.

1.1.4. Natural and artificial isotopes

There are four naturally occurring stable chromium isotopes, with mass numbers 50 (4.3 %), 52 (83.8 %), 53 (9.5 %), and 54 (2.4 %). Several radioactive isotopes are also known, all artificial: with the exception of ^{51}Cr, they exhibit very short half-lives, in general much shorter than 24 hours. ^{51}Cr, whose decay is by electron capture with emission of 0.32-MeV gamma rays and a half-life of 27.7 days, has been used as a tracer in medical research on blood: for example, $Na_2{}^{51}CrO_4$ has been employed to tag red blood cells (RBCs) and platelets in survival studies and blood volume measurements (Gray and Sterling, 1950; Najean et al., 1963; Pearson, 1963; Dever et al., 1989; Veillon et al., 1994); in addition, ^{51}Cr is commonly used in toxicokinetics investigations. ^{50}Cr is also suspected of being radioactive, but with such a long half-life (> 10^{17} years) that it is regarded as a stable isotope.

1.1.5. Redox chemistry

Aside from possible negative oxidation states, of no interest in this opinion, chromium can exist in oxidation states from Cr(I) (Cr^{1+}) to Cr(VI), with the trivalent and hexavalent states being largely predominant. Elemental chromium, Cr(0), seldom if ever occurs naturally. Cr(V) and Cr(IV), of which a few solid compounds are known, are observed as transient labile species in the reduction of Cr(VI) solutions; on the other hand, in solution they both can readily transform to Cr(III) and Cr(VI).

As is typical of transition metals, chromium compounds are characterized by an elaborate co-ordination chemistry (Cotton et al., 1999), whose principal morphologic features may be summarized as follows: an octahedral geometry is associated with a coordination number of 6 and with all the oxidation states from Cr(0) to Cr(V); Cr(V) also exhibits a tetrahedral geometry with a coordination number of 4, just like Cr(VI). Clear examples of octahedral and tetrahedral geometries are exhibited in Figures 2 and 3. As discussed later in the opinion (see Section 7.1), oxidation state and molecular geometry of chromium compounds have a strong bearing on cellular uptake. Much of chromium chemistry deals with Lewis acid-base coordination complexes, in which ligands (ions or molecules) bind to the coordinating metal (atom or ion): ligands act as electron-pair donors (Lewis bases) while the metal acts as an electron-pair acceptor (Lewis acid) owing to its valence-shell orbitals that can

accommodate electron pairs. Therefore, ligands must have at least one pair of electrons suitable for being donated to the metal. The metal-ligand bonding can have various degrees of covalent nature even when both chromium and ligands are formally ionic species.

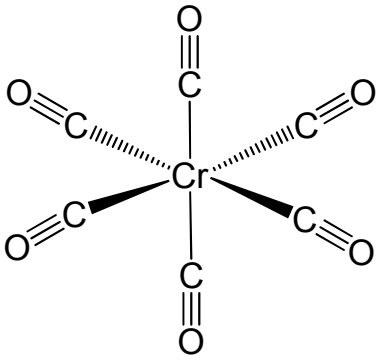

Figure 2: Chromium hexa-carbonyl

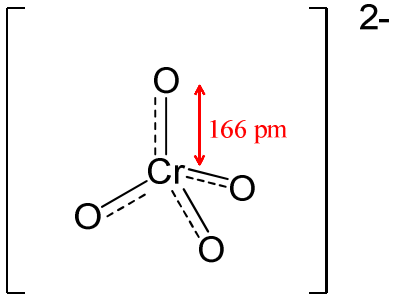

Figure 3: Chromate ion

DIVALENT CHROMIUM. All Cr(II) compounds are energetic reducing agents and under environmental conditions Cr(II) (chromous state) is relatively unstable. In aqueous media, the chromous ion is readily oxidized to the stable Cr(III) species ($Cr^{3+} + e^- \rightarrow Cr^{2+}$; $E^0 = -0.41$ V), for instance by the dissolved molecular oxygen, O_2 (Kotaś and Stasicka, 2000). Therefore, Cr(II) solutions can only be preserved if degassed (anaerobic conditions). The only coordination number observed for Cr(II) is six, in the form of a twisted octahedral geometry.

TRIVALENT CHROMIUM. Cr(III) is the most stable and important oxidation state of the element, in particular in relation to its aqueous chemistry (Kotaś and Stasicka, 2000). This state is characterized by the formation of a very large number of relatively kinetically inert complexes, in which Cr(III) is always hexacoordinate (octahedral geometry). This kinetic inertness allows many complex species to be isolated as solids and to persist for relatively long periods of time in solution, even if their thermodynamical condition is unstable. In aqueous media and in the absence of specific ligands, Cr(III) is present as $Cr(H_2O)_6^{3+}$ (hexa-aquachromium$^{(3+)}$, a moderately strong acid), $Cr(OH)_3$ (chromium trihydroxide), and their reaction products. Therefore, the aqueous compositions of these groups of substances are complex and depend on environmental conditions and their influence on processes such as hydrolysis, complexation, redox reactions, and adsorption. Even at naturally-occurring concentrations and substantially neutral pHs, Cr(III) compounds in aqueous systems may be actively oxidized to Cr(VI) by strong oxidants such as chlorine or hypochlorous acid, ozone, or potassium permanganate — used, for instance, in water purification treatments (Schroeder and Lee, 1975; Lai and McNeill, 2006; Saputro et al., 2011; Lindsay et al., 2012).

HEXAVALENT CHROMIUM. Cr(VI), or chromate, is the second most stable state: its compounds, whose aqueous chemistry is of particular relevance, primarily arise from anthropogenic sources (Shanker et al., 2005; Johnson et al., 2006). In addition to its occurrence in rare minerals, naturally occurring Cr(VI) has also been occasionally detected in groundwater (McNeill et al., 2012a). In its highest oxidation state, chromium forms oxy-compounds that are fairly potent oxidizing agents (Kotaś and Stasicka, 2000). In basic solutions (pH > 6.5), it exists predominantly as the yellow chromate ion (CrO_4^{2-}), exhibiting a coordination number of four and a tetrahedral geometry (Figure 3). As the pH is lowered (pH < 6), the solution of chromate ions turns orange owing to the formation of dichromate ions ($Cr_2O_7^{2-}$). In $Cr_2O_7^{2-}$ two chromium atoms are linked by an oxygen bridge and exhibit a slightly distorted tetrahedral geometry (Figure 4).

Acid solutions of dichromate are quite powerful oxidizing agents, the Cr(VI) reduction process yielding Cr(III). In basic solution, the chromate ion exhibits a much lower oxidizing power as the CrO_4^{2-} species undergoes a relative stabilization.

Cr(VI) compounds are reduced to the trivalent form in the presence of oxidizable substances (reductants). In natural waters, often characterized by a fair degree of acidity (Kotaś and Stasicka, 2000), Cr(VI) compounds are generally more stable as the concentration of reducing materials is relatively low. However, Fe(II) in solution or Fe(II)-bearing minerals, sulphides, and/or oxidizable organic matter may cause a reduction of Cr(VI) to Cr(III) (Schroeder and Lee, 1975; Fendorf, 1995; Loyaux-Lawniczak et al., 2001).

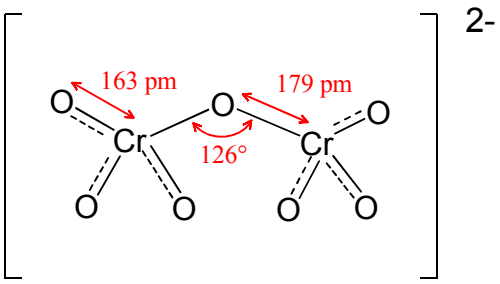

Figure 4: Dichromate ion

Conclusion

In conclusion, in aqueous media chromium generally occurs in the form of its two most stable oxidation states, Cr(III) and Cr(VI), both existing as complex groups of interrelated chemical species. As described, the distribution of species containing Cr(III) and Cr(VI) depends on the redox potential, the pH, the presence of oxidizing or reducing substances, the kinetics of redox reactions, the formation of Cr(III) complexes or insoluble Cr(III) compounds, and the total chromium concentration. In the environment, and specifically in aqueous media, the two forms are involved in rather complex equilibria, which may be easily altered if the ambient chemico-physical conditions are modified (for the technical problems in Cr(VI) analysis, see Section 3).

1.2. Environmental fate and sources of food and drinking water contamination

1.2.1. Environmental fate

In the atmosphere, chromium occurs from natural sources (e.g. volcanic emissions) as well as from many anthropogenic activities, including burning of fossil fuels and wood; the most important industrial sources of airborne chromium are associated with ferrochrome production. Both Cr(III), and Cr(VI) can be released into the air, although the latter to a lesser extent (WHO, 2003): due to analytical difficulties, chromium speciation data in air are very limited. In air, chromium is present in the form of aerosols that are removed by wet and dry deposition. Chromium particles of small aerodynamic diameter (< 10 µm) may remain airborne for long periods and undergo long-range transport. Under normal conditions, airborne Cr(0) and Cr(III) forms do not undergo any reaction, whereas Cr(VI) eventually reacts with dust particles or other pollutants to yield Cr(III) (U.S. EPA, 1998a, b).

As observed in the preceding Section, in the aquatic environment Cr(III) and Cr(VI) occur mostly as $Cr(OH)_n^{(3-n)+}$ and as CrO_4^{2-} or $HCrO_4^-$. In water, Cr(III) may form positive or negative ionic species at low or high pH values, respectively, whereas at intermediate pH values the neutral hydroxide form, $Cr(OH)_3^0$, is predominant. In surface waters, relatively high concentrations of Cr(VI) forms can be found locally (WHO, 2003). Surface runoff, deposition from air, and release of municipal and industrial waste waters are sources of chromium in surface waters. Cr(III) is lost from the aquatic environment primarily due to precipitation of hydrated Cr_2O_3 followed by sedimentation. The Cr(VI) anion species can persist in aquatic media, possibly for long periods, as water-soluble complexes; however, they will react with organic matter or other reducing agents to form Cr(III). Therefore, in surface waters rich in organic content, Cr(VI) will have a much shorter lifetime (U.S. EPA, 1998a, b).

In soil, Cr(III) predominates, likely as insoluble hydrated Cr_2O_3 forms: in addition to a direct release as a result of anthropogenic activities, trivalent chromium can easily arise from reduction of Cr(VI) species due to the presence of reductants. Chromium is lost from soil primarily due to physical processes. For instance, chromium-containing soil particles can be raised by air draughts and dispersed over long distances; likewise, runoff can remove from topsoil chromium ions and bulk precipitates of the metal. Flooding of soils and the subsequent decomposition of vegetal matter may also increase dissolution of soil-borne Cr_2O_3 through the formation of water-soluble chromium complexes which will possibly leach and percolate through soil (U.S. EPA, 1998a, b; WHO, 2003).

A study was conducted in 1991 to determine the levels of heavy metals and polycyclic aromatic hydrocarbons (PAHs) in soil along a busy road that runs through the Aplerbecker forest near the German town of Dortmund. Background concentrations of the metals were reached some 5-10 m away from the road. The concentration of chromium in the soil at the edge of the road showed a two- to four-fold increase relative to background levels, reaching up to 64 mg/kg (Münch, 1993). The thick vegetation structure of the forest and its barrier effect was discussed as a reason for the heavy accumulation of the metals and PAHs detected in the roadside soil.

The bioconcentration factor (BCF) for chromium in rainbow trout (*Salmo gairdneri*) was reported as 1. In bottom-feeder bivalves, such as the oyster (*Crassostrea virginica*), blue mussel (*Mytilus edulis*), and soft shell clam (*Mya arenaria*), chromium BCF values were found to be in the order of 10^2. Based on experimental observations, chromium is not expected to biomagnify in the aquatic food chain (U.S. EPA, 1998a, b; OEHHA, 2011; ATSDR, 2012). Higher chromium concentrations were found in plants growing in soils with high chromium contents compared with plants growing in normal soils: however, as only a small fraction of chromium is translocated from soil to the epigeal parts of edible plants, bioaccumulation of chromium from soil to the aforesaid plant parts is unlikely. There is no indication of chromium biomagnification along the terrestrial food chain.

1.2.2. Sources of food and drinking water contamination

Chromium can enter the food chain via the different environmental compartments, either as a result of natural presence or emission from anthropogenic activities. Food preparation with stainless steel containers, processors and utensils could represent an additional source for the presence of chromium in food (Stoewsand et al., 1979; Offenbacher and Pi-Sunyer, 1983; Kumpulainen, 1992).

Environmental levels

According to studies from the late 1970s onwards (WHO, 2000, 2003), air chromium concentrations in the range of 0.005-1.1 ng/m^3 were detected in various remote locations such as the Arctic and Antarctic poles, north Atlantic ocean, Shetland Islands, Norway, and northwest Canada; in remote European areas, concentrations up to 3 ng/m^3 were measured. Health Canada (1986) reported chromium concentrations in air samples from five remote areas in Canada between 0.32 and 25 ng/m^3, while in the USA chromium concentrations in urban air were reported from less than 10 to 50 ng/m^3. Most environment monitoring stations in the USA detected average chromium levels in ambient air of rural and urban areas below 300 ng/m^3 (median, < 20 ng/m^3), although occasional measurements could be higher (WHO, 2000, 2003). The mean concentration of chromium in air in the Netherlands appeared to vary in the range of 2 to 5 ng/m^3; in continental Europe, air chromium concentrations were found to span 1-140 ng/m^3, a range comprising urban area values (4-70 ng/m^3). In industrial European settings, air chromium concentrations were in the range 5-200 ng/m^3. The air chromium levels in Japan and Hawaii were found to be in the range 20-70 ng/m^3 (WHO, 2000). In general, in non-industrialized areas concentrations above 10 ng/m^3 were uncommon whereas in urban areas they were two to four times higher than regional background concentrations (WHO, 2003; OEHHA, 2011). As a result of smoking, chromium concentrations in indoor air (≈ 1000 ng/m^3) may be 10-400 times greater than outdoor concentrations (WHO, 2003). Chromium concentrations in rainwater showed a marked variability (for example, see: van Daalen, 1991; Neal et al., 1996; Kaya and Tuncel, 1997); however, on average they were found to be in the range 0.2-1 µg/L (WHO, 2003). Cr(VI) forms may be present in rainwater (Seigneur and Constantinou, 1995): for instance, chromium species were determined in several rainwater samples collected in North Carolina in 1999 through 2001 (Kieber et al., 2002). The

annual average concentrations of (total) chromium, particulate Cr, Cr(III), and Cr(VI) were estimated respectively as 4.6, 2.2, 0.8, and 1.2 nM (0.24, 0.11, 0.042, and 0.062 µg/L). Distinct seasonal and diurnal variability in the rainwater concentrations of the various chromium species were observed. Based on the results of a total global flux study, the authors concluded that essentially all chromium released into the atmosphere is removed via wet deposition and that about half this chromium is dissolved with similar concentrations of Cr(III) and Cr(VI) forms.

Natural chromium concentrations in seawater were reported to typially range between 0.04 and 0.5 µg/L; in the North Sea, a concentration of 0.7 µg/L was detected (WHO, 2003). The natural total chromium content of surface waters was reported to be approximately in the range 0.5-2 µg/L while dissolved chromium concentrations were generally in the range 0.02-0.3 µg/L; chromium concentrations in Antarctic lakes (range, < 0.6-30 µg/L) appeared to increase as depth increased (WHO, 2003). In most surface waters, chromium levels were by-and-large between 1 and 10 µg/L, in general reflecting the impact of industrial activity. In USA rivers and lakes, chromium concentrations from less than 1 to 30 and below 5 µg/L, respectively, were reported by OEHHA (2011); however, in U.S. surface waters levels up to 84 µg/L were also detected (WHO, 2003). In the 1960s, chromium concentrations in the Canadian Great Lakes averaged approximately 1 µg/L (range, < 0.2-19 µg/L), while concentrations in rivers were found between 2 and 23 µg/L. In central Canada, surface water concentrations in the period 1980-1985 ranged from less than 2 to 44 µg/L, while for the Atlantic region the concentrations fell between less than 2 and 24 µg/L (Health Canada, 1986). In the river Rhine, chromium levels were reported to be below 10 µg/L (WHO, 2003).

Chromium concentrations in groundwater are generally low (< 1 µg/L) (WHO, 2003). In the Netherlands, a mean concentration of 0.7 µg/L was measured (≤ 5 µg/L). In India, 50 % of 1 473 water samples from dug wells contained less than 2 µg/L. A 1976-1977 survey of Canadian drinking water supplies suggested that the maximum levels of chromium in unprocessed and treated waters were up to 14 and 9 (median, 2) µg/L, respectively (Méranger et al., 1979; Health Canada, 1986). Chromium concentrations in water samples taken from a large number of U.S. drinking water sources in 1974-1975 were on average below 2 µg/L (range 0.4-8.0 µg/L) (DHEW, 1970; WHO, 2003). Over the period 1984-1996, California water monitoring activities detected (total) chromium in about 9 % of the numerous sources surveyed, with levels up to a maximum of 1100 µg/L (mean, 23 µg/L; median, 17 µg/L) (OEHHA, 2011). In 2001 the California Department of Public Health (CDPH, then the California Department of Health Services, CDHS) added Cr(VI) to the list of unregulated chemicals for which monitoring is required (UCMR). Results of 2000-2012 UCMR monitoring from over 7000 drinking water sources vulnerable to contamination showed Cr(VI) at or above 1 µg/L (reporting detection limit) in about one-third of them (2432) with the following distribution breakdown (Cr(VI) concentration range, proportion of detections): 1-10 µg/L, 86.0 %; 11-20 µg/L, 10.2 %; 21-30 µg/L, 2.7 %; 31-40 µg/L, 0.7 %; 41-50 µg/L, 0.2 %; over 50 µg/L, 0.2 %. Detections concerned sources and not drinking water served to customers (CDPH, 2013). A Water Research Foundation project in 2004 surveyed more than 400 drinking water sources (before treatment) across the USA and found an average Cr(VI) concentration of 1.1 µg/L (median concentration below the 0.2 µg/L detection limit) (McNeill et al., 2012a, b). Cr(VI) was found in many drinking water systems by a nationwide survey carried out in 2005-2009 by the U.S. Environmental Working Group (EWG) (Sutton, 2010). Recently, the U.S. EPA (2010) indicated that for the nearly 186 000 records analysed in public drinking water supplies, 15.3 % of samples had detectable total chromium concentrations, with a median of 4.2 µg/L and a 90th percentile of 10 µg/L (min-max 0.009-5200 µg/L). Total dissolved chromium is the parameter most often determined in trace element analyses of environmental fresh waters and waters for human consumption: however, both the trivalent and hexavalent forms were shown to exist in surface waters. As water treatment facilities use strong oxidants to potabilise water, in drinking water chromium may easily be present in the hexavalent state (Schroeder and Lee, 1975; Health Canada, 1986).

Chromium levels in soils can vary up to three orders of magnitude, reflecting the composition of the parent rock from which the soils were formed and/or local anthropogenic sources (WHO, 1988, 2000). In ultramafic (or ultrabasic) and serpentine rocks, chromium (as Cr(III)) may be present at concentrations in the order of thousands of mg/kg, whereas in granitic rocks and coal the element is on

average found at a few mg/kg levels. Rare crocoite ($PbCrO_4$) is the only mineral where Cr(VI) occurs naturally. Soils from the weathering of basalt, serpentine and ultramafic rocks, and phosphorites may contain chromium at levels as high as 3500 mg/kg, whereas soils from degradation of granite or sandstone rocks normally have chromium only at levels of a few tens of mg/kg. Chromium concentrations in thousands of USA and Canadian soil samples were reported to range from 1 to 2000 and from 5 to 1500 mg/kg, respectively, with corresponding geometric means of 37 and 43 mg/kg (WHO, 1988; ATSDR, 2012). Examples of hot spots can be found, for instance associated with old chromite mining sites; chromium has also been detected at a very high level (43 000 mg/kg) in soil at the Butterworth Landfill site in Grand Rapid City, Michigan. The use of chromated copper arsenate (CCA) as an outdoor wood preservative may be a cause for soil contamination. In 1994 and 1995, chromium was detected in sediments obtained from the coastal waters of the eastern U.S. seashore at concentrations lower than 0.2 mg/g (Hyland et al., 1998).

Examples of Cr(VI) occurrence from incidental anthropogenic sources

As human exposure to toxic Cr(VI) compounds, several of which are quite soluble, is a matter of health concern, investigations and monitoring activities have been and are performed in different parts of the world, especially focused on assessing the chemical presence and levels in drinking water and its sources. From the generic examples described hereafter, drinking water seems to be the matrix of concern with respect to a potential human exposure deriving from an undetected accidental contamination.

An accidental release of Cr(VI) from a chemical plant into the atmosphere occurred in August 2011 in Kooragang Island (Newcastle, New South Wales). The aerosol emission carrying Cr(VI) was deposited downwind of the stack, mostly on and around the facility. The spill continued for approximately 20 minutes. The original Cr(VI) emission estimate of 10-20 kg was subsequently revised to an estimated 1 kg of Cr(VI) which, in fact, rained down over the Orica plant; another 35-60 g fell out over the suburb of Stockton (Orica, 2012), whose residents were therefore potentially exposed to the contaminated aerosol. Approximately 20 workers at the plant were exposed as well as 70 nearby homes in Stockton.

The contamination of drinking water in the southern California town of Hinkley ensued from a prolonged groundwater contamination (EWG, 2005; Sutton, 2010). At the center of the case was a facility called the Hinkley compressor station, part of a long natural gas pipeline. Between 1952 and 1966, the compressor station used water containing Cr(VI) compounds to fight corrosion in the machinery. Some Cr(VI)-contaminated wastewater, discharged to unlined ponds at the site, percolated into the groundwater, affecting a large area near the plant. Average background Cr(VI) levels in groundwater were recorded as 1.2 µg/L (total chromium 1.5 µg/L) with a peak of 3.1 µg/L (total chromium 3.2 µg/L) (PG&E, 2007; CA EPA, 2008).

A contaminated groundwater plume originating from unknown source(s) allegedly composed of hazardous substances that were released into the Edwards-Trinity aquifer was detected at Midland (Texas), a community of approximately 114 000 people. At the time of the report by Cook (2010), the plume had an extension of a few kilometres and was situated under approximately 105 ha of residential and commercial land. Based on the results of a domestic drinking water well, an extensive groundwater sampling was performed in 2009. The groundwater plume contained elevated concentrations of total chromium including Cr(VI), that exceeded the U.S. EPA maximum contaminant limit (MCL) of 0.1 mg/L for total chromium and Cr(VI) in many active domestic water wells: in particular, a large proportion of samples contained total chromium and/or Cr(VI) forms in the range 500-5000 µg/L.

According to Vasilatos et al. (2008), total chromium and Cr(VI) were measured in the Thiva-Tanagra-Malakasa basin, Eastern Sterea Hellas, Greece. In the area, which is known for a 40-year long industrial activity, chromium levels as high as 80 and 53 µg/L were found in the urban drinking water supplies of Oropos and Inofyta, respectively. The pollution of groundwater by Cr(VI) in the majority of water wells in the Thiva-Tanagra-Malakasa basin was related to the widespread industrial activity, the use of hexavalent chromium in various processes, and the discharges of Cr-containing wastes. In another study (Vasilatos et al., 2010), hexavalent chromium was detected in groundwater systems in

Eastern Sterea Hellas (central Euboea and Asopos valley), central Greece, at concentrations sometimes exceeding the Greek and EU drinking water regulatory limit for total chromium of 50 µg/L. Water contamination by Cr(VI) species in central Euboea was mainly linked to natural processes, although there were cases when it seemed of anthropogenic origin. In Asopos valley Cr(VI) presence was associated to industrial wastes.

While the presence of Cr(VI) and/or its precursors in drinking water is often evidence/consequence of anthropogenic activity, over the last years there have been several reports of naturally occurring Cr(VI) in groundwater (McNeill et al., 2012a). Although in most cases the Cr(VI) concentrations found appear to be in the order of a few µg/L or some tens of µg/L, values of a few hundreds of µg/L are not unusual.

Food preparation

Food preparation may increase food chromium content, the increase depending on the process (Stoewsand et al., 1979; Offenbacher and Pi-Sunyer, 1983; Kumpulainen, 1992): for instance, stainless steel utensils used in food preparation may contribute to chromium levels. Likewise, chromium may be present in acidic fruit juices as a result of the contact with stainless steel equipment or utensils. There are various factors that may affect the release of chromium into acidic foods coming in contact with stainless steel surfaces, such as: contact area, pH of the food product, food temperature during contact and duration of contact, agitation, presence of organic chelating constituents in the food (e.g. citric acid), and particular features of the metal alloy. However, large percentages of chromium can also be removed from foods during food processing other than preparation (Schroeder, 1971, 1974; Anderson, 1981). It can be observed that the forms of chromium leaching into foods during food preparation should contain mainly or exclusively the trivalent metal due to both the reducing characteristics of the environment and the fact that Cr(III) is its most stable oxidation state. The increased concentrations of chromium in foods possibly consequent to leaching, have the potential to contribute measurably to chromium dietary exposure (Stoewsand et al., 1979; Offenbacher and Pi-Sunyer, 1983).

1.2.3. Conclusions

Chromium occurs in environmental compartments with highly variable levels. Unlike the large availability of total chromium data, Cr(VI) speciation appears to have been carried out on a relatively limited basis. The metal presence is determined by natural as well as anthropogenic factors, the latter identifiable primarily with industrial sources.

Cr(III) and Cr(VI) can both be released into the air, the latter in general to a likely quite lesser extent. In air, chromium is present in the form of aerosols that are removed by wet and dry deposition. Chromium particles of small aerodynamic diameter (< 10 µm) may remain airborne for long periods and undergo long-range transport. Under normal conditions, airborne Cr(0) and Cr(III) forms do not undergo any reaction, whereas Cr(VI) eventually reacts with dust particles or other pollutants to yield Cr(III). In non-industrialized areas total chromium concentrations above 10 ng/m^3 are uncommon whereas in urban and industrialized areas they can be quite higher (from tens to hundreds of ng/m^3). As a result of smoking, chromium concentrations in indoor air have been reported as high as 1000 ng/m^3. In rainwater, chromium concentrations on average fall in the range 0.2-1 µg/L, some part of which may be accounted for by Cr(VI).

Surface runoff, deposition from air, and release of municipal and industrial waste waters are sources of chromium in surface waters. Cr(III) is lost from the aquatic environment primarily due to precipitation of hydrated Cr_2O_3 followed by sedimentation. In surface waters, high concentrations of Cr(VI) forms can be found locally. The Cr(VI) anion species can persist in aquatic media, possibly for long periods, as water-soluble complexes: however, they will react with organic matter or other reducing agents to form Cr(III). Therefore, in surface waters rich in organic content, Cr(VI) is expected to have a shorter lifetime. Although in surface waters total chromium may be present at levels greater than 50 µg/L, in general the element is detected at concentrations in the order of few tens of µg/L or lower, rivers being more contaminated than lakes and sea water.

Total chromium concentrations in groundwater and water from drinking water sources/supplies may range from quite less than 1 µg/L up to a few µg/L, although cases of a high chromium occurrence have also been reported. Cr(VI) appears to be occasionally present in the aforesaid types of water, at levels in the range from a few up to some tens of µg/L and possibly higher. The presence of Cr(VI) in drinking water and/or its precursors is often evidence/consequence of anthropogenic contamination. As water treatment facilities use strong oxidants to potabilise water, in drinking water chromium may easily be present in the hexavalent state.

1.3. Previous risk assessments

Chromium III

IARC evaluated chromium and chromium compounds in 1990 and concluded that metallic chromium and Cr(III) compounds are not classifiable as to their carcinogenicity to humans (Group 3) (IARC, 1990).

The U.S. Environmental Protection Agency (U.S. EPA, 1998a) established a reference dose (RfD) for metallic Cr(III) of insoluble salts of 1.5 mg/kg body weight (b.w.) per day based on a subacute and long-term feeding experiment in rats fed with chromic oxide pigment (Ivankovic and Preussmann, 1975). It was noted that the overall confidence in this RfD was low due to low confidence in the database and the lack of an observed effect level. As to its human carcinogenicity, trivalent chromium was classified as group D (not classified).

In 2003 the Scientific Committee on Food (SCF) issued an opinion on the 'Tolerable Upper Intake Level of Trivalent Chromium' and concluded that the limited oral toxicity data available in animals as well as in humans did not give enough information on a dose-response relationship, and therefore a tolerable upper intake level that is likely to pose no risk of adverse health effects could not be derived (SCF, 2003).

The UK Expert group on Vitamins and Minerals (EVM, 2003) concluded that there were insufficient data from human and animal studies to derive a safe upper level for Cr(III) although its oral toxicity appeared to be low (due also to low absorption). Based on a study of oral toxicity in rats administered with chromium chloride (Anderson et al., 1997), the EVM proposed that a total daily intake of about 0.15 mg/kg b.w. per day (or 10 mg/person) of Cr(III) would be expected to be without adverse health effects.

The UK Committee on Mutagenicity of Chemicals in Food (COM), at the request of the UK Food Standards Agency (FSA), reviewed all the available data pertaining to the mutagenicity of Cr(III), particularly Cr(III) picolinate. The evaluation of the COM (COM, 2004) led to the overall conclusion that, taken all together, the data from the *in vitro* genotoxicity assays suggested that Cr(III) picolinate was negative with respect to genotoxicity.

The Concise International Chemical Assessment Document (CICAD) (WHO/IPCS, 2009a) on inorganic trivalent chromium compounds, concluded that the key toxic endpoints for soluble inorganic Cr(III) salts were chronic respiratory toxicity on inhalation and contact sensitization of the skin, while oral toxicity was low. It was noted that there was no clear evidence of genotoxic and/or carcinogenic effects of trivalent chromium compounds, there were no effects on fertility and the widespread use of mainly organic Cr(III) complexes as food supplements at 10-fold or even higher dose levels than the suggested dietary intakes had not shown any consistent toxic effect.

The EFSA evaluated the safety and efficacy of chromium methionine as a feed additive for all species in 2009 (EFSA, 2009a). The EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP Panel) noted that on the basis of the available literature Cr(III) may be a genotoxic compound under *in vivo* conditions and then considered it prudent to avoid any additional exposure of the consumers resulting from the use of supplementary Cr in animal nutrition.

The EFSA evaluated the safety of chromium picolinate as a source of chromium added for nutritional purposes in food supplements in 2009 (EFSA, 2009b). The EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS Panel) concluded that the use of picolinate as a source of Cr(III)

in food supplements could amount to intake levels of 600 μg chromium per day that was well above the levels considered safe by the World Health Organization (WHO) for supplemental intake (i.e. 250 μg per day) (WHO, 1996b). The Panel indicated that although the amount of picolinate that would be consumed as a result of the proposed uses (4300 μg per day) would be safe, it could not be concluded that the use of Cr(III) picolinate was of no safety concern. It was also noted that there were diverging views and conclusions on the genotoxicity of Cr(III) and therefore its safety needed to be re-evaluated.

The EFSA evaluated the safety of chromium picolinate as a source of chromium added for nutritional purposes to foodstuff in 2010 (EFSA ANS Panel, 2010a). The ANS Panel noted that the genotoxicity studies suggested that *in vitro* at high concentrations chromium picolinate might cause DNA damage. Long-term carcinogenicity studies provided equivocal or no evidence of carcinogenic activity of chromium picolinate (Stout et al., 2009; NTP, 2010). The Panel noted that the margin of safety between the No-Observed-Adverse-Effect Level (NOAEL) of 2400 mg/kg b.w. per day chromium picolinate, resulting from the National Toxicology Programme (NTP) long-term study, equivalent to 2100 mg/kg b.w. per day picolinate, would amount to at least 4 orders of magnitude assuming a combined intake of picolinate from all sources. The ANS Panel concluded that the use of Cr(III) picolinate as a source of chromium would not be of concern provided that the amount of chromium does not exceed 250 μg/day as established by WHO for supplemental intake of chromium that should not be exceeded.

The EFSA evaluated the safety of trivalent chromium as a nutrient added for nutritional purposes to foodstuffs in 2010 (EFSA ANS Panel, 2010b). On the basis of the analysis of *in vivo* genotoxicity assays and long-term carcinogenicity studies, the ANS Panel concluded that the safety of Cr(III) as a nutrient added to foodstuffs is not of concern, provided that the intake of Cr(III) from these sources does not exceed 250 μg/day, which is the value established by the WHO for supplemental intake of chromium. In 2012, the ANS Panel assessed the use of other additives as a source of Cr(III) for nutritional purposes, namely a cellular bound chromium yeast (EFSA ANS Panel, 2012a) and Cr(III) lactate tri-hydrate (EFSA ANS Panel, 2012b). In both cases, the opinions did not focus specifically to the safety of Cr(III) and in line with the EFSA ANS Panel (2010b) opinion concluded that an intake of Cr(III) from these sources below 250 μg/day was not of concern.

In 2012, the Agency for Toxic Substances and Disease Registry (ATSDR) published a toxicological profile for chromium in humans and animals. In the case of Cr(III) the studies on oral toxicity were considered inadequate for establishing the exposure concentrations that are likely to be without appreciable risk of adverse effects (noncarcinogenic) (minimal risk level, MRL). Little or no information was identified regarding acute or intermediate-duration oral exposure to Cr(III) compounds. Several animal studies showed no adverse effects associated with chronic oral exposure to Cr(III) compounds (chromium acetate, chromium nicotinate, chromium oxide, chromium picolinate) even at very high daily doses, therefore an MRL was not derived (ATSDR, 2012).

Chromium VI

The US National Institute for Occupational Safety and Health (NIOSH, 2002) considered all Cr(VI) compounds to be potential occupational carcinogens. Occupational exposure to Cr(VI) compounds is associated with lung, nasal, and sinus cancer. Other local effects include nasal irritation and ulceration, and perforation of the nasal septum and eardrum. Dermal exposure to Cr(VI) compounds can cause skin irritation, ulceration, sensitization, and allergic contact dermatitis.

The WHO guideline value for chromium in water of 0.05 mg/litre appears to have been established in the first edition of the WHO drinking water guidelines in 1984/85, and the basis for its derivation is unclear. The second edition (WHO, 1993) and third edition of the guidelines (2003) both noted that different guideline values for CrIII and CrVI should be derived, but analytical methods favoured a guideline value for total Cr. They also noted that because of the carcinogenicity of CrVI by the inhalation route and its genotoxicity, the current guideline value of 0.05 mg/litre had been questioned, but the available toxicological data did not support the derivation of a new value. As a practical measure, 0.05 mg/litre, which was considered to be unlikely to give rise to significant risks to health,

was retained as the provisional guideline value until additional information became available and chromium could be re-evaluated.

In 2012, ATSDR published a toxicological profile for chromium in humans and animals. In the case of Cr(VI) compounds an oral MRL of 0.005 mg/kg b.w. per day was derived for intermediate (15-364 days) exposure based on haematological effects (microcytic, hypochromic anemia) in rats (NTP, 2008). An oral MRL of 0.001 mg/kg b.w. per day was derived for chronic exposure (> 1 year) by selecting as the critical effect nonneoplastic lesions of the duodenum as reported in a chronic drinking water study (NTP, 2008< ATSDR, 2012).

Chromium(VI) compounds have been evaluated by several IARC working groups in different years (1973, 1979, 1980, 1982, 1987, 1990 and 2012). IARC concluded that there was sufficient evidence in humans for the carcinogenicity of Cr(VI) compounds, with respect to the cancer of the lung and also cancer of the nose and nasal sinuses. There was sufficient evidence in experimental animals for the carcinogenicity of Cr(VI) compounds. Therefore, Cr(VI) compounds are carcinogenic to humans (Group 1) (IARC, 2012).

U.S. EPA assessed chromium in 1998 (U.S. EPA, 1998a) and is currently reviewing the health effects of Cr(VI) and may set new limits in drinking water if needed in the future[7].

The International Programme on Chemical Safety (IPCS) published an assessment of the risk to human health and the environment of inorganic chromium(VI) compounds (WHO/IPCS, 2013). This evaluation is based principally on the *Toxicological profile for chromium* prepared by ATSDR in 2000 and on its update published in 2008. The IPCS derived an oral TDI for non-cancer effects of 0.9 µg chromium(VI)/kg b.w. per day taking into account the data relative to diffuse epithelial hyperplasia in the duodenum observed in female mice after exposure to sodium dichromate dihydrate in drinking-water. This TDI was based on a $BMDL_{10}$ of 0.094 mg/kg b.w. per day calculated by ATSDR (ATSDR, 2012) and the application of an uncertainty factor of 100. Concerning the neoplastic effects observed in the oral cavity in rats and small intestine in mice, IPCS noted that genotoxic mechanisms may be involved in the mode of action and there are no reasons for excluding a similar mode of action in humans. However, it was recognized that ther is a high degree of uncertainty on the relevance of these effects to humans because the processes and factors that determine absorption and metabolism in rodents and humans are not fully understood. Therefore, no hazard characterisation for neoplastic effects was performed.

1.4. Dietary reference values

Chromium has been viewed as an essential element with a role in the maintenance of carbohydrate, fat, and protein metabolism. Safe and adequate dietary intakes have been established by some institutional bodies.

In 1989, the US National Research Council (NRC), Food and Nutrition Board established an 'estimated safe and adequate daily dietary intake' range for chromium. For adults and adolescents that range was 50 to 200 µg per day (NRC, 1989).

The UK Committee on Medical Aspects of Food Policy (COMA) suggested that an adequate and safe level of intake lay above 25 µg/day chromium for adults and between 0.1 and 1.0 µg per day for children and adolescents (COMA, 1991). COMA also noted that no adverse effects were observed for intakes ranging between 1000 to 2000 µg Cr(III) per day.

The Institute of Medicine (IOM) of the National Research Council (NRC) determined the adequate intakes (AI) for chromium for different age groups (IOM, 2001). The AI ranged from 0.2-5.5 µg/day for infants to 35 µg/day for males between 19 and 50 years old. The suggested intakes were 29-30 µg/day during pregnancy and 44-45 µg/during lactation.

However, it should be noted that on the basis of the currently available data it is questionable whether chromium is an essential element. In its opinion on nutrient and energy intakes, the Scientific

[7] http://water.epa.gov/drink/contaminants/basicinformation/chromium.cfm

Committee for Food was unable to define a specific physiological requirement for Cr(III) (SCF, 2003). In a recent review Vincent pointed out that the mechanism of action of Cr(III) as an essential element has not been identified yet and the reports of clinically relevant chromium deficiency in humans are rare and controversial (Vincent, 2010). The role of Cr(III) as an essential element is currently under evaluation by the EFSA Panel on Dietetic Products, Nutrition and Allergies (EFSA NDA Panel, in preparation).

2. Legislation

EU Council Directive 98/83/EC[8] 'on the quality of water intended for human consumption' sets a parametric value for total chromium at 50 µg/L (Annex I, Part B 'Chemical parameters'); at the same time, it also indicates the minimum performance characteristics to be warranted by the method used for the analysis. As the aforesaid maximum level is for unspeciated chromium, the water could virtually contain toxic Cr(VI) up to the maximum concentration allowed and still be compliant with chromium regulatory requirement for potability. As known, within the Directive scope, water intended for human consumption refers to:

> 'all water … intended for drinking, cooking, food preparation or other domestic purposes, … from a distribution network, from a tanker, or in bottles or containers';
>
> 'all water used in any food-production undertaking for the manufacture, processing, preservation or marketing of products or substances intended for human consumption …'.

In the EU, the concentration limit for chromium in natural mineral waters is regulated by the Commission Directive 2003/40/EC[9]. In this Directive, chromium is listed in Annex I amongst the constituents naturally present in natural mineral waters, with a Maximum Limit of 50 µg/L (as total chromium).

In the USA, total chromium in drinking water is regulated in the Title XIV of the Public Health Service Act (Safe Drinking Water Act) with a federal drinking water standard of 0.1 mg/l (U.S. EPA, online).

There are currently no maximum levels in the EU legislation for chromium - either Cr(III), Cr(VI), or total - in foodstuffs.

In general, chromium in food contact materials (FCM) is not regulated at the EU level, and in particular in metal and alloys used for FCM. However, the Council of Europe recently published a practical guide on metals and alloys used for food contact materials and articles, and which sets out a specific release limit of 0.25 mg/kg (EDQM, 2013).

Several Cr(VI) compounds and salts are included in the list of substances subject to authorisation for their placing on the market under Annex IV of the REACH Regulation (EC) No 1907/2006[10].

Chromium is listed in the EC Regulation 1925/2006[11] amongst the minerals which may be added to food in the form of the following Cr(III) salts: chromium chloride and its hexahydrate, chromium sulphate and its hexahydrate. Following a decision of the European Commission[12], chromium

[8] Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption. OJ L 330, 5.12.1998, p. 1-28.

[9] Commission Directive 2003/40/EC of 16 May 2003 establishing the list, concentration limits and labelling requirements for the constituents of natural mineral waters and the conditions for using ozone-enriched air for the treatment of natural mineral waters and spring waters. OJ L126, 22.5.2003, p. 34-39.

[10] Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC. OJ L 136, 29.5.2007, p. 3-280.

[11] Regulation (EC) No 1925/2006 of the European Parliament and of the Council of 20 December 2006 on the addition of vitamins and minerals and of certain other substances to foods. OJ L 404, 30.12.2006, p. 26-38.

[12] Commission Decision of 27 May 2011 authorising the placing on the market of Chromium Picolinate as a novel food ingredient under Regulation (EC) No 258/97 of the European Parliament and of the Council. 2011/320/EU, OJ L 143, 31.5.2011, p. 36-37.

picolinate was authorised as a novel food ingredient under Regulation (EC) No 258/97 of the European Parliament and of the Council.

3. Sampling and methods of analysis

3.1. Sample collection and storage

There are no specific guidelines for the sampling of foods to be analysed for their total chromium and chromium species content. Therefore, basic rules for sampling of trace elements should be followed. For example, requirements are laid down in Commission Regulation (EC) No 333/2007[13] amended by Commission Regulation (EU) No 836/2011[14] for methods of sampling and analysis for the official control of some trace elements in certain foodstuffs. This Regulation contains *inter alia* a number of provisions concerning methods of sampling depending on the size of the lot, packaging, transport, storage, sealing and labelling. The primary objective is to obtain a representative and homogeneous laboratory sample with no secondary contamination.

The EN 13804:2013 standard does not deal with sampling issues but it details processes involved from receipt of the laboratory sample to the end result. Both laboratory samples and test samples shall be stored in such a way that the composition and sample mass does not change as a result of, for instance, drying out, evaporative loss, spoilage or decay.

In speciation analysis of food samples, only borosilicate or quartz glass should be used for handling and storage. Some glassware may cause contamination with chromium (EN 13804:2013). Dilution shall be done only immediately before the analysis. Parameters with a strong influence in speciation analysis are:

a) Temperature: Storage temperature shall be low enough to prevent microbial activity resulting in reactions e.g. methylation and biodegradation. For Cr species, keep samples at 4 °C or lower.

b) pH: The pH of the media may strongly affect the stability of the inorganic species. Samples intended for species analysis shall not be changed in their acidity for preservation purposes. The pH has different effects on the stability of Cr(III) and Cr(VI).

c) Light: Light may cause instability of organometallic compounds by photodegrading. When analysing organometallic compounds storage shall be done in the dark or in opaque containers.

d) Storage time: Generally, storage should be kept as short as possible.

Minimum frequency of sampling and analysis for water intended for human consumption is laid down in Council Directive 98/83/EC. For water, sampling, preservation and handling are described in different parts of EN ISO 5667 standard (EN ISO 5667-1:2007; EN ISO 5667-3:2012; EN ISO 5667-5:2006).

For total chromium analysis, water samples are collected in acid cleaned polyethylene (PE), polypropylene (PP), perfluoroethylene/propylene (FEP), polytetrafluoroethylene (PTFE), polyethylene high density (PE-HD) perfluoroalkoxy polymer (PFA) containers and acidified to pH 1 to pH 2 with HNO_3 before storage. Samples remain stable for a maximum of 6 months (EN ISO 5667-3:2012).

Water samples for Cr(VI) analysis are collected in acid cleaned plastics or borosilicate glass containers and analysed preferably within 24 hours to a maximum of 4 days (EN ISO 5667-3:2012).

[13] Commission Regulation (EC) No 333/2007 of 28 March 2007 laying down the methods of sampling and analysis for the official control of the levels of lead, cadmium, mercury, inorganic tin, 3-MCPD and benzo(a)pyrene in foodstuffs OJ L 88, 29.3.2007, p. 29-38.

[14] Commission Regulation (EU) No 836/2011 of 19 August 2011 amending Regulation (EC) No 333/2007 laying down the methods of sampling and analysis for the official control of the levels of lead, cadmium, mercury, inorganic tin, 3-MCPD and benzo(a)pyrene in foodstuffs. OJ L 215, 20.8.2011, p. 9-16.

3.2. Methods of analysis

3.2.1. Food sample preparation

The analyst must ensure that samples do not become contaminated during sample preparation. Wherever possible, apparatus and equipment that comes into contact with the sample should not contain chromium and should be made of inert materials, e.g. titanium or ceramic knives, agate mortar or ball mill for size reduction and homogenisation instead of stainless steel or iron equipment. These should be acid cleaned to minimise the risk of contamination (EN 13804:2013). Food samples are commonly treated in the same way as is done before consumption (washed, peeled, removal of non-edible parts). Examples of sample preparation procedures for some foodstuffs are given in EN 13804:2013.

3.2.2. Instrumental techniques

3.2.2.1. Total chromium analysis

The methods of analysis of total chromium in water and food samples have been reviewed by Gomez and Callao (2006). Spectroscopy techniques flame or graphite furnace atomic absorption spectrometry (FAAS, GFAAS), inductively coupled plasma atomic emission or mass spectrometry (ICP-AES or ICP-MS) are the main techniques used followed by spectrophotometric techniques (ultra-violet (UV)-visible absorption, fluorimetry or chemiluminescence).

The limit of detection (LOD) ranged from 0.5 ng/L to 8.6 µg/L in water samples depending on the preconcentration technique used (Gomez and Callao, 2006), and from 0.5 µg/L to < 250 µg/L if no pre-concentration technique is used (Table 3).

Table 3: LOD for total chromium in waters according to the analytical method used

Detection technique	Preconcentration technique (Y/N)	LOD (µg/L)	Reference
Chemiluminescence	Y	0.0005	Paleologos et al. (2003)
UV-Visible	N	17	Monteiro et al. (2002)
FAAS	N	85	Monteiro et al. (2002)
FAAS	N	[a] < 250	EN 1233: 1996 or ISO 9174:1998
FAAS	Y	8.6	Narin et al. (2008)
FAAS	Y	2.6	Saracoglu et al. (2002)
GFAAS	N	[a] < 2.5	EN 1233: 1996 or ISO 9174:1998
GFAAS	Y	0.020	Zhang et al. (1999)
GFAAS	N	0.67	Monteiro et al. (2001)
GFAAS	N	1.1	Monteiro et al. (2002)
GFAAS	Y	0.2	Pereira et al. (2004)
GFAAS	N	0.5	EN ISO 15586: 2004
GFAAS	Y	0.3	Minami et al. (2005)
GFAAS	Y	0.1	Water Research Foundation (2012)
ICP-OES	Y	1.3	Li et al. (2003)
ICP-OES	N	0.5-2.5	EN ISO 11885: 2009
ICP-OES	N	0.2-7	Water Research Foundation (2012)
ICP-MS	N	0.5	EN ISO 17294-2: 2003
ICP-MS	N	0.08	Water Research Foundation (2012)
GC/ICP-MS	Y	0.020	Yang et al. (2004)

LOD: limit of detection; UV: ultraviolet; FAAS: Flame atomic absorption spectrometry; GFAAS: Graphite furnace atomic absorption spectrometry; ICP-OES: Inductively coupled plasma optical emission spectrometry; ICP-MS: Inductively coupled plasma mass spectrometry; GC: Gas chromatography.
(a): no LOD indicated, estimation based on optimal working range given.

In foods, the LOD ranged from 0.23 µg/kg by ICP-MS to 90 µg/kg by FAAS (Table 4).

Table 4: LOD for total chromium in foods according to the analytical method used

Detection technique	Preconcentration technique (Y/N)	LOD (µg/kg)	Reference
FAAS	Y	90	Yebra-Biurrun and Cancela-Pérez (2007)
GFAAS	N	[a] < 8	EN 14082:2003
GFAAS	N	20 - 80	EN 14083:2003
GFAAS	N	[b] 28	Cubadda et al. (2003)
GFAAS	N	20	Hammer et al. (2005)
GFAAS	N	1	Reczajska et al. (2005)
GFAAS	N	5	Figueiredo et al. (2007)
ICP-AES	N	[a] < 0.5	Pehlivan et al. (2008)
ICP-MS	N	[b] 13	Cubadda et al. (2003)
ICP-MS	N	3	Hammer et al. (2005)
ICP-MS	N	12	Dufailly et al. (2006)
ICP-MS	N	0.23	D'Ilio et al. (2008)
ICP-MS	N	12	Kadar et al. (2011)

LOD: limit of detection; FAAS: Flame atomic absorption spectrometry; GFAAS: Graphite furnace atomic absorption spectrometry; ICP-AES: Inductively coupled plasma atomic emission spectrometry; ICP-MS: Inductively coupled plasma mass spectrometry.
(a): no LOD indicated, estimation based on quantified values given;
(b): given in µg/L and calculated with a sample weight of 0.3 g and a final volume of 50 mL.

After pressure digestion of the food samples, inductively coupled plasma - mass spectrometry (ICP-MS) with a collision/reaction cell technology (CCT) to reduce ArC interferences, is increasingly being used, due to its multielement capacity and its sensitivity (Hammer et al., 2005; Dufailly et al., 2006; D'Ilio et al., 2008; Kadar et al., 2011).

3.2.2.2. Chromium speciation analysis

The methods of analysis for chromium species have been reviewed by several authors, mainly in water or environmental matrices (Swietlik, 1998; Sarzanini, 1999; Camara et al., 2000; Kotaś and Stasicka, 2000; Yalcin and Apak, 2004; Unceta et al., 2010; Amouroux et al., 2011; Namiesnik and Rabajczyk, 2012; Rakhunde et al., 2012; Water Research Foundation, 2012) and rarely in food (Gomez and Callao, 2006). Analytical techniques generally used for Cr speciation can be separated into two groups. The first group so-called 'off line methods' brings together methods that can determine Cr(VI) or Cr(III) after a pretreatment of the sample and the second group so-called 'on line methods' corresponds to on-line hyphenated techniques allowing the determination of both species simultaneously. When applied to solid samples, these methods require a previous extraction step.

Chromium speciation analysis in food is influenced by the nature of the matrix and by the analytical method used. Consequently, the main difficulty is to preserve the initial distribution of chromium species in the sample because of losses and/or cross-species transformations that may occur (Novotnik et al., 2013). Extraction is one of the most critical steps, because two conflicting issues need to be addressed: obtaining high extraction efficiency and minimising losses (Unceta et al., 2010). Once in solution, pH, exposure to light, the type of storage container and high storage temperatures may affect the stability of chromium species (EN 13804:2013). Only a few methods were optimised for the speciation of chromium in some foodstuffs.

A fluorimetric detection of Cr(VI) combined with flow injection analysis (FIA), has been developed for analysis of tomato juice samples (Paleologos et al., 1998). Cr(VI) (LOD: 50 µg/L) and total chromium is measured and Cr(III) (LOD: 20 µg/L) is determined by difference.

A speciation method for chromium in cow's milk by solid-phase extraction/dynamic reaction cell inductively coupled to plasma mass spectrometry (SPE/DRC-ICP-MS) was developed by Ambushe et al. (2009). Pre-concentration and separation procedures of Cr(VI) were adapted from the work of Lameiras et al. (1998). Ion-exchange columns were used to separate Cr(VI) from Cr(III). The LODs were 0.091 and 0.085 µg/L for total Cr and Cr(VI), respectively against 0.20 (total Cr) and 0.15 µg/L (Cr(VI)) by electrothermal atomic absorption spectrometry (ETAAS) (Lameiras et al., 1998). The method of Lameiras et al. (1998) was also adapted for the selective determination of Cr(VI) in powdered milk infant formulas with a LOD of 1.8 µg/L in reconstituted milk samples by ETAAS (Soares et al., 2000).

A selective alkaline extraction (with 0.01 M NaOH) of Cr(VI) and quantification by GFAAS was developed to analyse bread samples (Soares et al., 2010). LOQs were 4.95 and 5.60 µg/kg for total chromium and Cr(VI), respectively. Another selective alkaline extraction of Cr(VI) with 0.1 M Na_2CO_3 and quantification of this species (LOQ of 70 µg/kg) by GFAAS was developed to analyse tea samples (Mandiwana et al., 2011). Cr(VI) represents up to 10 % of the average total chromium in bread and and 3-21 % of water soluble Cr(VI) in tea samples.

The recent developments in speciated isotope-dilution mass spectrometry (SIDMS; i.e. spiking the samples with isotopically enriched species) with hyphenated ICP-MS techniques has dramatically improved the quality and accuracy of the data on speciation analysis. The use of isotopically enriched Cr species (i.e. spikes) as tracers overcame the traditional problems related to non-quantitative recoveries and the formation of chromium artefacts that can occur during the extraction and derivatisation steps (Amouroux et al., 2011; Novotnik et al., 2012a,b). Using this technique and high performance liquid chromatography coupled to ICP-MS detection (SID-HPLC-ICP-MS) to follow Cr(VI) and Cr(III) species interconversions during the extraction procedures (LOQs of about 0.1 µg/L for both species), Novotnik et al. (2013) repeated the experiments of Soares et al. (2010) and Mandiwana et al. (2011) mentioned above. In contrast to their results, no Cr(VI) was found in these samples and it was experimentally proven that added $^{53}Cr(III)$ was not oxidised in tea infusions and extracts of bread samples, while almost all added $^{50}Cr(VI)$ was reduced in tea infusions due to the presence of antioxidants. In conclusion, these results emphasized the significance of the use of adequate analytical methodologies in the evaluation of Cr(VI) contents in foodstuffs and other biological and environmental matrices and that results on Cr speciation that are not supported by

speciation analysis allowing the determination of both species simultaneously should be treated with caution (Novotnik et al., 2013).

A large number of papers are dealing with methods of analysis of chromium species in water. Chromium can be separated from interfering materials by precipitation, chelation extraction, or ion chromatography. The purified chromium can then be quantified by a variety of techniques. The most established ones are briefly summarized in Appendix A.

Spectrophotometric and colorimetric methods are still widely used for chromium speciation in water samples (Swietlik, 1998; Kotaś and Stasicka, 2000; Namiesnik and Rabajczyk, 2012; Water Research Foundation, 2012). Both methods are mainly based on determination of Cr(VI) as a coloured complex with 1,5-diphenylcarbazide. Cr(III) can be similarly measured as the diphenylcarbazide complex after oxidation to Cr(VI). Despite its simplicity, the 1,5-diphenylcarbazide method suffers from the presence of interfering compounds which can react with 1,5-diphenylcarbazide, resulting in the formation of complexes that absorb at the same analytical wavelength. Ion chromatography (IC) can be used to separate Cr(VI) from these positive interferences before the derivatization with 1,5-diphenylcarbazide (LOD ranging from 1 ng/L to 0.3 µg/L for Cr(VI)) (U.S. EPA 218-7, 2011; Water Research Foundation, 2012). Some recent methods also based on colorimetric reactions used different reagent solutions with similar LOD (range 0.2 ng/L to 1 µg/L) (Li et al., 2006; Jamaluddin and Reazul, 2011; Amin and Kassem, 2012; Kanwal et al., 2012).

Electroanalytical methods are also employed for the direct determination of Cr(VI) or Cr(III). The most common method is differential pulse adsorptive stripping voltammetry (DPAdSV) or catalytic adsorptive stripping voltammetry (CAdSV), because of their low cost and high sensitivity. LOD varied between 2 µg/L without preconcentration and 2-16 ng/L when using a deposition step in which the target analyte is preconcentrated on to the working electrode (Swietlik, 1998; Dominguez and Arcos, 2002; Bobrowski et al., 2004; Lin et al., 2005; Zhu et al., 2007; Abbasi and Bahiraei, 2012).

Atomic spectrometric techniques such as FAAS, ETAAS and ICP-AES have been used for chromium speciation after a separation or isolation technique that provides selectivity for one species relative to the other (Vercoutere et al., 1996; Kotaś and Stasicka, 2000; Namiesnik and Rabajczyk, 2012). For samples with low levels of chromium, the use of a preconcentration technique with ETAAS (LOD of 0.021 µg/L) (Liang and Sang, 2008) is more suitable than FAAS detection (LOD of 0.2-6.1 µg/L) (Cespon-Romero et al., 1996; Tuzen and Soylak, 2006; Duran et al., 2007; Aydin and Soylak, 2007; Saygi et al., 2008; Bulut et al., 2009; Matos et al., 2009; Uluozlu et al., 2009; Zeng et al., 2012).

Many of the methods mentioned above have the disadvantage that one of the species is determined as the difference between total Cr (often obtained after reduction or oxidation) and the other chemical form of the element. So, on-line separation often coupled with UV-Vis (LOD of 5 ng/L for Cr(III) and 7-20 ng/L for Cr(VI)) (Kaur and Malik, 2009), chemiluminescence (LOD of 0.05 µg/L for Cr(III) and 0.1 µg/L for Cr(VI)) (Beere and Jones, 1994), FAAS (LOD of 30 µg/L for Cr(III) and 20 µg/L for Cr(VI) or 0.5 µg/L for Cr(VI) with a preconcentration technique) (Posta et al., 1993), ICP-AES (LOD of 1000 µg/L for Cr(III) and 2000 µg/L for Cr(VI)) (Byrdy et al., 1995) are increasingly used in order to minimize contamination and losses of Cr species or redox conversion (Swietlik, 1998; Sarzanini, 1999; Cornelis et al., 2003). In recent years, owing to its high sensitivity and selectivity, ICP-MS has received most attention as a detection technique for chromium (Sarzanini, 1999). Coupled to ICP-MS, ion chromatography is the most widely used separation method. Anion-exchange columns and anion exchange columns having also cation exchange capacities have been explored to separate Cr(III) and Cr(VI) species, with ICP-MS detection limits ranging from 0.1 to 1.0 µg/L (Byrdy et al., 1995; Barnowski et al., 1997; Pantsar-Kallio and Manninen, 1997; Donais et al., 1999; Seby et al., 2003). The use of complexing agents, high salt concentration and eluents with carbon or chlorine generate ArC and ClOH polyatomic interferences that may disturb measurement of the most abundant chromium isotopes ^{52}Cr and ^{53}Cr. To overcome the spectral interferences caused by carbon, most interferences can be removed by using an ICP-MS equipped with a CCT (LOD of about 0.010-0.050 µg/L for Cr(III) and Cr(VI)) (McSheehy et al., 2006; Sakai and McCurdy, 2007; Agilent, 2011; Wolf et al., 2011). Another advantage of using ICP-MS is to correct analytical biases by SIDMS. The method developed by Ma and Tanner, (2008) in natural waters indicated that the

percentage of conversion from Cr(III) (LOD of 0.4 µg/L) to Cr(VI) (LOD of 0.04 µg/L) increased from 5.9 % to 9.3 % with increase of the concentration of Cr(VI) and Cr(III) from 1 to 100 µg/L, while the reverse conversion from Cr(VI) to Cr(III) was observed within a range between 0.9 % and 1.9 %. The equilibrium constant for the conversion was found to be independent of the initial concentrations of Cr(III) and Cr(VI) and in the range of 1.0 (at pH 3) to 1.8 (at pH 10).

3.2.3. Analytical quality assurance: performance criteria, reference materials, validation and proficiency testing

Some performance criteria (limits of detection and quantification (LOD/LOQ), method bias and recovery, measurement uncertainties and analytical quality assurance) for the determination of total chromium and chromium species content in food are laid down in the EN 13804, 2013. The LOD and LOQ will vary with the analytical technique, the sample mass, the laboratory and the food matrix.

For the determination of chromium in water intended for human consumption, EU Council Directive 98/83/EC indicates that the performance characteristics for the method of analysis used must, as a minimum, be capable of measuring concentrations equal to the parametric value with a trueness, precision and limit of detection that must not exceed 10 % of the parametric value (i.e. 5 µg/L).

To demonstrate the trueness (i.e. systematic error) and precision (i.e. random error) of trace element data, one of the important criteria is the reporting of correct (and precise) data for the chromium content of certified reference materials that closely match the matrix of the samples under investigation (Jorhem, 2004). Several standard or certified reference materials (SRMs and CRMs) are available for total chromium (Appendix B, Table B1). There is a current need for CRMs certified for different chromium species in water and other foodstuffs.

Two fully validated, European standardised methods are available for the determination of total chromium in food by graphite furnace atomic absorption spectrometry (GFAAS) after pressure digestion wih a LOQ of about 0.04 to 0.16 mg/kg according to the sample weight (EN 14083:2003) or by atomic absorption spectrometry (FAAS or GFAAS), but GFAAS is recommended) after ash drying (EN 14082:2003). Four standardised methods are available for the determination of total chromium in water by flame or graphite furnace atomic absorption spectrometry (FAAS or GFAAS (EN 1233:1996 or ISO 9174:1998, EN ISO 15586: 2004), by inductively coupled plasma optical emission spectrometry (ICP-OES) (EN ISO 11885:2009) or mass spectrometry (ICP-MS) (EN ISO 17294-2:2003). Similar sensitivity can be obtained by GFAAS, ICP-OES and ICP-MS methods (LOD of 0.5 µg/L).

No standardised methods are available for determination of Cr(VI) in food while two methods are suitable for various types of water and based on colorimetric reactions with 1,5-diphenylcarbazide. Namely, the continuous flow analysis (CFA) and spectrometric detection method (EN ISO 23913:2006) and the photometric method (EN ISO 18412:2006) can be applied for drinking water in the concentration range of 2 to 20 µg/L and 2 to 50 µg/L, respectively.

A number of proficiency testing schemes (PTS) are regularly organised by several providers for total chromium in food and for both total chromium and Cr(VI) in water to demonstrate and maintain analytical quality assurance. However, no PTS are available for Cr(VI) in food.

Between 2010 and 2012, Food Analysis Performance Assessment Scheme (FAPAS) organized several proficiency tests on the determination of total chromium in food e.g. in infant cereal (FAPAS® reports 07183, 07165), infant formula (FAPAS® report 07177, 07159), soft drinks (FAPAS® report 07155) and milk powder (FAPAS® report 07138). The results indicate that most of the participating laboratories, although applying different methods, are capable of reliably analysing total chromium (range 67-98 % satisfactory results, 42 to 60 participants) at the level of interest.

Between 2012 and 2013, FAPAS organized several proficiency tests on the determination of total chromium in potable water (LEAP® Scheme reports CHEM107, 109, 111V2 and 112). 88-95 % of 17 to 25 participants obtained satisfactory results at the level of interest (range 9.91-41.4 µg/L).

In 2011-2012, the Bureau Interprofessionnel d'Etudes Analytiques (Bipea) organised three different proficiency tests in feed water (Bipea reports n°2010-2011 – 0415; n°2011-2012 – 0448; n°2012-2013 – 0123). For 57 out of 65 participants, 88 to 96 % of the results for total chromium (assigned values ranging from 7.5 to 273 µg/L) and 79 to 95 % of the 38-46 results for Cr(VI) (assigned values ranging from 98 to 241 µg/L) were considered satisfactory. At low Cr(VI) concentration level, no assigned value could be given, as 38 out of 39 participants indicated results < 1 to < 50 µg/L (Bipea report n°2012-2013 – 0123).

3.3. Conclusions

In summary, several analytical techniques are suitable for the determination of total chromium and chromium species in foods and waters:

For total chromium, F- or GF-AAS, and increasingly ICP-MS with a collision/reaction cell technology to reduce ArC interferences have been used. Two European standardised methods for the determination of total chromium in food by GFAAS are available (EN 14082:2003; EN 14083:2003) while four standardised methods are available in water by F- or GF-AAS or ICP-(OES or MS) techniques (EN 1233:1996 or ISO 9174:1998; EN ISO 17294-2:2003; EN ISO 15586:2004; EN ISO 11885:2009).

For Cr(VI), no standardised methods are available in food while two exist for water, based on colorimetric reactions with 1,5-diphenylcarbazide and spectrometric detection (EN ISO 23913:2006; EN ISO 18412:2006). For food, although the use of NaOH-Na_2CO_3 solutions with hot plate extraction seems to be the more widespread procedure, chromium species transformation can still occur. Modern analytical techniques, such as HPLC–ICP-MS, and the use of speciated isotope dilution (SID) are a suitable tool for correction of these interconversions in both foods and waters while delivering more accurate and precise results (Ma and Tanner, 2008; Unceta et al., 2010; Novotnik et al., 2013).

Several SRMs and CRMs are available for total chromium and none are also available for chromium species. There is a current need for CRMs in water and other foodstuffs certified as to chromium species. To demonstrate and maintain analytical quality assurance, regular proficiency testing schemes are available for total chromium in food and water as well as for Cr(VI) in water. However, none is available for Cr(VI) in food.

4. Occurrence of chromium in food and drinking water

4.1. Previously reported occurrence results

There is a very large number of data in the literature as regards total chromium in food, and significantly less for Cr(VI). All the analytical results are reported on a wet weight basis unless otherwise specified or there is lack of information.

In general, food was reported to contain chromium at (unspeciated) concentrations ranging from less than 10 µg/kg to more than 1000 µg/kg, although most fresh foods had chromium levels from a few up to tens or possibly hundreds of µg/kg (Schroeder, 1971, 1974; Anderson, 1981; Kumpulainen, 1992; OEHHA, 2011). The highest concentrations (> 100 µg/kg) were found in (roughly descending order): condiment and spices, cocoa, molasses and raw sugar, nuts, dry corn, seafood, and butter and oil. Meat, grains and cereals, starch, polished rice, vegetables, fruits, and milk and dairy products were in general seen to have lower chromium concentrations (< 100 µg/kg). Chromium content in a given food type can vary substantially: for instance, in whole cereals variations were seen to occur among different types of cereals, but also within cereals of the same type reflecting the area of origin (Plessi and Monzani, 1990).

4.1.1. Total Chromium in food

Analyses of the total chromium concentrations in foods performed in 20 different countries and available from the literature (51 articles published between 1980 and 2007) have been recently

reviewed by Thor et al. (2011). Across the articles reviewed, a total of 1382 chromium values were reported for 856 foods. Means, standard deviations, medians, and range chromium values for the most commonly consumed foods in the USA, for which two or more analytical values were identified in the literature (23 publications), are listed in Appendix C.

Another recent review on chromium in food products indicated that the largest quantities in breakfast food products occur in raw cereal products (non-roasted buckwheat (0.82 mg/kg) or brown rice bread (0.86 mg/kg)) and herbs such as garlic (0.99 mg/kg dry weight (d.w.)) or mint (1.11 mg/kg d.w.). The lowest chromium contents were observed in raw and ultra high temperature (UHT)-processed milk samples (Sykula-Zajac and Pawlak, 2012).

The content of total Cr determined in 72 samples of 17 different spices and aromatic herbs of the Spanish diet ranged from not detectable to 1.42 mg/kg d.w., the highest Cr concentrations were in dried garlic samples and Cr presence was detected in 95 % of samples (Garcia et al., 2000). In some basic foods of the Spanish diet, total Cr ranged from not detected (nd) to 0.040 mg/kg in olive oils, from 0.004 to 0.079 mg/kg in seafood, from 0.007 to 0.456 mg/kg in cereals and vegetables (maximum in sweet corn), and from nd to 0.065 mg/kg in dairy products, except in curd and custard (range 0.500-0.625 mg/kg) (Lendinez et al., 2001). The total Cr content in 36 different types of three commercial brands of breakfast cereals, accounting for 85 % of the Spanish market, ranged from 0.09 to 0.55 mg/kg (mean content of 0.23 mg/kg) (Mateos et al., 2003). Eleven different types of infant formulae (cow's milk and soy protein based, number of samples, n = 104) marketed in Spain were also analysed for total Cr content (Sola-Larranaga and Navarro-Blasco, 2006). The results are expressed as µg/L according to the manufacturer's dilution instructions. In general, the infant formulae contain a higher chromium concentration than that found in human milk (reference range: 0.20-8.18 µg/L), particularly in the case of hypoallergenic (mean 18.16 µg/L), lactose-free (11.37 µg/L), pre-term (mean 11.48 µg/L) and soya (mean 10.43 µg/L) formulae. The mean of the other types of infant formulae (adapted, type 1 and 2, functional 1 and 2, follow-up and toddler) ranged from 6.29 to 9.68 µg/L. A comparison with results from various countries (Austria, Belgium, France, Italy, Spain, UK, Nigeria, USA) found in the literature between 1983 and 2005 indicated a wide variability of chromium content in infant formula with values reported in some surveys considerably higher (range 1.9-174 µg/L), probably due to the limited number of samples or insufficient control of contamination in sample handling.

Chromium content of various Greek foods (n = 532) indicated that in average, meat, fish and seafood, cereals and pulses were higher sources of chromium (> 0.100 mg/kg on average, range 0.02-0.45 mg/kg) than fruits, milk, oils and fats and sugar (range 0.01 - 0.28 mg/kg) (Brakatos et al., 2002).

Average chromium concentrations in dairy products from sheep milk collected in eight farms in two regions of Southern Italy ranged from 0.14 (in milk) to 0.47 mg/kg d.w. (in mature cheese) (Anastasio et al., 2006). In 2010, the analysis of total Cr in the flesh and hepatopancreas of 320 cephalopod mollusks sampled in the Southern Adriatic Sea indicated that total Cr was uniformly distributed among the various families (0.38-0.43 mg/kg in the flesh) (Storelli et al., 2010). Another Italian study indicated that chromium ranges in 54 wild boar samples were 0.069-0.692 mg/kg (mean 0.133 mg/kg) in meat and < 0.012 (LOD)-0.626 mg/kg (mean 0.146 mg/kg) in liver (Danieli et al., 2012).

In France, of the 1319 food samples analysed for the Second total diet study (TDS), the highest mean levels were found in the food group 'fat and oil' (0.810 mg/kg), followed by 'sweeteners, honey and confectionery' (0.574 mg/kg) and 'ice cream' (0.365 mg/kg) (Noël et al., 2012). For the remaining food groups, concentrations ranged from 0.056 mg/kg (drinks) to 0.299 mg/kg (meats and offal). For all groups, these concentrations were between 2.4 and 13 times (fat and oil) higher than those of the First TDS ('sweeteners, honey and confectionery' (0.161 mg/kg), 'cereals and cereal products' (0.124 mg/kg) and 'ice cream' (0.107 mg/kg), the other groups contained less than 0.100 mg/kg on average) (Leblanc et al., 2005), which could be related to the use of stainless steel, aluminium or cast iron kitchen equipment (not used in the First TDS) and the sample grinding equipment. In a specific study of fish and other seafood from the French market (n = 159), chromium was found at an average level of 0.220 mg/kg in fish and 0.228 mg/kg in seafood (Guérin et al., 2011). Amongst fish, eel and anchovy had the highest levels of Cr (0.573 and 0.450 mg/kg, respectively) and amongst seafood,

tarama contained the highest level (0.850 mg/kg) followed by spider crab, surimi, whelk, mussel, crab and periwinkle (0.269-0.421 mg/kg).

In Poland, chromium content was determined in a total of 272 samples of some fresh fruits, vegetables and wheat grains collected in 2001 in three various agricultural areas and of juices, wines and beers purchased on the domestic market (Reczajska et al., 2005). Chromium content ranged from < 1 to 183 µg/kg and the highest mean values were observed in samples of wheat grains (39 µg/kg), strawberries (32 µg/kg) and cucumbers (19 µg/kg) and the lowest in juices (< 1 µg/L).

The Cr levels in 49 rice samples sold on the Swedish market ranged between <0.003 and 0.033 mg/kg (average 0.008 mg/kg) with no significant difference between brown and white rice (Jorhem et al., 2008).

In the 2006 UK total diet study (TDS) (Rose et al., 2010), total Cr was detected in various food groups but the concentrations of bread, offal, poultry, beverages and milk were below the LODs of 0.003-0.02 mg/kg. The sugars and preserves group contained the highest chromium concentration (0.08 mg/kg). These concentrations were below those observed in the 1997 UK TDS (ranging from 0.01 in milk group to 0.23 mg/kg in meat products group) (Ysart et al., 2000). Also in the UK, total Cr was detected at concentrations at or above the LOD (0.002-0.01 mg/kg depending on sample weight taken) in a wide range of commercial weaning foods and formulae (n = 201) (FSA, 2006). The mean concentration was 0.05 mg/kg (mean in food type ranging from 0.01 in growing up milk to 0.098 mg/kg in cereal bars/rice cakes) and the maximum value of 0.62 mg/kg was found in a sample of dessert.

In Hungarian foodstuffs, the total Cr content was 1.31 mg/kg in a brewer's yeast product, 0.119 mg/kg in broccoli purchased from a local farm (Debrecen, Hungary) and ranged from 0.010 to 0.028 mg/kg in wines, from 0.0006 to 0.014 mg/kg in beers, from 1.25 to 1.36 mg/kg in egg-yolks, from 0.009 to 0.092 mg/kg in cereal milling products, and from 0.377 to 12 mg/kg in five spice samples (Kovacs et al., 2007).

The mean total chromium content determined in six different species of edible vegetable oils (17 samples analysed) from some food supply markets in Turkey ranged from 0.5 µg/kg in sunflower oil to 1.0 µg/kg in almond oil (Pehlivan et al., 2008).

Total Cr was present at quantifiable levels in 93.5 % (n = 367) of the duplicate beverage samples (dairy, fruit, vegetable and other beverages except drinking water) collected from up to 80 individuals in Maryland, USA in 1995-1996, with a mean concentration of 0.029 mg/kg (median 0.015 mg/kg and range 0.002-2.62 mg/kg) (MacIntosh et al., 2000).

The highest level of total Cr were found in muskmelon, brinjal and mango (range 1.04-1.07 mg/kg) while the lowest levels were found in potato and garlic (0.15 mg/kg) in 20 samples of fruits and vegetables purchased from local markets of Karachi, Pakistan (Parveen et al., 2003). Levels of chromium determined in several sweet, sour and bitter tasting fruits, vegetables and medicinal plants purchased from the Pakistan local markets ranged from 0.02 in white sugar (refined) to 2.20 mg/kg in banana (mean of 0.69 mg/kg; n = 25), from 1.50 in Tamarind (Imli) to 62.3 mg/kg in Sour mango powder (Aamchoor) (mean of 22.5 mg/kg; n = 13) and 0.17 in salt blush root (tooth brush tree) to 1.56 mg/kg in Gurmar buti (mean of 0.61 mg/kg; n = 21), respectively (Tirmizi et al., 2007).

The geometric mean concentrations of total Cr determined in 90 samples of vegetables (leafy vegetables, fruit, root, grain and cereal), derived products (sugar, coffee, manioc flour, wheat flour, corn flour, and pasta) and animal products (meat, fish, milk) most frequently consumed by adult inhabitants of Rio de Janeiro city, Brazil, ranged from 0.0024 to 0.230 mg/kg (Santos et al., 2004). The foodstuffs that presented the highest concentrations were banana and coffee (range 0.175-0.270 mg/kg).

The total Cr concentrations found in 24 samples of raw cow's milk collected from eight dairy farms close to mines in Gauteng and North West Provinces of South Africa ranged from 0.186 to 0.371 mg/kg d.w. and total Cr was detectable in all the samples (Ataro et al., 2008).

4.1.2. Chromium speciation in food

The mean values found for total Cr in 34 samples of Spanish wild mushrooms were 1.14 mg/kg d.w. for cap (ranging from 0.02 to 13.84 mg/kg d.w.) and 1.11 mg/kg d.w. for stalk (ranging from 0.04 to 6.50 mg/kg kg d.w.) (Figueiredo et al., 2007). For Cr(VI), the mean values were reported to be 0.103 for cap (range <0.0085-0.580 mg/kg d.w.) and 0.143 mg/kg kg d.w.stalk (range < 0.0085-0.81 mg/kg d.w.). The percentage of Cr(VI) relative to total Cr was, in mean values, 9.0 and 12.9 %, for cap and stalk, respectively.

In Portugal, total Cr and Cr(VI) were determined in 60 UHT milk samples (Lameiras et al., 1998). The mean total Cr values found for the monitored samples were 0.95, 1.26 and 2.70 µg/L in skimmed, half-fat (simple and supplemented together) and whole milk, respectively. The Cr(VI) values found (< 0.15-1.20 µg/L) were reported to be, in terms of mean values (< 0.15-0.68 µg/L), about 2-4 times lower than those for total chromium. Also in Portugal, Cr(VI) levels in 20 commercial brands of powdered milk infant formulas were reported to range from <10 to 75 µg/kg, with mean values of 24, 12, and 33 µg/kg for 7 infant formulas, 5 follow-up milks, and 8 dietetic milks, respectively (Soares et al., 2000). Finally, Soares et al. (2010) found mean total Cr values of 47.3 and 50.9 µg/kg d.w. in 76 white bread and 76 whole bread samples, respectively. The mean values found for Cr(VI) were reported to be 5.65 µg/kg d.w. for white bread and 6.82 µg/kg d.w. for whole bread and represent slightly above 10 % of the total chromium contents but these data were characterized by a large variability possibly reflecting the effect of a relatively high LOQ (5.60 µg/kg d.w.).

In South Africa, the content of total Cr in pasteurised cow's milk of eight different commercial brands was in the range of 33.2 to 57.1 µg/L (Ambushe et al., 2009). These milk samples contained 1.31 to 3.28 % Cr(VI) (range 0.61-1.39 µg/L). The contents of total Cr found for five brands were within the same range as the results (range 0.186-0.371 mg/kg d.w.) reported by Ataro et al. (2008). Also in South Africa, the concentration of Cr(VI) in black teas was reported to be between 0.03 and 3.15 mg/kg (with an average of 1.07 mg/kg), in green tea ranged between 0.03 and 0.14 mg/kg (with an average of 0.09 mg/kg) and in herbal tea was below the LOD, thereby indicating that Cr(VI) levels increase in the order of herbal tea, green tea and black tea (Mandiwana et al., 2011). It was also found that up to 17.5 µg Cr(VI) could be consumed per unit cup of black tea (200 mL) when standard tea bag (2.0 g) or 2.0 g leaf was used for the preparation of tea. Similarly, black teas were found to contain higher total Cr content (0.28-14.0 mg/kg with an average of 4.38 mg/kg) than green teas (0.22-0.95 mg/kg with an average of 0.70 mg/kg) and herbal teas (0.68-1.24 mg/kg with an average of 0.95 mg/kg). Water soluble Cr(VI was reported to represent 2.6-20.5 % of the average total Cr in tea samples and up to 100 % of total Cr(VI).

However, the work of Novotnik et al. (2013) that recently repeated the experiments of Soares et al. (2010) and Mandiwana et al. (2011) mentioned above indicated that previous Cr(VI) findings were analytical artefacts as no Cr(VI) was detected in any of the samples analysed using a speciated isotopic dilution technique to follow Cr(VI) and Cr(III) species interconversions during the extraction procedures. It was experimentally demonstrated that added ^{53}Cr(III) was not oxidised in tea infusions and extracts of bread samples, while almost all added ^{50}Cr(VI) was reduced in tea infusions due to the presence of antioxidants. Partial reduction of ^{50}Cr(VI) was observed even in highly alkaline bread extracts (pH 12), exhibiting the high reducing potential of bread constituents. In addition, according to the Kovacs et al. (2007) study, Cr(III) in considerable quantity does not convert to Cr(VI) during the heating process (at the temperature of baking and toasting bread) because the organic substances of the flour ensure reductive medium. Moreover, if there were Cr(VI) compounds in the bread they would reduce to Cr(III) as well at high temperature during toasting.

4.1.3. Chromium in breast milk

Several studies presented total Cr occurrence data in breast milk samples (Appendix D). In European studies, the mean total Cr concentrations ranged from 0.14 to 1.80 µg/L, except in the study of Wappelhorst et al. (2002) on a relatively low number of subjects (n = 19), which reported a mean and median values of 10.8 µg/L (range 3.1-19.4 µg/L).

Outside Europe, the mean concentrations are also generally well below 2 µg/L, except in three studies (in Egypt, Nigeria and Japan) where the mean concentrations found are largely higher (range 17-110 µg/L; Carter et al., 1968; Okolo et al., 2001; Yamawaki et al., 2005). However, in the more recent Japan study (Yoshida et al., 2008), the mean concentration found were also below 2 µg/L and according to the authors, the results of the Yamawaki et al. (2005) study were not reliable, since no evaluation of analytical values using standard reference materials was performed. It is likely that the high levels observed in the other studies are also related to analytical bias poorly mastered by adequate internal quality controls. Recently, Sola-Larrañaga and Navarro-Blasco (2006) reported a range of chromium concentration in human milk of 0.20-8.18 µg/L.

4.1.4. Total chromium and/or hexavalent chromium in drinking water

Examples of chromium occurrence in drinking water are reported hereafter from the reviews of WHO (2003) and McNeill et al. (2012a,b), while a summary of chromium in environmental water and drinking water sources is available in Chapter 1. Approximately 18 % of the population of the USA in 1987 were exposed to drinking water total Cr levels between 2 and 60 µg/L and < 0.1 % to levels between 60 and 120 µg/L. Cr(VI) was measured in the tap water of 31 out of 35 cities sampled - several of which have a population greater than 1 million - at concentrations in the range 0.03-12.9 µg/L. Therefore, in most cities drinking water was found to exceed California's 2011 Public Health Goal (0.02 µg/L) (OEHHA, 2011). On the whole, eight, 15, and eight of the cities tested by the EWG were found to have drinking water with Cr(VI) oncentrations respectively above 1, between 0.1 and 1, and below 0.1 µg/L.

Out of the 138 445 results on total chromium extracted from the French SISE-EAUX (Health and Environment Information System on Water) database for the period 1 January 2001 to 31 March 2011, 133 191 (96.2 %) are below the LOQ (ranging from 1 to 10 µg/L, median LOQ of 5 µg/L and average LOQ of 4 µg/L) and 14 cases of non-compliance in total Cr were reported, ranging from 51 to 199 µg/L, with a median of 63 µg/L (ANSES, 2012).

4.1.5. Conclusions

Staple foods are particularly low in total chromium. Processed meats, whole grain products, pulses and spices are the main sources of chromium, whilst dairy products and most fruit and vegetables, contain only small amounts. Scarce studies have analysed the chromium speciation in some food samples (milk, mushrooms, bread, tea) and concluded that the percentage of Cr(VI) relative to total Cr is, in average, generally below 10 % (range 1.31-12.9 %). However, Novotnik et al. (2013) showed the absence of Cr(VI) in bread and tea samples using a more accurate and precise speciation method (a speciated isotopic dilution technique to follow Cr(VI) and Cr(III) species interconversion during the extraction procedure). These results indicated that previous Cr(VI) findings were probably due to analytical artefacts. The assumption that Cr(III) cannot be oxidised to Cr(VI) when baking or toasting bread, because of the reductive nature of the organic substances of the flour, was also reported by Kovacs et al. (2007). According to Novotnik et al. (2013), the data confirmed that Cr(VI) does not exist in foodstuffs of plant origin and provided some conclusive evidence that the same can be expected for foods of animal origin.

Cold and hot common beverages, such as coffee, tea, orange juice, etc., were investigated as potential electron donors in the reduction of Cr(VI) to Cr(III) (see, for instance: Kerger et al., 1996; Kim et al., 2012). Laboratory studies were carried out under conditions mimicking real situations, with excess reduction capacities of the beverages. According to Kerger et al. (1996), the reduction process would be thermodynamically favoured and, given enough time, all Cr(VI) would turn into Cr(III). However, the experimental rates of reaction presented a remarkable variability: from very fast (disappearance of Cr(VI) in few minutes) to quite slow, with a partial survival of Cr(VI) species for hours. Apparently, there were no reliable indications to predict how Cr(VI), if present, would behave as a function of time. In the relatively complex matrices tested, the redox reaction kinetics depended on a considerable array of factors, largely unknown or uncharacterized. In the end, the aforesaid papers demonstrated that Cr(VI) in certain beverages may undergo a complete fast reduction to Cr(III), whereas in other cases a fraction of Cr(VI) may survive long enough for a potential uptake. Nothing well-founded can

be said as to Cr(VI) in food, although the concentrations of reductants may be expected to be higher than in beverages, a condition that might shorten the life of Cr(VI) species, if present, especially if food is cooked.

The CONTAM Panel noted that there is a lack of data on the presence of Cr(VI) in food, and decided to consider all the reported analytical results in food as Cr(III). This assumption is based on the outcome of the recent speciation work by Kovacs et al. (2007) and Novotnik et al. (2013), the fact that food is by-and-large a reducing medium that would likely determine Cr(VI) to be lowered to Cr(III), and that oxidation of Cr(III) to Cr(VI) would not be favoured in such a medium, In conclusion, it can be considered that all the chromium ingested via food is in the trivalent form, in contrast to drinking water where chromium may easily be present in the hexavalent state, not only due to anthropogenic contamination events, but also because water treatment facilities use strong oxidants to make water potable (Section 1.2).

Finally, it should be noted that the published data on total chromium in foods and on total chromium and Cr(VI) in waters are in the same range as those reported to EFSA and supports the findings and evaluation reported below in Section 4.2.

4.2. Current occurrence results

4.2.1. Data collection summary

The Dietary and Chemical Monitoring (DCM) unit published a call for available data on nickel and chromium (trivalent and hexavalent) levels in food and drinking water[15]. European national food authorities and similar bodies, research institutions, academia, food and feed business operators and any other stakeholders were invited to submit analytical data. The data submission to EFSA followed the requirements of the EFSA Guidance on Standard Sample Description for Food and Feed (EFSA, 2010a).

By the end of February 2013 and before applying any data quality criteria, 81 247 analytical results on chromium were available in the EFSA database. A total of 53 828 results were reported as chromium, 27 325 as total chromium, four as chromium and derivatives and two as Cr(III). Despite the specific request for Cr(VI), only 88 analytical results were received on this chromium species, all in bottled water. Out of the 81 247 available analytical results, a total of 27 138 were for food, 52 735 for drinking water, 1374 for feed.

Almost 80 % of the samples were collected in Germany. After Germany, Cyprus, Slovakia and Ireland were the countries where the highest numbers of samples were collected. Data reported covered all years from 2000 to 2012, with the analytical data well distributed over the different years.

In order to guarantee an appropriate quality of the data used in the exposure assessment the initial dataset was carefully evaluated applying several data cleaning and validation steps (e.g. exclusion of duplicates and samples without complete information).

4.2.2. Data collection on food, drinking water and unprocessed grains of unknown end-use

All samples were classified according to the FoodEx classification system (EFSA, 2011a). FoodEx is a food classification system developed by the DCM Unit in 2009 with the objective of simplifying the linkage between occurrence and food consumption data when assessing the exposure to hazardous substances. It contains 20 main food groups (first level), which are further divided into subgroups having 140 items at the second level, 1261 items at the third level and reaching about 1800 endpoints (food names or generic food names) at the fourth level. Although drinking water is considered as food in the FoodEx classification system (EFSA, 2011a), in this Scientific Opinion drinking water is dealt with independently from the other food categories.

[15] Available online at: http://www.efsa.europa.eu/en/dataclosed/call/120426.htm

4.2.2.1. Data collection on food (excluding drinking water)

Before applying any data quality criteria 27 138 analytical results on chromium were reported on food (17 958 as unspecified chromium, 9176 as total chromium and four as chromium and derivatives). No data on chromium speciation were reported.

Based on the evidence discussed in Section 4.1. the CONTAM Panel decided to assume that chromium analytical data reported in food refer to Cr(III). However, certain foods are prepared with water to be consumed (coffee, tea infusions, and dry infant and follow-on food), and an incomplete reduction of the Cr(VI) present in this water into Cr(III) may happen if the foods are ingested immediately after their preparation. In these cases, the occurrence data on Cr(III) reported for dry foods were used to estimate the exposure to Cr(III) while the occurrence data on Cr(VI) reported for different types of drinking water were used to estimate the exposure to Cr(VI).

Of the reported data, 64 results were not considered for dietary exposure assessment as they belonged to the category 'Grain as crops', whose final use is unknown. Then, samples reporting neither LOD nor LOQ values were excluded together with those reported as 'suspect samples' related to the sampling strategy (939 and 337 samples, respectively). Finally, ten samples with exceptionally high concentrations of chromium as compared with the rest of the samples in the same food category were excluded (2-3 orders of magnitude higher). They were two samples of 'Grains and grain-based products', one unspecified (12 100 μg/kg) and one of 'Wheat rolls, white' (13 500 μg/kg), and eight samples of beer with reported values between 5100 and 9000 μg/kg. The decision to exclude the eight samples of beer was based on diverse pieces of evidence. The reported values were three orders of magnitude higher than those reported for the rest of the samples in the food category 'Beer and beer-related beverage' (average value of this category = 8.3 μg/kg, n = 493). This average value was similar to those reported in the literature for the presence of chromium in beer, 0.48-56 μg/kg (Anderson and Bryden, 1983), 0.6-13.6 μg/kg (Kovács et al., 2007) or 6-8 μg/kg (Reczajska et al., 2005). Based on this, and the fact that no plausible explanation for the high concentrations was found and the data provider could not confirm these eight analytical results, it was decided to exclude the eight samples of beer from the final dataset.

After these steps a total of 25 788 analytical results remained in the database.

Almost 50 % of the results were left-censored data and, therefore, special attention should be paid to the LODs and LOQs reported. To avoid overestimation of the exposure calculations at the upper bound (UB) and underestimation at the lower bound (LB) it was decided to establish a cut-off value for the reported LOQs above which samples were excluded from the final dataset. In order to establish an appropriate LOQ cut-off the distribution of the LOQs reported for the different foods at different FoodEx levels was evaluated (FoodEx levels 1, 2 and 3). Those analytical results, within one specific food category and at the selected FoodEx level, that reported LOQs above the 95th percentile of the LOQ distribution were excluded. In Table 5 are shown the different cut-offs selected for the different food groups. It can be seen that in most of the cases the cut-off was applied at FoodEx level 1. However, specific cut-offs were applied for some specific food groups at FoodEx level 2 and 3 when needed (Table 5). In addition, an exception was made for the food category 'Food for infants and small children'. For this category, the LOQ cut-off selected was the 75th percentile (50 μg/kg) instead of the 95th percentile (1000 μg/kg) as the samples with LOQ = 1000 μg/kg were all left-censored data. The exclusion of these data avoided the bias of the occurrence values in this food category since the quantified values showed average chromium concentrations of 75 μg/kg.

Following this approach a total of 1 159 analytical results were excluded (4.5 % of the total), among them 226 corresponding to the food groups 'Vegetable and vegetable products (including fungi)' and 'Starchy roots and tubers'. Among the excluded samples only 110 were quantified results.

Table 5: LOQ cut-offs established in food samples analysed for chromium and classified as described in FoodEx classification (EFSA, 2011a). Selection of the different cut-offs was based on the evaluation of the distribution of LOQs among the different food samples at the appropriate FoodEx level. Food samples reporting LOQs above the shown values were excluded from the final dataset.

FoodEx Level 1	FoodEx Level 2	FoodEx Level 3	Selected LOQ cut-off (µg/kg)	Number of samples eliminated/Total
Grains and grain-based products			200	126/4 034
Vegetables and vegetable products (including fungi)			70	84/2 996
	Vegetable products		70	4/36
		Sun-dried tomatoes	90	-/3
		Hops (dried), incl. hop pellets and unconcentrated powder	90	-/3
	Fruiting vegetables		70	15/491
		Chilli pepper (Capsicum frutescens)	90	-/11
	Cocoa beans and cocoa products		250	15/268
	Tea and herbs for infusions (Solid)		250	-/231
	Coffee beans and coffee products (Solid)		90	13/66
	Fungi, wild, edible		90	-/151
	Fungi, cultivated		90	12/509
		Shiitake mushroom (Lentinus edodes)	140	-/25
Starchy roots and tubers			70	83/714
Legumes, nuts and oilseeds			150	35/1 202
Fruit and fruit products			150	64/1 512
Meat and meat products (including edible offal)			100	67/2 155
Fish and other seafood			100	51/1 236
	Water molluscs		150	-/380
Milk and dairy products			150	16/624
Eggs and egg products			30	2/82
Sugar and confectionary			200	73/1 199
Animal and vegetable fats and oils			200	39/225
Fruit and vegetable juices			50	58/1 274
Non-alcoholic beverages (excepting milk-based beverages)			40	8/387
	Tea (Infusion)		250	1/21
Alcoholic beverages			70	70/1 664
Herbs, spices and condiments			1000	10/617
		Cinnamon (Cinnamonum verum syn. C. zeylanicum)	5000	-/4
Food for infants and small children			50	228/927
Products for special nutritional use			2000	24/2 064
	Food for weight reduction		1000	44/135
Composite food (including frozen products)			60	12/307
Snacks, desserts, and other foods			200	5/235

The final food dataset includes 24 629 results, which are shown grouped by sampling country in Figure 5 and by year of analysis in Figure 6. Most of the foods were sampled in Germany (77.5 %) and, within these samples, 73.8 % also reported Germany as the country of origin of the food. Samples were collected in a total of 10 different countries. All analytical results were expressed as whole weight.

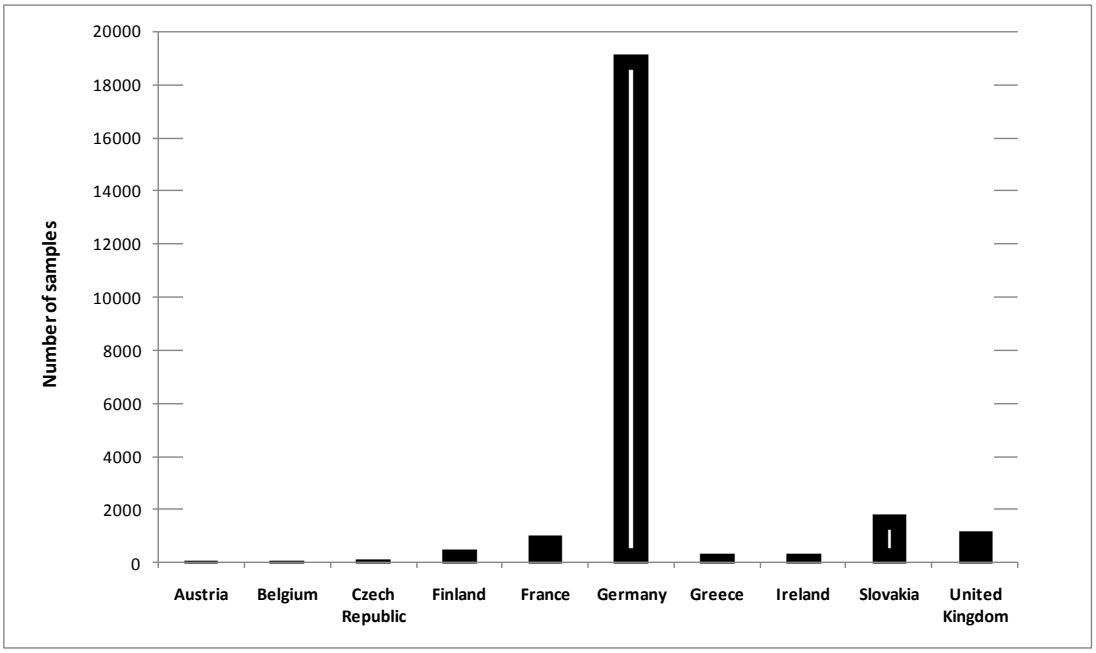

Figure 5: Distribution of food samples analysed for chromium across different European countries.

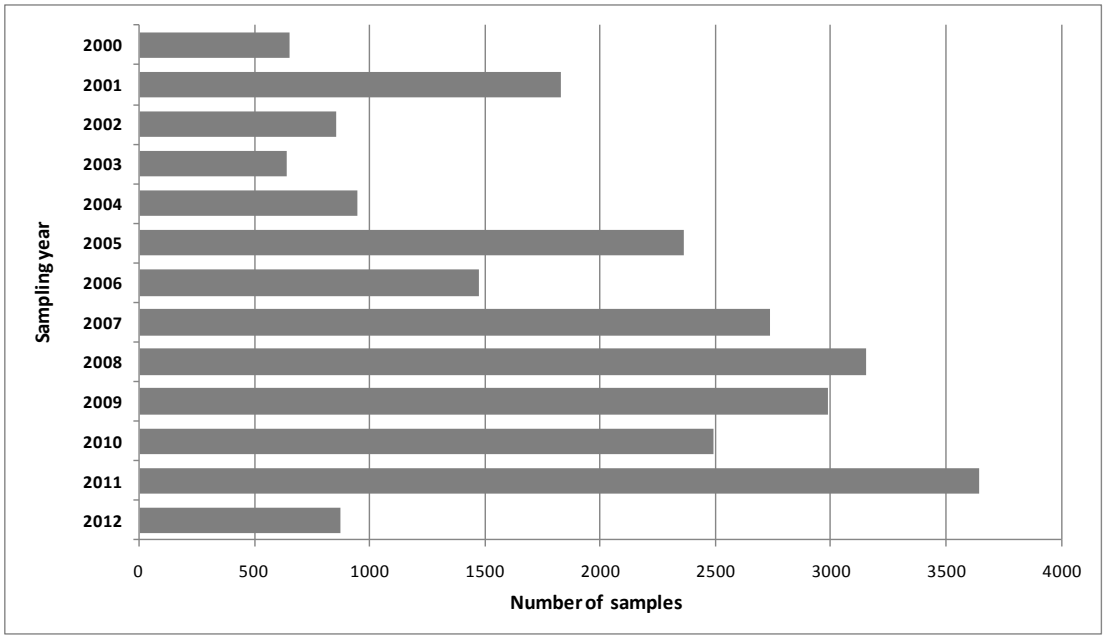

Figure 6: Distribution of food samples analysed for chromium over the sampling years.

4.2.2.2. Data collection on drinking water

In the FoodEx classification system (EFSA, 2011a) the different types of water (bottled water, tap water, water ice and well water) are grouped under the generic name 'Drinking water'. Therefore, the generic term 'Drinking water' as used in this opinion includes both water intended for human consumption (Council Directive 98/83/EC) and natural mineral waters (Commission Directive 2003/40/EC). Bottled water as used in this opinion includes natural mineral water, but also spring water and other bottled drinking water, products that must comply with Council Directive 98/83/EC.

Before applying any data quality criteria 52 735 analytical results on drinking water were present in the database. Since 88 samples reported data for both total chromium and Cr(VI), results for 52 647 different drinking water samples were available. The same procedure followed for the food samples was applied to water, eliminating the samples reported as 'suspect samples' and those without neither LOD nor LOQ. In this step, a total of 5 211 were excluded of the dataset (4 929 without neither LOD nor LOQ and 282 as suspect samples).

Legislation establishes performance characteristics for the methods used to analyse the presence of chromium in water intended for human consumption (Council Directive 98/83/EC) and in natural mineral waters (Commission Directive 2003/40/EC). Based on the concentration limits described in both pieces of legislation (50 µg/L) a maximum LOD of 5 µg/L is established for the analytical methods in use. As all the samples reported LOQs but only 40 % LODs, it was decided that the cut-off value should be applied to the LOQ. A cut-off value of 10 µg/L for total chromium was selected taking into account legislation and the available literature on chromium analysis in water. A total of 1292 samples were eliminated (136 quantified). No cut-off value was applied to the analytical data on Cr(VI).

After applying the selected cut-off of 10 µg/L a total of 46 234 analytical results (46 146 on total chromium and 88 on Cr(VI)) on drinking water were included in the final dataset. Information on the country of sampling and the sampling year is provided in Figures 7 and 8, respectively. As for food, most of the samples were collected in Germany (~ 82 %) followed by Cyprus (~ 11 %). Water samples adequately representing the years between 2000 and 2012 were collected (a minimum of 1 000 analytical data/year).

For all the different types of drinking water samples reported, i.e., bottled water, tap water, water ice, and well water, it was difficult to predict the amount of Cr(VI) present. The CONTAM Panel decided to consider all chromium present in drinking water as Cr(VI) (worst case scenario) based on two facts. First, the samples where both Cr(VI) and total chromium were quantified (71 out of 88 samples) showed an average ratio Cr(VI)/total chromium of 0.97. In addition, as previously mentioned in this scientific opinion, tap water is usually treated with different oxidizing agents to make it potable, and this would promote the presence of Cr(VI) instead of Cr(III).

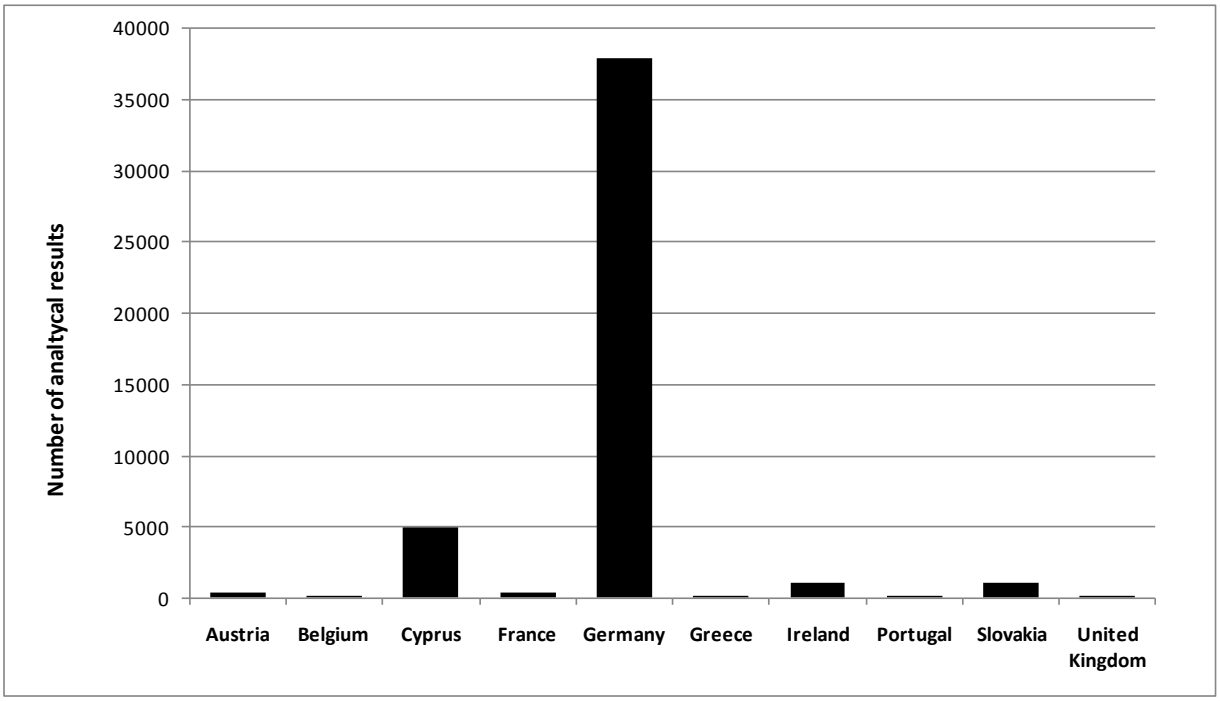

Figure 7: Distribution of drinking water samples analysed for chromium across different European countries.

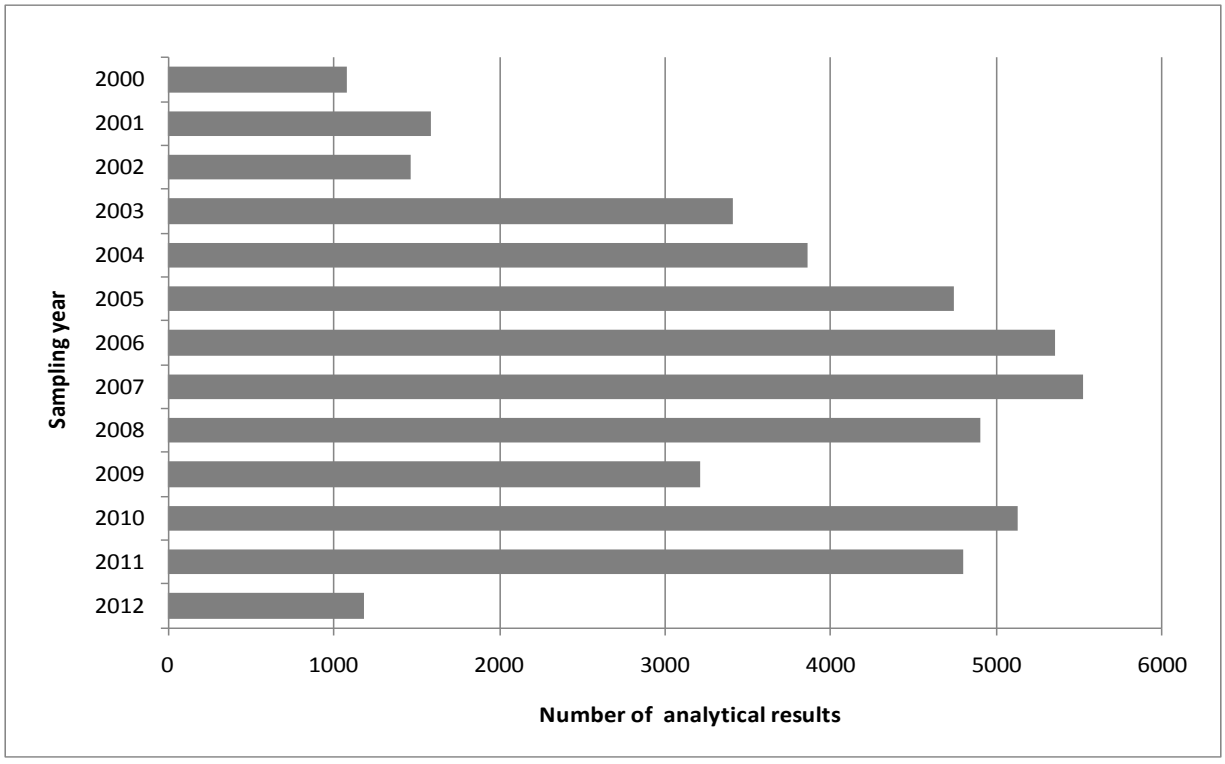

Figure 8: Distribution of analytical results for chromium in drinking water over the sampling years.

4.2.3. Analytical methods used

4.2.3.1. Analytical methods used in food analysis

Different analytical methods were reported for the analysis of food samples. However, 45 % of the analytical results did not report information on the analytical method used. Atomic absorption spectrometry (AAS) techniques were the most reported representing 33.5 % of the total followed by ICP-MS with 21.7 % of the total. Among the reported methods the highest sensitivity was associated with ICP-MS. Regarding the final food dataset selected for exposure assessments (24 629 samples), 49.1 % of the data were left-censored. Figure 9 shows quantified and left-censored data divided by food categories at FoodEx Level 1. The food groups 'Snacks, desserts, and other foods' and 'Non-alcoholic beverages (excepting milk based beverages)' reported the highest number of left-censored data (83.5 % and 80.2 %, respectively)¨. In contrast the food groups 'Legumes, nuts and oilseeds', 'Herbs, spices and condiments' and 'Composite food (including frozen products)' reported the lowest number of left-censored data (27.9 %, 26.8 % and 20.3 %, respectively).

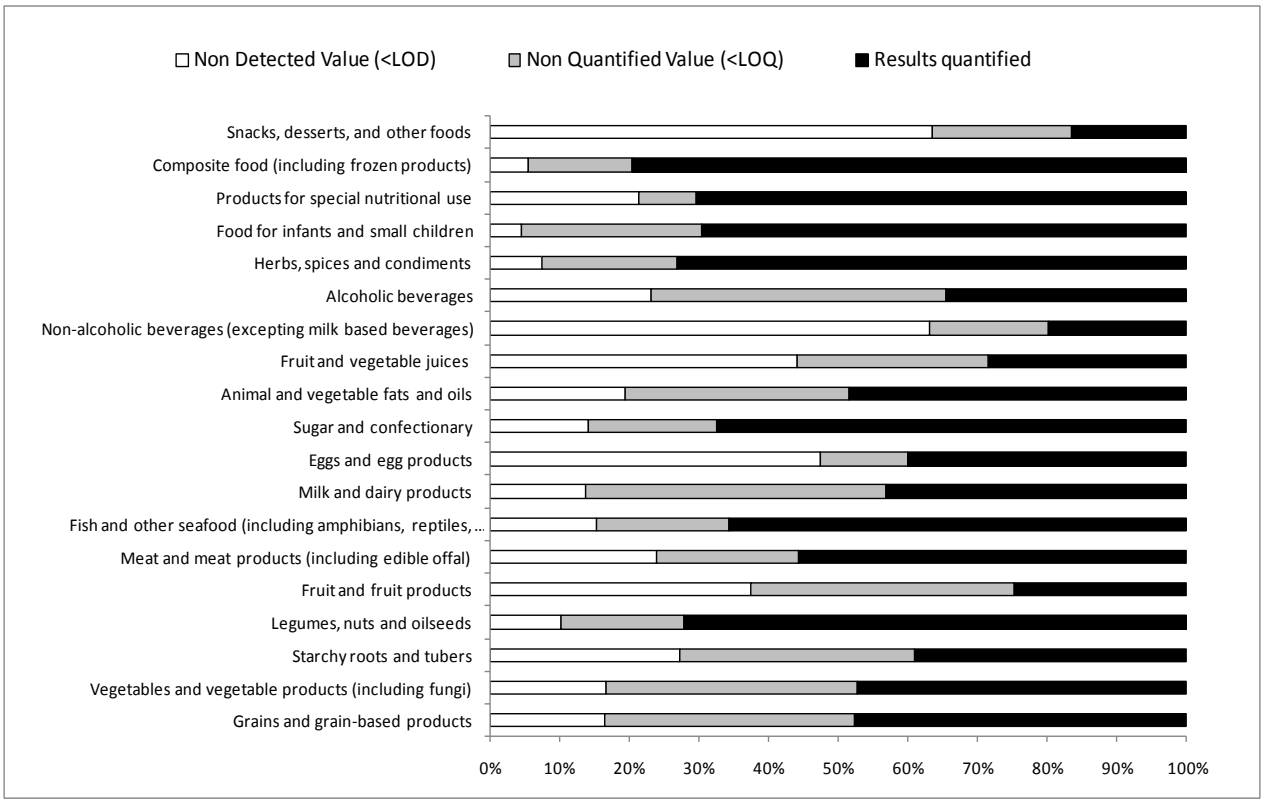

Figure 9: Percentage of analytical results below LOD, below LOQ and quantified in the final food dataset across the different food categories (FoodEx Level 1).

4.2.3.2. Analytical methods used in drinking water

In most of the cases (61 %), information on the analytical method was not reported. When reported, the main methods used for water analysis were AAS and ICP-MS techniques representing 26.5 % and 11.3 % of the total, respectively. By applying the selected LOQ cut-off of 10 µg/L a total of 1 292 samples were eliminated. After applying the cut-off, the left-censored data accounted for 91.3 % of the reported results.

4.2.4. Occurrence data by food category and by type of drinking water

The left-censored data were treated by the substitution method as recommended in the 'Principles and Methods for the Risk Assessment of Chemicals in Food' (WHO/IPCS, 2009b). The same method is indicated in the EFSA scientific report 'Management of left-censored data in dietary exposure assessment of chemical substances' (EFSA, 2010d) as an option in the treatment of left-censored data. The guidance suggests that the LB and UB approach should be used for chemicals likely to be present in the food (e.g. naturally occurring contaminants, nutrients and mycotoxins). At the LB, results below the LOQ and LOD were replaced by zero; at the UB the results below the LOD were replaced by the LOD and those below the LOQ were replaced by the value reported as LOQ.

4.2.4.1. Occurrence data in food (excluding drinking water)

As explained in Section 4.2.2.1, the reported occurrence data on food was considered as Cr(III). For those foods prepared with water before their consumption (coffee, tea infusions, and dry infant and follow-on food), the occurrence values provided for the dry foods were used either as such or with dilution factors (later described in Section 6.1) depending on how the consumption data were reported. Occurrence values provided for their corresponding prepared foods (e.g. twenty samples of tea beverage) were excluded.

Table 6 shows the summary statistics of chromium concentrations in the final dataset of food samples aggregated at FoodEx Level 1. Despite the important number of left-censored data present, no big differences are observed between LB and UB values in most of the food categories. A plausible explanation is that in general sensitive methods were used, and where high LOQs were reported they were linked to quantified samples, therefore, without relevance on the UB. In addition, by applying the LOQ cut-offs described in Section 4.2.2.1, the differences between LB and UB were reduced in several food groups, such as 'Beer and beer-like beverage' or 'Cereal-based food for infants and young children'.

At FoodEx level 1 all the food groups were well represented, with a maximum of 4 647 samples in the food group 'Vegetables and vegetable products (including fungi)' and a minimum of 80 samples in the food group 'Eggs and egg products'. Regarding the occurrence values, five food groups at FoodEx Level 1 showed the highest occurrence values: 'Products for special nutritional use', 'Herbs, spices and condiments', 'Sugar and confectionery', 'Vegetables and vegetable products (including fungi)', and 'Animal and vegetable fats and oils' (see Table 6). In all cases, a detailed evaluation of the occurrence data at different FoodEx levels was indispensable prior to their use to estimate the dietary exposure to chromium.

The highest occurrence was reported in the food group 'Products for special nutritional use'. In this food group, where Cr(III) is in some cases intentionally added, were reported average values of 12 129 μg/kg (n =2 131 LB = UB). A great heterogeneity of occurrence values was observed for the different food subgroups at lower FoodEx levels. Some of the highest values were reported for the food subgroup 'Combination of vitamins and minerals supplements' with a LB = 23 441 μg/kg and UB = 23 514 μg/kg (n = 582).

The group 'Herbs, spices and condiments' was reported on 611 occasions, with mean concentrations of 1627 μg/kg and 1665 μg/kg at the LB and UB, respectively. The high concentration reported in this food group is clearly driven by the presence of four samples of 'Cinnamon (*Cinnamonum verum* syn. *C. zeylanicum*)', all quantified and with an average value of 84 250 μg/kg. High levels of chromium in cinnamon have been described in the literature (Gul and Safdar, 2009). Other spices are also reported to contain high concentrations of chromium, such as 'Paprika powder' (LB = 3200 μg/kg and UB = 3271 μg/kg, n = 71) and 'Pepper, black and white (*Piper nigrum*)' (LB = 2609 μg/kg and UB = 2611 μg/kg, n = 105). High concentrations of chromium in spices and aromatic herbs have been reported by different authors (García et al., 2000; Divrikli et al., 2006; Kovacs et al., 2007; Sykula-Zajac and Pawlak, 2012).

A total of 1 126 samples were reported for the food group 'Sugar and confectionery'. Mean occurrence values were 625 μg/kg and 639 μg/kg at the LB and UB, respectively (Table 6). As

described for most of the food groups at FoodEx level 1, a broad range of concentrations was reported among the different food subgroups. The highest concentrations were observed for 'Chocolate (cocoa) products, unspecified' (1428 μg/kg, n = 421), together with 'Chocolate bars' (886 μg/kg, n = 5). The high chromium levels detected in the chocolate products notably influenced the mean occurrence values in the food group 'Sugar and confectionery'.

Another food group that stands out among the others due to its chromium levels was 'Animal and vegetable fats and oils' (LB = 263 μg/kg and UB = 301 μg/kg, n = 186). The main subgroups responsible for the high levels of chromium reported for this food group were the high levels found in some vegetable oils such as 'Sunflower oil' (LB = 592 μg/kg and UB = 680 μg/kg, n = 57) and 'Rapeseed oil' (LB = 425 μg/kg and UB = 427 μg/kg, n = 11). Other food samples with reported high levels of chromium were animal fats such as 'Pork lard (Schmaltz)' (LB = 263 μg/kg and UB = 264 μg/kg, n = 20) and 'Butter' (LB = 176 μg/kg and UB = 179 μg/kg, n = 23). Results reported in the literature on chromium levels in this type of foods are somehow contradictory, probably due to the low number of samples analysed. While in the second French TDS study (Nöel et al., 2012) high concentrations of chromium were reported in oils (1000 μg/kg), butter (640 μg/kg) or margarine (590 μg/kg), a previous study in Turkey found very low levels in different types of vegetable oils included sunflower oil (Pehlivan et al., 2008).

The most reported food group was 'Vegetables and vegetable products (including fungi)' with a total of 4 647 samples. Although this food group at FoodEx level 1 showed relatively high concentrations of chromium, an exhaustive evaluation of the different food subgroups provided a different conclusion. Only very specific food commodities within this food group presented high concentrations of chromium while the rest showed, in general, relatively low concentrations (below 100 μg/kg). Foods contained in the food subgroups 'Cocoa beans and cocoa products' and 'Tea and herbs for infusions (Solid)' reported the highest concentrations. Particularly high were the values reported for 'Cocoa powder' (n= 239) with average values of 4345 μg/kg (LB = UB), although all cocoa-related products had high concentrations of chromium. It is also important to mention that chocolate-containing foods had higher concentrations of chromium as compared to other food commodities at the same FoodEx level. This was the case for 'Croissant, filled with chocolate' (358 μg/kg, LB = UB) or 'Chocolate and chocolate products for diabetics' (1226 μg/kg, LB = UB). Regarding 'Tea and herbs for infusions (Solid)', apart from one sample of 'Maté (*Ilex paraguariensis*)' with reported concentration of 6930 μg/kg, 'Ginseng root (*Panax ginseng*)' and 'Camomile flowers (*Matricaria recutita*)' had the highest concentrations of chromium with 1327 μg/kg and 1150 μg/kg, respectively (LB = UB). Both 'Cocoa beans and cocoa products' and 'Tea and herbs for infusions' have been reported in the literature as possessing high amounts of chromium. As an example, different cocoa samples and chocolate products were recently analysed for chromium values in the range 260-6260 μg/kg (Sager, 2012). Similarly, high values of chromium have been reported in different herbs and infusions in a study carried out in Turkey (Başgel and Erdemoğlu, 2006), with concentrations in the range of 340-1220 μg/kg.

In the remaining food subgroups from 'Vegetables and vegetable products (including fungi)' there were only few foods for which high concentrations of chromium were reported. Examples of these foods are samples of 'Sea weeds' (441 μg/kg, LB = UB), 'Chilli pepper (*Capsicum frutescens*)' (1137 μg/kg, LB = UB.), 'Shiitake mushroom (*Lentinus edodes*)' (LB = 345 μg/kg and UB = 364 μg/kg) or 'Sun-dried tomatoes' (423 μg/kg, LB = UB) (see Appendix E for details).

Table 6: Summary statistics of chromium concentration (µg/kg) in the different food samples at FooodEx level 1. Values were rounded off to the nearest whole number (0 decimal places).

	N	LC (%)	Concentration (µg/kg)					
			LB/UB	Mean	P25	P50	P75	P95
Alcoholic beverages	1 594	66	LB	16	0	0	12	60
			UB	34	11	25	50	60
Animal and vegetable fats and oils	186	52	LB	263	0	0	80	1730
			UB	301	20	50	200	1730
Composite food (including frozen products)	295	16	LB	67	13	42	82	193
			UB	69	20	42	82	193
Eggs and egg products	80	60	LB	22	0	0	39	94
			UB	29	9	9	39	94
Fish and other seafood (including amphibians, reptiles, snails and insects)	1 565	34	LB	98	0	43	123	380
			UB	109	28	54	124	380
Food for infants and small children	699	30	LB	52	0	34	72	194
			UB	64	32	50	72	194
Fruit and fruit products	1 448	75	LB	22	0	0	0	116
			UB	39	9	17	40	120
Fruit and vegetable juices	1 216	72	LB	10	0	0	6.1	52
			UB	24	12	12	40	52
Grains and grain-based products	3 910	52	LB	103	0	0	70	260
			UB	135	30	60	100	260
Herbs, spices and condiments	611	27	LB	1627	0	137	1200	5800
			UB	1665	70	186	1200	5800
Legumes, nuts and oilseeds	1 167	14	LB	163	0	69	190	663
			UB	177	33	95	190	663
Meat and meat products (including edible offal)	2 088	44	LB	53	0	19	70	205
			UB	64	18	40	77	205
Milk and dairy products	608	57	LB	27	0	0	30	140
			UB	55	10	30	75	150
Non-alcoholic beverages (excepting milk based beverages)	399	80	LB	45	0	0	0	64
			UB	58	12	12	15	64
Products for special nutritional use	2 131	30	LB	12129	0	410	11100	50800
			UB	12219	182	620	11100	50800
Snacks, desserts, and other foods	230	84	LB	43	0	0	0	185
			UB	104	60	60	60	200
Starchy roots and tubers	631	61	LB	29	0	0	20	120
			UB	38	8	15	29	120
Sugar and confectionery	1 126	33	LB	625	0	187	913	2600
			UB	639	40	197	913	2600
Vegetables and vegetable products (including fungi)	4 647	53	LB	307	0	0	68	2540
			UB	319	10	30	70	2540

N: number of samples; LC: left-censored; LB: lower bound; UB: upper bound; P25/50/75/95: 25th/50th/75th/95th percentile.

4.2.4.2. Occurrence data in drinking water (water intended for human consumption and mineral waters)

The submitted occurrence data on the different types of water (see Table 7 below) were grouped under the generic name 'Drinking water' (including unspecified drinking water, bottled water, tap water, water ice and well water) that includes the types of water defined by legislation, i.e. water intended for human consumption and natural mineral waters. Bottled water, as used in this opinion, includes not only natural mineral waters, but also spring water and other bottled drinking water (see Section 4.2.2.2.)

As explained in Section 4.2.2.2. the chromium content reported in water was assumed to be all Cr(VI). For the 88 samples of bottled water where Cr(VI) and total chromium were reported only Cr(VI) was considered. The 88 samples with data reported on Cr(VI) showed an average value of 4.7 µg/L (LB = UB), with 12 samples below the LOQ (13.6 %). Out of the 76 samples quantified for Cr(VI),

71 corresponded to still mineral water and 5 to unspecified bottled water. The minimum concentration of Cr(VI) reported in the quantified samples was 0.1 µg/L (LB = UB), and the maximum 36.0 µg/L (LB = UB).

Among the 46 146 samples available. tap water samples were the most reported (60.6 %) with mean occurrence values of 0.2 µg/L and 1.9 µg/L at the LB and the UB, respectively (Table 7). Taking into account all bottled water samples (13 162) the mean occurrence values ranged between 0.3 µg/L for carbonated mineral water (LB) and 3.4 µg/L at the UB reported for unspecified bottled water. Overall, mean occurrence values at the LB ranged between 0.1 µg/L for water ice and 2.0 µg/L for unspecified drinking water. At the UB values ranged between 1.9 µg/L for tap water and 3.9 µg/L for well water.

Table 7: Summary statistics of chromium concentrations (µg/L) in the different types of drinking water (water intended for human consumption and mineral waters). Concentration values (LB and UB) were reported to one decimal place.

			N	LC	Concentration (µg/L)					
					LB/UB	Mean	P25	P50	P75	P95[(a)]
Drinking water	Bottle water	Bottled water (unspecified)	1 617	84	LB	0.9	0.0	0.0	0.0	6.7
					UB	3.4	1.0	4.0	5.0	6.77
		Carbonated mineral water	7 839	94	LB	0.3	0.0	0.0	0.0	1.2
					UB	2.8	2.0	2.0	3.0	10.0
		Still mineral water	3 706	88	LB	0.7	0.0	0.0	0.0	4.0
					UB	3.2	2.0	2.0	5.0	7.0
		Drinking water (unspecified)	3 174	55	LB	2.0	0.0	0.0	1.8	9.3
					UB	2.2	0.3	0.5	2.0	9.3
		Tap water	27 971	96	LB	0.2	0.0	0.0	0.0	0.0
					UB	1.9	1.0	1.0	2.0	5.0
		Water ice (for consumption)	21	95	LB	0.1	0.0	0.0	0.0	-
					UB	2.4	0.3	0.3	5.0	-
		Well water	1 818	86	LB	1.0	0.0	0.0	0.0	4.0
					UB	3.9	2.0	5.0	5.0	5.0

N: number of samples; LC: left-censored; LB: lower bound; UB: upper bound; P25/50/75/95: 25th/50th/75th/95th percentile.
(a): The 95th percentile estimates obtained on dietary surveys/age classes with less than 60 observations may not be statistically robust (EFSA, 2011b). Those estimates were not included in this table.

Different LOQ cut-offs other than 10 µg/L were assessed on the occurrence values to try to minimize the gap between LB-UB in the exposure calculations. However, no significant improvements were obtained without compromising the number of samples and, after all, 10 µg/L was considered as the most adequate cut-off.

5. Food consumption

5.1. EFSA's Comprehensive European Food Consumption Database

The EFSA Comprehensive European Food Consumption Database (Comprehensive Database) was built in 2010 based on information provided by EU Member States and the food consumption data for children obtained through an EFSA Article 36 project (Huybrechts et al., 2011). The Comprehensive Database version 1 contains results from a total of 32[16] different dietary surveys carried out in 22 different Member States covering more than 67 000 individuals (EFSA, 2011b). The Comprehensive Database includes individual food consumption data concerning infants (2 surveys from 2 countries), toddlers (8[16] surveys from 8 countries), children (16[16] surveys from 14 countries),

[16] When counting the total number of available dietary surveys and those for 'Toddlers' and 'Other children', the three Germans surveys named as Donald 2006, Donald 2007, and Donald 2008 are counted here as only one survey since they were carried out using the same methodology (Dietary record). For more details on these surveys see Table C1 in the Appendix C.

adolescents (14 surveys from 12 countries), adults (21 surveys from 20 countries), elderly (9 surveys from 9 countries) and very elderly (8 surveys from 8 countries).

The CONTAM Panel considered that chronic exposure to chromium (Cr(III) in food and Cr(VI) in drinking water) had to be assessed. As suggested by the EFSA Working Group on Food Consumption and Exposure (EFSA, 2011b), dietary surveys with only one day per subject were not considered as they are not adequate to assess repeated exposure. Similarly, subjects who participated only one day in the dietary studies, when the protocol prescribed more reporting days per individual, were also excluded for the chronic exposure assessment. Thus, for chronic exposure assessment, food consumption data were available from 26 different dietary surveys carried out in 17 different European countries (Appendix F).

Within the dietary studies, subjects were classified in different age classes as follows: Infants (< 12 months old), Toddlers (≥ 12 months to < 36 months old), Other children (≥ 36 months to < 10 years old), Adolescents (≥ 10 years to < 18 years old), Adults (≥ 18 years to < 65 years old), Elderly (≥ 65 years to < 75 years old) and Very elderly (≥ 75 years old)

Overall, the food consumption data gathered at EFSA in the Comprehensive Database are the most complete and detailed data currently available in the EU. However, it should be pointed out that different methodologies were used between surveys to collect the data and thus direct country-to-country comparisons can be misleading. Similarly to what is described for the occurrence data, consumption records are also codified according to the FoodEx classification system. Further details on how the Comprehensive Database is used are published in the Guidance of EFSA (2011b).

6. Exposure assessment in humans

6.1. Chronic exposure to trivalent chromium via the food

Reported analytical results for total chromium in food were assumed to be as Cr(III) as explained in Section 4.2.2.1.

Despite their high content in water, in water-based foods classified (following FoodEx classification) as ‘Fruit and vegetable juices’, ‘Soft drinks’, and ‘Alcoholic beverages’ the analytical results reported as total chromium were assumed to be Cr(III), as it is assumed that the Cr(VI) present in water is completely reduced to Cr(III).

In order to consider only the content of Cr(III), in foods such as coffee, tea infusions, and dry infant and follow-on food only the occurrence values reported for the dry foods (therefore only Cr(III)) were considered. Depending on how the consumption was reported the dilution factors detailed below were applied or not.

Due to the limited and incomplete consumption information in the Comprehensive database on fortified foods, foodstuffs for particular nutritional use (PARNUTS) and food supplements, the CONTAM Panel decided to exclude the food group ‘Products for special nutritional use’ from the dietary exposure calculations. In Section 6.1.3. a particular scenario is described evaluating the potential additional contribution of this type of food products to the dietary exposure to Cr(III).

Different assumptions were done before assessing the dietary exposure. When food categories were not represented they were either excluded from the exposure assessment or, when possible, assigned an occurrence value derived from similar food commodities. In general, when less than 10 samples were reported for one specific food group, the average occurrence value of all samples contained in the immediate upper FoodEx level was used. Dilution factors were also used to match the occurrence values reported in dry samples with their respective liquid consumption amounts. An average dilution factor of 18 was used to match occurrence value in coffee beans with the different type of coffees, except for ‘coffee expresso’ where the dilution factor was 7 and for ‘instant coffee’ where it was 63. Other dilution factors used were 100 for tea and herbal leaf varieties, 60 for cocoa powder, and 8 for follow-on and infant formulae (EFSA, 2011a,b; USDA, 2013).

For calculating the chronic dietary exposure to Cr(III), food consumption and body weight data at the individual level were accessed in the Comprehensive Database. Occurrence data and consumption data were linked at the lowest FoodEx possible. In addition, the different food commodities were grouped within each food category to better explain their contribution to the total dietary exposure to Cr(III). For each country, exposure estimates were calculated per dietary survey and age class (see Section 5.1.1). Chronic exposure estimates were calculated for 26 different dietary surveys carried out in 17 different European countries. Not all countries provided consumption information for all age groups and in some cases the same country provided more than one consumption survey.

6.1.1. Mean and high dietary exposure to trivalent chromium

The mean and the high (95th percentile) chronic dietary exposures to Cr(III) were calculated separately for each dietary survey using consumption data recorded at the individual level and for both LB and UB mean concentrations. Minimum, median and maximum exposure estimates across dietary surveys and age groups are reported in Table 8. Detailed mean and 95th percentile dietary exposure estimates calculated for each of the 26 dietary surveys are presented in Appendix G. In accordance with the specifications of the EFSA Guidance on the use of the Comprehensive database (EFSA, 2011b), 95th percentile estimates for dietary surveys/age classes with less than 60 observations are not considered since they may not be statistically robust. Mean chronic dietary exposure values, across the different dietary surveys and age classes, ranged from 0.6 µg/kg b.w. per day (minimum LB) to 5.9 µg/kg b.w. per day (maximum UB). The 95th percentile dietary exposure ranged from 1.1 µg/kg b.w. per day (minimum LB) to 9.4 µg/kg b.w. per day (maximum UB).

Table 8: Summary statistics of the chronic exposure assessment (µg/kg b.w per day) for Cr(III) across European dietary surveys. Estimates were rounded up to one decimal place.

Mean dietary exposure (µg/kg b.w per day)						
	Lower bound (LB)			Upper bound (UB)		
	Min	**Median**	**Max**	**Min**	**Median**	**Max**
Infants	1.5	-[(a)]	2.2	1.9	-[(a)]	3.6
Toddlers	2.3	2.4	4.6	3.1	3.7	5.9
Other children	1.6	2.4	3.5	2.1	3.3	4.9
Adolescents	0.9	1.2	2.1	1.2	1.6	2.5
Adults	0.8	0.9	1.2	1.0	1.2	1.6
Elderly	0.6	0.8	1.0	1.0	1.1	1.4
Very Elderly	0.7	0.8	1.2	1.0	1.1	1.5
95th percentile dietary exposure[(b)] (µg/kg b.w per day)						
	Lower bound (LB)			Upper bound (UB)		
	Min	**Median**	**Max**	**Min**	**Median**	**Max**
Infants	4.8	-[(c)]	-[(c)]	9.4	-[(c)]	-[(c)]
Toddlers	3.4	4.5	5.9	4.5	6.7	9.0
Other children	2.9	4.2	7.3	3.7	5.6	7.9
Adolescents	1.7	2.4	4.1	2.3	2.9	4.8
Adults	1.2	1.5	2.0	1.7	2.0	2.6
Elderly	1.1	1.3	1.5	1.6	1.7	2.0
Very Elderly	1.2	1.6	1.8	1.7	2.0	2.3

b.w. : body weight.
(a): Not calculated since estimates were only available from two dietary surveys;
(b): The 95th percentile estimates obtained on dietary surveys/age classes with less than 60 observations may not be statistically robust (EFSA, 2011b). Those estimates were not included in this table.
(c): Not calculated since estimates were only available from one dietary survey.

6.1.2. Contributions of different food groups to chronic exposure to trivalent chromium by age class

The dietary exposure to Cr(III) and the contribution of different foods is presented divided by age class and individual dietary survey. It is important to mention that some dietary surveys (DIPP and FINDIET 2007) reported the consumption data at the disaggregated level (e.g. reporting the amount of flour instead of the amount of bread), which could have influence on the contribution of specific food categories to the dietary exposure to Cr(III). Before calculating the dietary exposure, the available foods were grouped to explain their contribution to the total exposure to Cr(III). Although some food commodities such as tea, coffee and cocoa are described in more than one food category (described within 'Vegetables and vegetable products' but also within 'Non-alcoholic beverages'), they were all grouped as 'Non-alcoholic beverages' when describing the contribution to the dietary exposure to Cr(III). Appendix E shows the different groups that were created.

6.1.2.1. Infants and toddlers

The results for infants should be cautiously interpreted as only two dietary surveys are available. Mean dietary exposure to Cr(III) for infants ranged between 1.5 µg/kg b.w. per day and 3.6 µg/kg b.w. per day (minimum LB and maximum UB). The 95th percentile dietary exposure for the single qualifying study was 4.8 µg/kg b.w. per day (LB) and 9.4 µg/kg b.w. per day (UB).

In the infant population the exposure to Cr(III) came basically from two different food categories. 'Foods for infants and small children' made the highest contribution (34-53 % of the total) followed by 'Milk and dairy products' (13-18 % of the total) (Figure 10).

As in previous scientific opinions, a mean consumption of human milk of 800 mL per day and a maximum of 1200 mL per day (EFSA CONTAM Panel, 2011) was considered representative for a breast-fed infant of three months and 6.1 kg b.w. For the occurrence value, an average Cr(III)

concentration of 2 µg/L in human milk was selected. This selection was based on the upper value of the range of mean total Cr concentrations described among most of the European studies on breast milk samples, 0.14-1.80 µg/L (see Section 4.1.3 and Appendix D). The mean dietary exposure for an infant of 6.1 kg exclusively fed with human milk was estimated to be 0.3 µg/kg b.w. per day, while for the same infant with high consumption the dietary exposure would be 0.4 µg/kg b.w. per day.

Nine dietary surveys were available for 'Toddlers'. This age class showed the highest exposure to Cr(III). The mean dietary exposure to Cr(III) ranged from 2.3 µg/kg b.w. per day to 5.9 µg/kg b.w. per day (minimum LB and maximum UB across European dietary surveys, respectively). The 95th percentile dietary exposure estimates ranged from a minimum LB of 3.4 µg/kg b.w. per day to a maximum UB of 9.0 µg/kg b.w. per day.

In the toddler population the exposure to Cr(III) was in general mainly due to 'Foods for infants and small children' (1-26 % of the total, median = 9 %), 'Milk and dairy products' (9-25 % of the total, median = 14 %), and 'Bread and rolls' (0.3-12 % of the total, median = 10 %) (Figure 10). In some dietary surveys 'Chocolate (cocoa) products' also made an important contribution to the dietary exposure to Cr(III). The contribution of the food group 'Vegetables and vegetable products (including fungi)' ranged between 3 % and 8 % of the total (median = 6 %). In one survey the reported consumption of cocoa powder led to the food group 'Non-alcoholic beverages' to contribute up to 45 % of the total exposure to Cr(III). However, the consumption data from this dietary survey refers only to 17 individuals and, therefore, this value could not be representative.

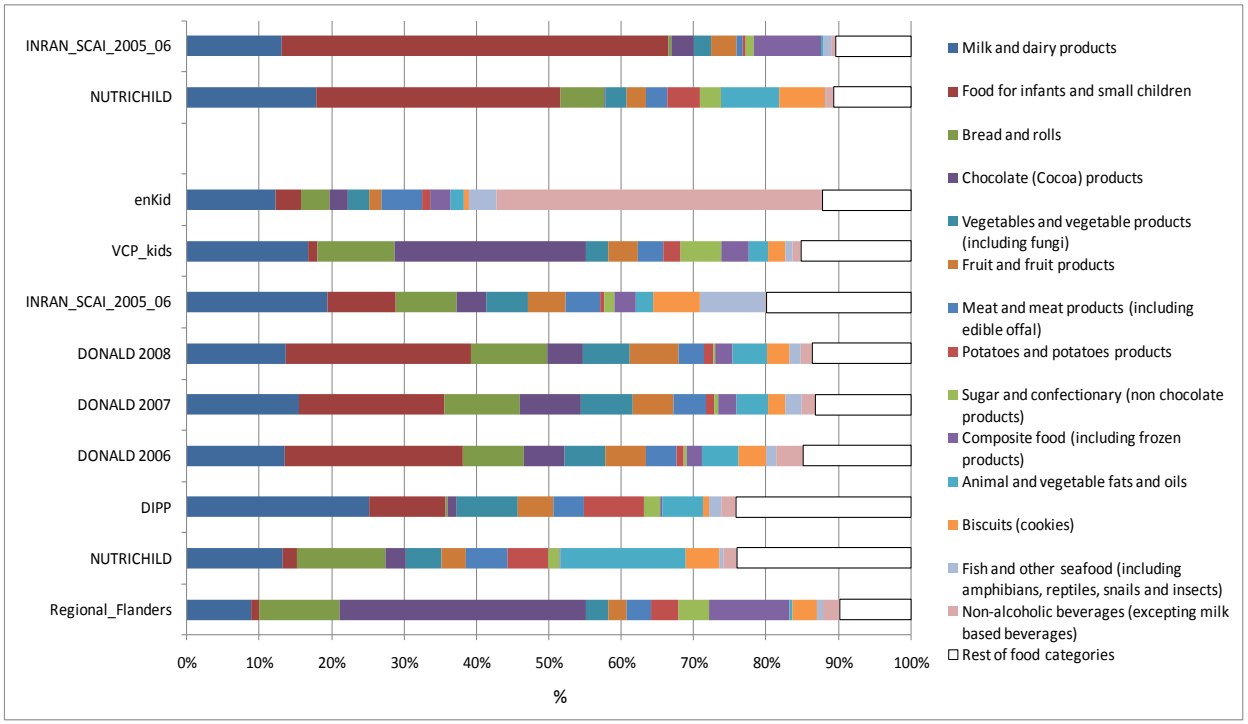

Figure 10: Main food groups contributing (%) to the chronic dietary exposure to Cr(III) for the age classes 'Infants' (from the top the two first surveys) and 'Toddlers'. Data are presented by individual dietary surveys across Europe using LB estimations. The names on the left refer to the names of the different surveys (see Appendix F for more details).

6.1.2.2. Other children

A total of 17 dietary surveys were available to evaluate the chronic dietary exposure to Cr(III) in the age class 'Other children'. The mean dietary exposure ranged from 1.6 µg/kg b.w. per day to 4.9 µg/kg b.w. per day (minimum LB and maximum UB, respectively). The 95th percentile dietary

exposure estimates ranged from a minimum LB of 2.9 µg/kg b.w. per day to a maximum UB of 7.9 µg/kg b.w. per day.

In general, the main contributors to the exposure in the age class 'Other children' were the food groups 'Milk and dairy products' (7-22 % of the total, median = 11 %), 'Chocolate (Cocoa) products' (4-32 % of the total, median =12 %) and 'Bread and rolls' (1-19 % of the total, median = 10 %) (Figure 12). Apart from the high contribution to the exposure of composite food in some countries, the food group 'Vegetables and vegetable products (including fungi)' contributed among the different dietary surveys only between 1 % to 10 % of the total exposure to Cr(III) (median = 4 %). As for 'Toddlers', the reported consumption of cocoa powder was responsible of the high contribution of 'Non-alcoholic beverages' in the two dietary surveys with the highest values (31 % and 40 %).

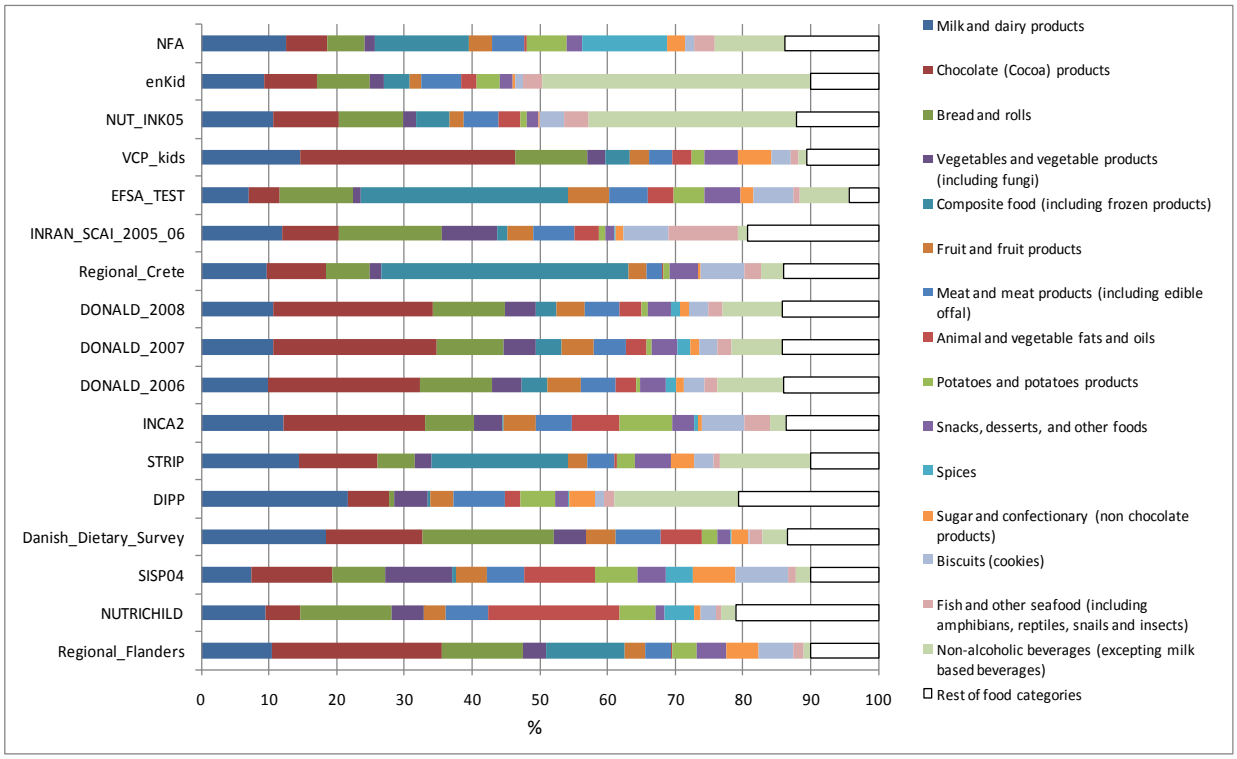

Figure 11: Main food groups contributing (%) to the chronic dietary exposure to Cr(III) for the age class 'Other children'. Data are presented by individual dietary surveys across Europe using LB estimations. The names on the left refer to the names of the different surveys (see Appendix F for more details).

6.1.2.3. Adolescents

A total of 12 dietary surveys were available to estimate the chronic exposure to Cr(III) in 'Adolescents'. The minimum value for the mean dietary exposure at the LB was 0.9 µg/kg b.w. per day, while the maximum estimated value at the UB was 2.5 µg/kg b.w. per day. For the 95th percentile dietary exposure the values ranged between 1.7 µg/kg b.w. per day (minimum LB) and 4.8 µg/kg b.w. per day (maximum UB).

In general, the main foods contributing to the dietary exposure to Cr(III) in 'Adolescents' were the same as described for 'Toddlers'. The main food groups were 'Bread and rolls' (6-20 % of the total, median = 13 %), 'Chocolate (Cocoa) products' (4-30 % of the total, median = 9 %) and 'Milk and dairy products' (6-17 % of the total, median 8 %). In addition, 'Non-alcoholic beverages' was also a major source of exposure to Cr(III) (1-37 % of the total, median 8 %) with an important contribution of cocoa powder used to prepared cocoa drinks (particularly in specific surveys such as enKid and

NUT_INK05). The range of contribution of 'Vegetables and vegetable products (including fungi)' to the exposure across the different surveys varied between 1 % and 10 % (median = 4 %).

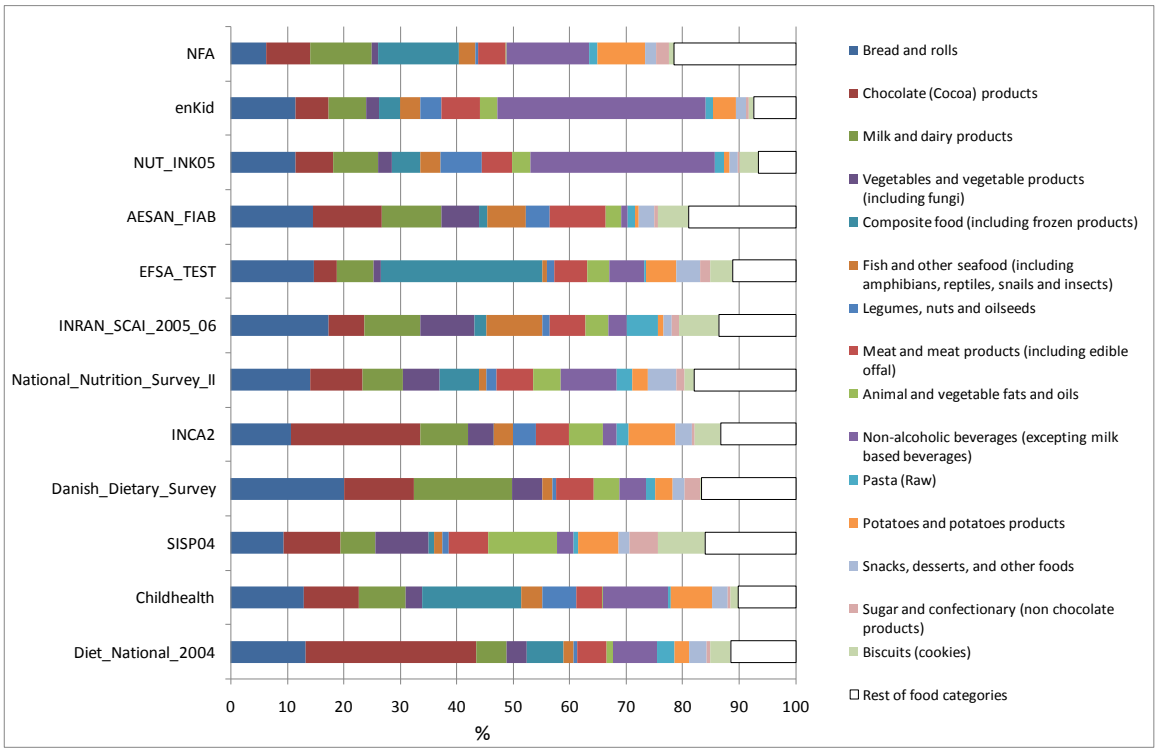

Figure 12: Main food groups contributing (%) to the chronic dietary exposure to Cr(III) for the age class 'Adolescents'. Data are presented by individual dietary surveys across Europe using LB estimations. The names on the left refer to the names of the different surveys (see Appendix F for more details).

6.1.2.4. Adults

The adult populations showed lower exposure to Cr(III) than the younger populations. Considering the 15 dietary surveys available for this age class, the mean dietary exposure to Cr(III) in the European adult population varied between 0.8 µg/kg b.w. per day and 1.6 µg/kg b.w. per day (minimum LB and maximum UB). The 95th percentile dietary exposure ranged from 1.2 µg/kg b.w. per day (minimum LB) and 2.6 µg/kg b.w. per day (maximum UB).

Regarding the contribution of the different food categories to the exposure to Cr(III) in the adult population, the food category 'Bread and rolls' made, in general, the highest impact on the exposure (0.4-18 %, median 14 %). As occurred in the previous age classes the other food groups making an important contribution to the exposure to Cr(III) were 'Milk and dairy products' (5-15 % of the total, median 8 %), 'Non-alcoholic beverages' (2-15 % of the total, median 7 %), and 'Chocolate (Cocoa) products' (2-18 % of the total, median = 6 %). Compared to the younger population, the food categories 'Meat and meat products (including edible offal)' (5-10 % of the total, median 7 %) and 'Potatoes and potatoes products' (1-13 % of the total, median 5 %) played a more important role in the exposure to Cr(III). Similar contribution to the exposure to Cr(III) was observed for 'Vegetables and vegetable products (including fungi)' across the different surveys with values between 2 % and 13 % (median = 6 %).

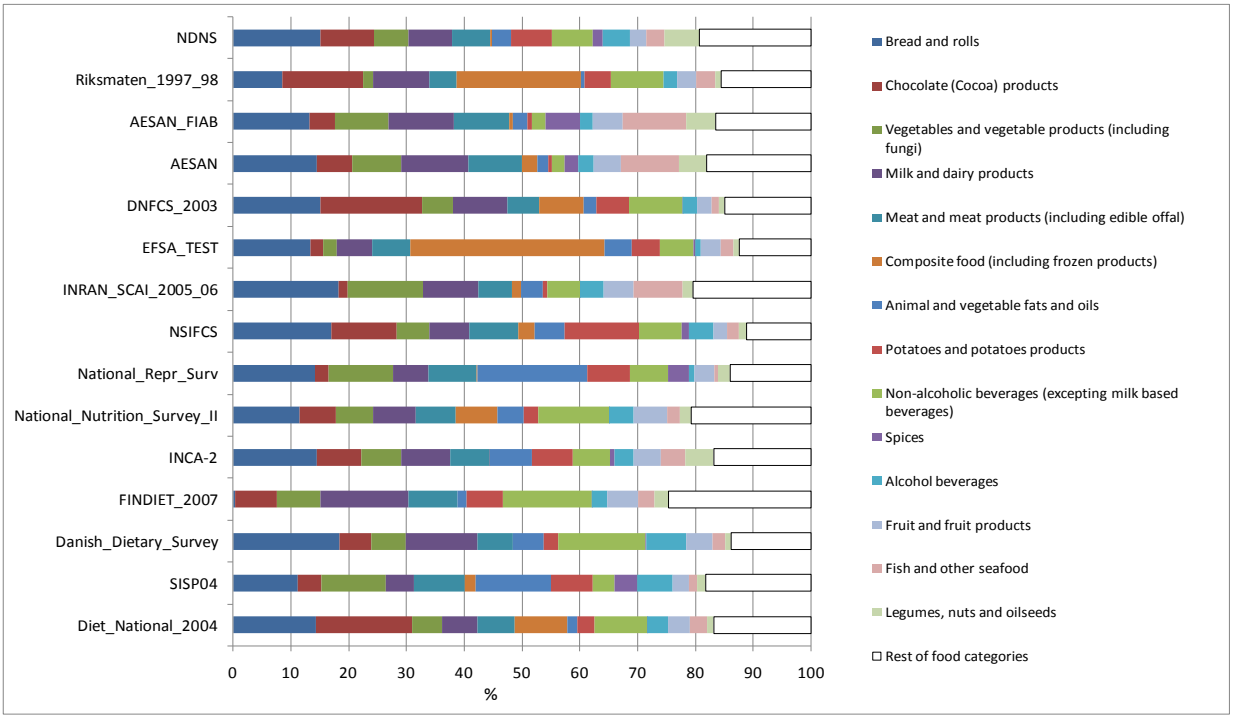

Figure 13: Main food groups contributing (%) to the chronic dietary exposure to Cr(III) for the age class 'Adults'. Data are presented by individual dietary surveys across Europe using LB estimations. The names on the left refer to the names of the different surveys (see Appendix F for more details).

6.1.2.5. 'Elderly' and 'Very elderly'

A total of seven and six dietary surveys across Europe were available for the age classes 'Elderly' and 'Very elderly', respectively. For the 'Elderly' population the mean dietary exposure to Cr(III) ranged between 0.6 µg/kg b.w. per day and 1.4 µg/kg b.w. per day (minimum LB and maximum UB). The 95th percentile dietary exposure ranged from 1.1 µg/kg b.w. per day (minimum LB) to 2.0 µg/kg b.w. per day (maximum UB). Very similar values were obtained for the 'Very elderly' population. Mean dietary exposure varied between 0.7 µg/kg b.w. per day and 1.5 µg/kg b.w. per day (minimum LB and maximum UB), while the 95th percentile dietary exposure ranged between 1.2 µg/kg b.w. per day (minimum LB) and 2.3 µg/kg b.w. per day (maximum UB).

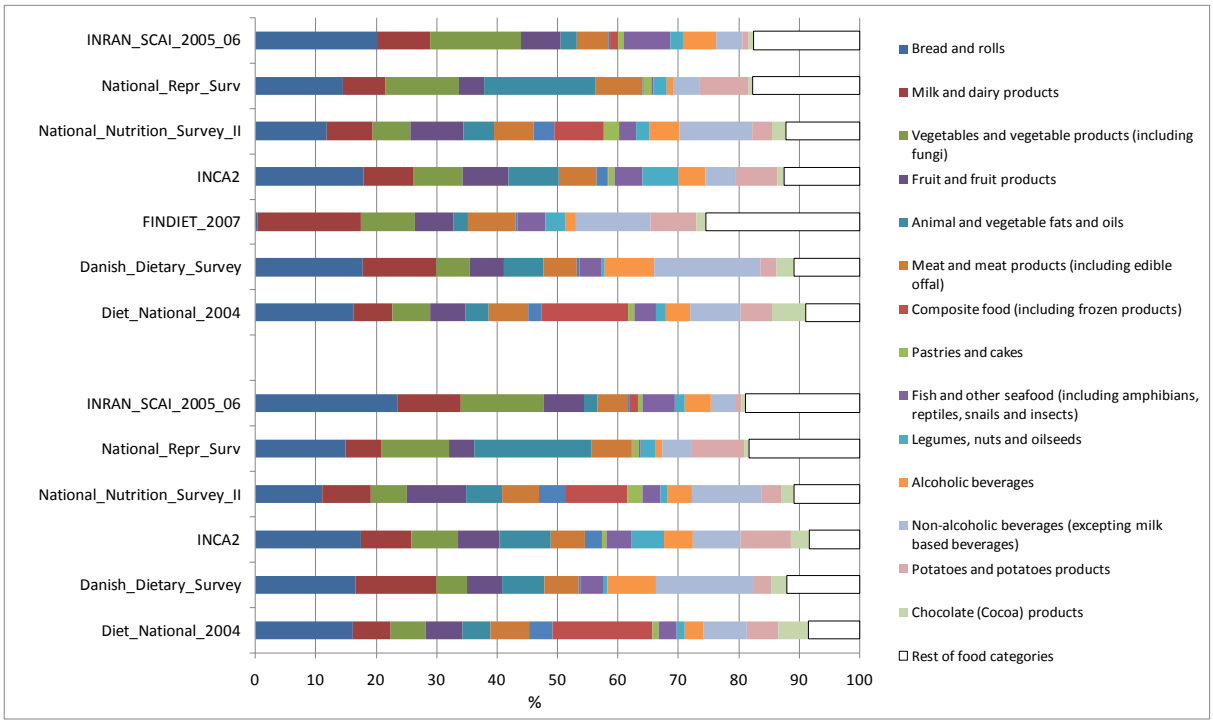

Figure 14: Main food groups contributing (%) to the chronic dietary exposure to Cr(III) for the age classes 'Elderly' (from the top the seven first surveys) and 'Very elderly'. Data are presented by individual dietary surveys across Europe using LB estimations. The names on the left refer to the names of the different surveys (see Appendix F for more details).

There were hardly any differences between 'Elderly' and 'Very elderly' populations regarding the main contributors to the dietary exposure to Cr(III). Compared with the other age classes one of the main differences was that the food group 'Chocolate (cocoa) products' lost its relevance in the contribution to the exposure. However, and similarly to the other age classes the food groups 'Bread and rolls', 'Milk and dairy products' and 'Non-alcoholic beverages' had a significant contribution to the exposure to Cr(III). Especially important was the food group 'Bread and rolls' with contribution median values of 16 % (0.4-20 %) in the 'Elderly' population and also 16 % (11-23 %) in the 'Very elderly' population. The contribution of 'Non-alcoholic beverages' ranged between 4 % and 17 % of the total (median = 8 %) in 'Elderly' population and between 4 % and 16 % (median = 8 %) in 'Very elderly' population. 'Vegetables and vegetable products (including fungi)' became one of the most important contributors to the exposure to Cr(III), especially in the 'Elderly' population (6-15 % of the total, median 8 %), although also in the very 'Elderly' age class (5-14 % of the total, median 7 %). In both age classes the food group 'Fruit and fruit products' was an important source of Cr(III) as compared with other age classes ('Elderly' (4-9 %, median = 6 %) and 'Very elderly' (4-10 %, median = 6 %)).

6.1.2.6. Conclusions

In conclusion, apart from the food group 'Foods for infants and small children', which was an important source of Cr(III) in 'Infants' and 'Toddlers', the main contributors to the dietary exposure to Cr(III) among the different age classes were the food categories 'Bread and rolls', 'Milk and dairy products', 'Chocolate (cocoa) products' (except for 'Elderly' and 'Very elderly' populations) and 'Non-alcoholic beverages'. Other food groups that were important contributors to the dietary exposure to Cr(III) were 'Meat and meat products' (mainly for 'Adolescents', 'Adults', 'Elderly' and 'Very elderly' population), and 'Fruit and fruit products' (for 'Elderly' and 'Very elderly' population). 'Vegetables and vegetable products (including fungi)' contributed to the exposure to Cr(III) with

median values that ranged between 4 % in 'Adolescents' and 'Other children', and 8 % in the 'Elderly' population.

Whereas the high contribution of 'Chocolate (cocoa) products' is mainly due to their high Cr(III) levels, for other foods it is more due to the fact that they are highly consumed (e.g. bread and rolls). As can be seen in Appendix E, 'Chocolate (cocoa) products' refers to a group where Cr(III) concentration up to 1500 µg/kg were reported (minimum 500 µg/kg). In contrast, the group 'Bread and rolls' includes foods with relatively low levels of Cr(III) (LB range = 30-110 µg/kg) but consumed everyday and in, usually, higher amounts than the chocolate products. A similar situation occurs with 'Milk and dairy products' where chromium concentrations are even lower (LB range = 13-62 µg/kg). The important contribution of the food group 'Non-alcoholic beverages' in the dietary exposure to Cr(III) is mainly due to the fact that both cocoa powder used to prepare cocoa drinks and cocoa beverages were included in this group. Relatively high concentration of Cr(III) is reported for cocoa powder and as consequence for cocoa beverages (see Appendix E). Additional contribution of 'Non-alcoholic beverages' to the exposure to Cr(III) is also made by the presence of coffee and tea (reported both as solid and liquid) in this food group.

The case of 'Vegetables and vegetable products (including fungi)' is slightly different as compared with the previous food groups. In this case we refer to a food group that covers a large and heterogeneous amount of foods with very different Cr(III) concentration. The large amount of foods included in this category is, probably, the reason for their contribution to the overall exposure to Cr(III).

6.1.3. Dietary exposure for specific groups

Vegetarians

The Comprehensive Database contains only very limited data on food consumption of people who declared they were vegetarian at the time of the survey. Considering the surveys with at least 15 adult vegetarians, the available data were grouped in five dietary surveys (FI/2, 39 individuals; FR, 15 individuals; DE/4, 237 individuals; SE/1, 18 individuals and UK, 77 individuals). When comparing the five surveys with data on both general and vegetarian population, virtually the same values were observed for mean dietary exposure (0.8 - 1.3 µg/kg b.w. per day versus 0.7 - 1.4 µg/kg b.w. per day (minimum LB- maximum UB), respectively), and for the 95th dietary exposure (1.3 - 2.2 µg/kg b.w. per day versus 1.5 - 2.2 µg/kg b.w. per day (minimum LB - maximum UB), respectively.)

Consumers of fortified foods, foodstuff for particular nutritional use (PARNUTS) and food supplements

Overall, the Comprehensive database contains limited information on the consumption of fortified foods, foodstuffs for particular nutritional use (PARNUTS) and food supplements. Only some of the surveys registered and consequently reported the consumption of the above mentioned products. Moreover, the FoodEx classification used in the Comprehensive Database does not allow to correctly specify those susceptible to contain Cr(III). This could lead to inaccurate exposure estimations. Based on these facts, the CONTAM Panel decided to use the exposure to Cr(III) calculated in the Scientific opinion on the safety of chromium picolinate adopted in 2010 by the ANS Panel (EFSA ANS Panel, 2010a). The ANS Panel proposed use levels per serving equal to 12 µg of Cr(III) from fortified foods and 300 µg of chromium from PARNUTS. No use levels were proposed for food supplements, although the ANS Panel noted that levels up to 600 µg/day Cr(III) could be consumed from these supplements.

Using one (typical intake) and three (upper intake) servings per day the combined exposure from supplemental intake from PARNUTS and fortified foods was calculated. For food supplements 600 µg/day Cr(III) was used for both typical and upper intake. The combined exposure from supplemental intake in adults (i.e. from fortified foods, PARNUTS and food supplements) would be

between 910 μg/day for a typical intake and 1540 μg/day for upper intake. Comparing to the maximum mean dietary exposure to chromium (LB-UB) calculated in the current opinion for adults (1.2-1.6 μg/kg b.w. per day, 86.5-112.6 μg/day) the typical exposure due to supplemental intake would be 8-11 times higher than that obtained from food intake. The exposure from the upper supplemental intake would also be 8-11 times higher than the maximum dietary exposure (LB-UB) in the 95th exposed population (2.0-2.6 μg/kg b.w. per day, 144.7-190.2 μg/day).

6.2. Exposure to hexavalent chromium via drinking water (water intended for human consumption and mineral waters)

Reported analytical results for chromium in drinking water were assumed to be all Cr(VI) as explained in Section 4.2.2.2. For the main scenario on the exposure to Cr(VI), food was excluded. However, in a further scenario, it was considered that in certain foods prepared with water before their consumption (such as coffee, tea infusions, and dry infant and follow-on food) an incomplete reduction of the Cr(VI) present in water into Cr(III) may happen if the foods are ingested immediately after their preparation. As explained in Section 6.1., the chromium present in water-based foods classified (following FoodEx classification) as 'Fruit and vegetable juices', 'Soft drinks' and 'Alcoholic beverages' was assumed to be Cr(III). Therefore, these food groups are not considered in the estimation of the exposure to Cr(VI).

As carried out with the exposure to Cr(III), the mean and the high (95th percentile) chronic exposures to Cr(VI) were calculated separately for each dietary survey using consumption data recorded at the individual level and for both LB and UB mean concentrations. In most of the reported data on drinking water, consumption refers to 'Tap water' (63.3 %) followed by bottled water (27.7 %).

Minimum, median and maximum exposure estimates across dietary surveys and age groups are reported in Table 9. Mean chronic exposure values, across the different dietary surveys and age classes, ranged from 0.7 ng/kg b.w. per day (minimum LB) to 159.1 ng/kg b.w. per day (maximum UB). The 95th percentile dietary exposure ranged from 2.8 ng/kg b.w. per day (minimum LB) to 320.2 ng/kg b.w. per day (maximum UB). As observed in Table 9 the maximum exposure to Cr(VI) through the consumption of drinking water was estimated in the youngest population ('Infants' and 'Toddlers').

As mentioned for the exposure assessment to Cr(III) via food, the presence of only two surveys for infants implies that the exposure to Cr(VI) via drinking water in this age class should be cautiously interpreted. In the two surveys for infants, the reported data on water consumption mostly refer to the water used to reconstitute infant food (infant and follow-on food) since the consumption data on these foods were predominantly reported disaggregated. Contrarily, in the toddler population most of the data on water consumption refer to water consumed as such.

In all other age classes, a broad range of values between minimum LB and maximum UB in the mean and high exposure calculations is observed. Apart from small variations in the consumption pattern among surveys, the main reason for this broad range is the difference between LB and UB estimates for 'Tap water' (LB = 0.2 μg/L, UB = 2 μg/L), the type of drinking water mostly reported in the Comprehensive Database. In addition, in a few dietary surveys only a small number of individuals reported water consumption since the surveys were mainly focused on nutrient intake (Greece, Cyprus, Latvia and Hungary where the percentage of consumers was less than 50 %). As a consequence, the calculated exposure to Cr(VI) via water may have been slightly underestimated in certain cases, specially for the minimum mean exposure estimates. However, the exposure estimates for both average population and highly exposed population are adequate for risk characterisation since consumption values up to 1.5 L in average population and up to 2.5 L for high consumers were reported across the different surveys and age classes.

Table 9: Summary statistics of the chronic exposure assessment (ng/kg b.w per day) for Cr (VI) across European dietary surveys through the consumption of drinking water as such (water intended for human consumption and mineral waters). Surveys from Greece (age class 'Other children') and from Cyprus (age class 'Adolescents') and those with a percentage of consumers less than 50 % were excluded (Latvia and Hungary). Estimates were rounded up to one decimal place.

Mean exposure (ng/kg b.w. per day)						
	Lower bound (LB)			Upper bound (UB)		
	Min	**Median**	**Max**	**Min**	**Median**	**Max**
Infants	14.2	-[(a)]	33.2	106.2	-[(a)]	159.1
Toddlers	7.5	15.3	39.6	34.8	82.2	96.6
Other children[(d)]	0.7	7.9	26.6	7.4	49.0	60.8
Adolescents[(d)]	0.9	4.0	10.2	8.8	26.9	44.2
Adults[(d)]	1.4	4.9	10.9	9.5	24.8	43.7
Elderly[(d)]	1.9	4.0	8.4	21.0	23.3	33.4
Very Elderly[(d)]	1.3	4.8	7.4	15.3	26.2	33.0
95th percentile exposure[(b)] (ng/kg b.w per day)						
	Lower bound (LB)			Upper bound (UB)		
	Min	**Median**	**Max**	**Min**	**Median**	**Max**
Infants	49.8	-[(c)]	-[(c)]	-[(c)]	-[(c)]	320.2
Toddlers	16.1	101.3	113.3	126.5	185.4	239.3
Other children[(d)]	2.8	22.2	76.0	28.1	108.9	150.9
Adolescents[(d)]	2.9	11.5	29.7	28.5	64.1	110.3
Adults[(d)]	4.3	13.7	29.3	32.3	60.2	108.3
Elderly[(d)]	4.8	11.6	24.1	50.7	59.4	89.8
Very Elderly[(d)]	10.5	13.8	21.0	51.8	64.1	87.4

b.w.: body weight; LB: lower bound; UB: upper bound; P95: 95th percentile.

(a): Not calculated since estimates were only available from two dietary surveys;

(b): The 95th percentile estimates obtained on dietary surveys/age classes with less than 60 observations may not be statistically robust (EFSA, 2011b). Those estimates were not included in this table.

(c): Not calculated since estimates were only available from one dietary survey.

(d): Surveys from Greece (age class 'Other children'), Cyprus (age class 'Adolescents'), Latvia (age classes 'Other children', 'Adolescents' and 'Adults') and Hungary (age classes 'Adults', 'Elderly' and 'Very elderly'were excluded (see Table G2 in appendix)

A separate scenario was applied to estimate the exposure to Cr(VI) through the consumption of bottled water, as specified in the terms of reference. In this scenario, the occurrence values on Cr(VI) reported for the three types of bottled water (unspecified, carbonated and still mineral water) were combined with the available consumption data on bottled water. The summary statistics of the exposure assessment (ng/kg b.w. per day) to Cr (VI) under this scenario are shown in Table 10.

Table 10: Summary statistics of the chronic exposure assessment (ng/kg b.w. per day) for Cr (VI) across all European dietary surveys through the consumption of bottled water. Dietary surveys with no data on consumption of bottled water were not included. Estimates were rounded up to one decimal place.

Mean exposure (ng/kg b.w per day)						
	Lower bound (LB)			Upper bound (UB)		
	Min	**Median**	**Max**	**Min**	**Median**	**Max**
Infants	7.3	-[(a)]	32.4	28.2	-[(a)]	149.8
Toddlers	< 0.1	6.9	13.5	< 0.1	26.4	62.5
Other children	< 0.1	4.1	11.6	< 0.1	16.2	44.8
Adolescents	< 0.1	1.4	9.6	< 0.1	6.3	35.7
Adults	< 0.1	0.7	10.1	< 0.1	2.8	37.8
Elderly	< 0.1	3.4	7.8	< 0.1	15.7	28.9
Very Elderly	0.1	3.6	6.6	0.5	17.4	24.6
95th percentile exposure[(b)] (ng/kg b.w per day)						
	Lower bound (LB)			Upper bound (UB)		
	Min	**Median**	**Max**	**Min**	**Median**	**Max**
Infants	38.3	-[(c)]	-[(c)]	-[(c)]	-[(c)]	143.8
Toddlers	0.0	27.6	39.9	0.0	109.8	148.7
Other children	0.0	20.0	33.0	0.0	76.9	126.4
Adolescents	0.0	10.3	29.0	0.0	38.2	107.9
Adults	0.0	4.0	28.5	0.0	16.3	106.3
Elderly	0.6	10.2	23.7	5.4	47.0	88.1
Very Elderly	5.1	10.6	19.7	18.8	49.7	75.8

(a): Not calculated since estimates were only available from two dietary surveys;
(b): The 95th percentile estimates obtained on dietary surveys/age classes with less than 60 observations may not be statistically robust (EFSA, 2011b). Those estimates were not included in this table.
(c): Not calculated since estimates were only available from one dietary survey.

Similarly to what is observed in Table 9 for drinking water as such, the maximum exposure to Cr(VI) through the consumption of bottled water was estimated in the youngest populations ('Infants' and 'Toddlers') (Table 10). No exposure through the consumption of bottled water were reported in several dietary surveys, while the maximum estimates were 149.8 ng/kg b.w. per day (UB) for 'Infants' mean consumers and 148.7 ng/kg b.w. per day (UB) for 'Toddlers' at the 95th percentile exposure. As expected, the estimated exposure to Cr(VI) through consumption of bottled water was lower than that estimated through the consumption of all types of water, mainly due to the small amount of consumption data reported for bottled water (27.7 % of the total). However, in those dietary surveys with relatively high consumption of bottled water, chromium occurrence levels higher in bottled water than in other types of water led to Cr(VI) exposure levels similar to those estimated in the scenario on all types of water (e.g. 9.6 ng/kg b.w. per day for the mean exposure (LB) in 'Adolescents' through the consumption of bottled water and 10.2 ng/kg b.w. per day for all types of water).

Further scenarios, as commented above, considering the additional contribution of the water used to prepare certain foods before their consumption (such as coffee, tea infusions, and dry infant and follow-on food) were evaluated using a worst-case scenario with no reduction of Cr(VI) into Cr(III). To address this additional contribution to the exposure to Cr(VI), the consumption data reported for the prepared foods were linked to the occurrence data in tap water (for coffee and tea) and still mineral water (infant and follow-on food) (see occurrence values in Table 7). When the consumption of coffee and tea infusions were reported as solid, appropriate conversion factors were applied to tap water

occurrence values (a factor of 100 for tea infusions and a general factor of 18 for coffee) (EFSA, 2011b; USDA, 2013).

In infants there was no substantial increase on the exposure to Cr(VI) since in this age class the reported data on water consumption mostly referred to the water used in the food preparation (94 %). In 'Toddlers', since most of the consumption data on infant and follow-on food were reported as consumed (80%), the additional contribution of the water used to prepare these foods had not been considered previously. For this age class, a slight increase of the exposure to Cr(VI) was observed in most of the dietary surveys. However, since most of the data on infant and follow-on food as consumed were reported by one dietary survey (95 %), the highest increase of the exposure to Cr(VI) was calculated from this dietary survey (mean exposure at LB increased from 8.2 ng/kg b.w per day to 20.6 ng/kg b.w per day).

In the other age classes, the consumption of tea and coffee was the main reason of the increase of the exposure to Cr(VI) in several dietary surveys. Particularly important was the increase, as compared to the initial scenario, in some countries (e.g. LB mean exposure from 1.9 ng/kg b.w per day to 4.2 ng/kg b.w per day in the elderly population or LB mean exposure from 1.6 ng/kg b.w per day to 3.3 ng/kg b.w per day in the adult population). The additional contribution to the exposure to Cr(VI) of the water used in the preparation of the other mentioned foods was negligible either because not many consumption data were reported in the consumption database (instant soup and dehydrated fruit juice) or because of the low amounts consumed (evaporated and dried milk).

The CONTAM Panel noted that if even a small proportion of total chromium in food was in the form of Cr(VI), it could contribute substantially to Cr(VI) exposure.

6.3. Previous dietary exposure assessments

Different estimations of the dietary exposure to chromium have been made in the past in different countries. Unlike in this Scientific Opinion, the estimates were in most of the cases reported without considering the body weight, i.e. as total intake per day (µg/day or mg/day).

In order to compare the exposure estimates from this opinion with those reported in the literature, the dietary exposure to Cr(III) in this Opinion was also calculated as total intake (see Table G3 in Appendix). As can be seen in this Table, mean dietary exposure in 'Adults' varied between 54.1 and 112.6 µg/day (minimum LB-maximum UB) and 86.2-190.2µg/day (minimum LB-maximum UB) in the 95th exposure. For 'Toddlers', mean dietary exposure ranged between 23.6-85.7 µg/day (minimum LB-maximum UB) and 37.6-122.1 µg/day (minimum LB-maximum UB) in the 95th exposure. For 'Other children', mean dietary exposure ranged between 40.2-106.5 µg/day (minimum LB-maximum UB) and 65.0-179.0 µg/day (minimum LB-maximum UB) in the 95th exposure.

A wide range of estimates of dietary exposure to chromium are reported in the literature. In principle, those dietary intakes reported before 1980 are not reliable due to the analytical problems associated with the determination of this element. Compared with the exposure estimates calculated in this Scientific Opinion, lower mean dietary exposures to chromium have been reported in the adult population in two UK TDS, with values of 22-29 µg/day in 2006 and 46 µg/day in 2000 (Rose et al., 2010), in the USA (25-33 µg/day) (Anderson and Kozlovsky, 1985) or in Brasil (23 µg/day) (Santos et al., 2004). Similar values to those found in this scientific opinion were reported in Poland (values among 60-90 µg/day) (Marzec, 2004) or in the first French TDS with mean dietary intakes in the adult population of 77 µg/day (97.5th percentile=126 µg/day), and 68 µg/day for children aged 3-14 (97.5th percentile = 124 µg/day) (Leblanc et al., 2005).

Other mean chromium intakes reported in the literature are those estimated for the adult population in the 1997 UK TDS with 100 µg/day (97.5th percentile= 170 µg/day), or in different Spanish regions where the dietary chromium intake ranged between 120 µg/day (Barberá et al., 1989) and 124.6 µg/day (Schuhmacher et al., 1993). More recently, García et al., 2001, found similar mean intake levels (100 µg/day) ranging from 9.4 µg/day to 205.2 µg/day. Higher dietary exposure to chromium has been reported in the second French TDS (Arnich et al., 2012). The mean exposure to chromium of the French population was estimated as 277 µg/day in 'Adults' (95th

percentile = 413 µg/day) and 223 µg/day in children (95th percentile= 333 µg/day). This high exposure levels seemed to be related to the use of stainless steel equipment for milling samples. Similar high values were reported in 1994 UK TDS, with exposure estimates of 340 µg/day.

Recently, long-term dietary exposure to chromium in young children (1-14 years old) living in 12 different European countries was estimated (Boon et al., 2010). The consumption data used in that study represent the basis of the existing data for the young population in the Comprehensive database. For children 1 to 10 years of age, the long-term exposures to chromium using LB concentrations ranged from 1.8 to 5.1 µg/kg bw per day for median consumers, and from 3.4 to 16 µg/kg bw per day for 99th percentile consumers. Exposure levels in younger children were higher than in older children within this age group. The LB estimates for children aged 11 to 14 years were 1.2 to 1.9 µg/kg b.w. per day for median consumers and 2.3 to 4.5 µg/kg bw per day for 99th percentile consumers. Using UB concentrations, the exposures at the median and 99th percentile levels were on average a factor 1.4 higher for both age groups.

On the other hand, there are not many studies in the literature that report the contribution of drinking water to the total exposure to chromium. When talking about total chromium, it is accepted, in general, that the contribution of drinking water to the total exposure is quite limited (1.9 - 7 %), and only when total chromium levels are above 25 µg/L the contribution could be substantial (WHO, 2003). However, the CONTAM Panel noted that the contribution of drinking water to total chromium refers to Cr(VI), whereas total chromium also includes Cr(III) and that therefore this comparison is not relevant for the risk assessment.

6.4. Non-dietary exposure

Occupational exposure

Chromite is the only significant Cr ore, containing up to 55 % of chromic oxide. Chromium metal is produced either electrolytically after chemical treatment of high-carbon ferrochromium or by reduction of Cr compounds. Sodium chromate and dichromate are produced by roasting chromate ore, followed by chemical treatment for removing impurities and further processing to obtain other Cr compounds.

Ferrochromium and Cr metal are the most significant classes of Cr used in the alloy industry, e.g. to produce stainless steel. Exposure to Cr compounds also occurs in metal engineering, refractory, and chemical industries. Cr and its salts find a wide range of applications in the chemical industry, graphics industry, artistic paints, anticorrosion paints, electroplating, other steel alloys such as armoured steel, stainless steel welding, and a multitude of other uses. The tanning industry was for many years an important consumer of Cr. There are millions of stainless steel welders worldwide and stainless steel welding may, at present, be the most common sources of human exposure to Cr in the workplace. The wide range of uses of Cr has resulted in exposure to Cr compounds for numerous workers.

Potentially hazardous exposures are incurred in the production of dichromates, in the use of chromates in the chemical industry, in the stainless steel industry, in the manufacture of alloys, in refractory work, and in Cr-electroplating. In the last industry, health hazards are related to the Cr-containing mist. Chromium inhaled as Cr(VI) is partially reduced to Cr(III) after being deposited in the airways (Goldoni et al., 2006). A fraction of the Cr may be transported in the Cr(VI) form by the mucous escalator to the pharynx and subsequently being swallowed—the size of this fraction depending on the inhaled aerosols particle distribution and the efficacy of the escalator. Hence, inhalation of Cr(VI) may lead to ingestion of Cr(VI).

Exposure to Cr during welding of stainless steel may constitute a health hazard, both because Cr is a constituent in stainless steel and acid-stable steel (i.e., 18-21 % Cr) and because Cr-containing electrodes are used. Whenever mild steel is covered by Cr-containing anticorrosive paints, welding on mild steel may also entail a Cr(VI)-related health hazard to welders. When using the manual metal arc (MMA) method for welding on ship sections, average Cr(VI) levels in the work atmosphere are

around 140 µg/m^3, with Cr(VI) accounting for approximately 50 % of the total Cr. The fraction of Cr(VI) is much lower during stainless steel welding when applying the MIG/MAG method (Karlsen et al., 1996).

Air levels of Cr in chromate industry have been reported to achieve concentrations up to 1 mg/m^3. Most concentrations reported in the literature are in the range from 0.26 to 0.51 mg/m^3, but modern plants show levels < 0.1 mg/m^3. Most Cr concentrations recorded after personal sampling during 8 hours in a ferrochromium manufacturing plant were in the range of 0.02-0.05 mg/m^3. In a review, Cr workplace concentrations up to 5 mg/m^3 in the Cr-plating industry was mentioned, but most exposure levels reported were in the range of 0.1-0.2 mg/m^3. In modern plants, values are often < 10 µg/m^3 (IARC, 1990).

ECB (2005) performed an occupational exposure assessment for industrial production and use of different Cr(VI) salts and reported detected workplace concentrations to Cr(VI) to range between 0.01 and 760 µg/m^3 for various tasks. In the occupational risk assessment, 20 µg/m^3 was taken as reasonable worst case exposure concentration for chromium salt manufacturing.

Other background exposures

ATSDR estimated that for the general population, oral exposure via food and water is by far the most important contribution to the exposure to chromium (ATSDR 2012).

Non dietary exposure to chromium in the general population can occur mainly via inhalation, and less importantly via ingestion and dermal contact (ATSDR, 2012).

Chromium can be present in air mainly as a result of anthropogenic activities. Total Cr concentrations in air were reported to range from 5 to 525 ng/m^3 in USA urban and non urban areas during the period 1997-1984 (ATSDR, 2012). Cr(VI) was detected in ambient air from residential sites in the approximate range 0.1 - 2 ng/m^3 (ATSDR, 2012).

In its risk assessment on chromium compounds, ECB (2005) estimated the potential Cr(VI) concentrations in the proximity of a chromate salt production site and a metal treatment site to be up to 4.3 and 0.71 µg/m^3, respectively. According to ECB, assuming an absorption rate of 100 % for Cr(VI) species via inhalation, a daily volume of 20 m^3 of inhaled air, and that Cr(VI) is not reduced following its emission from the manufacturing plant, the daily uptake could be up to 86 µg per day (corresponding to a daily dose of 1.2 µg/kg b.w. per day, assuming a 70 kg b.w.) for the adult general population living in the vicinity of a chromate production site. However, this was considered a worst case scenario in view of the selected assumptions.

Consumer products including wood preservatives, cement, cleaning materials, textiles, and leather tanned with chromium may represent an additional source of exposure for the general population (ATSDR, 2012).

In particular cigarette tobacco has been reported to contain 0.39 mg/kg of Cr (Schroeder et al., 1962), but there have been no published estimates of the inhaled amount of Cr from smoking. Later values of 0.24-14.6 mg/kg(Al-Badri et al., 1977), or 0.24 to 6.3 mg/kg (IARC, 1980) have been reported, and more recently total Cr has been determined as a component of cigarette tobacco, ranging from 0.45 to 3.13 mg/kg (Freitas de Sousa Viana et al., 2011). Moreover, the Cr oxidation state upon inhalation is not known, though the high temperature of the cigarette when it burns could oxidise Cr to Cr(VI). Increased Cr concentrations have been found in lung tissue from smokers either affected or not from lung cancer (Pääkkö et al., 1989; Akslen et al., 1990; Adachi et al., 1991; Kuo et al., 2006; De Palma et al., 2008). Cr has also been determined in smokeless tobacco aerosols (Borgerding et al., 2012).

Based on reports, chromium levels in mainstream cigarette smoke ranges from 0.0002 to 0.5 µg per cigarette (Smith et al., 1997). It is known that Cr accumulates in tissue, especially in the lung. Concentrations of about 4.3 mg/kg (dry weight) are found in lung tissues of smokers compared with 1.3 mg/kg in non smokers, increasing with age and smoking time (Pääkkö et al., 1989).

ATSDR noted that neither the chemical form nor the amount of chromium in tobacco smoke is known, and that people who use tobacco products may be exposed to higher-than-normal levels of chromium (ATSDR, 2012).

The CONTAM Panel could not quantify the contribution of non-dietary exposure to Cr(III) or Cr(VI) due to the existing uncertainties on the levels of exposure via inhalation, the absorption rates of different chromium compounds via the respiratory system and the relevance of different chromium species for non-dietary exposure.

The CONTAM Panel concluded that exposure via the diet likely represents the most important contribution to the overall exposure to Cr in the general population. Inhalation of Cr compounds present in particular in cigarette smoke may contribute to the overall exposure levels but the currently available information dose not allow quantification of its relative contribution.

7. Hazard identification and characterisation

7.1. Toxicokinetics

Several previous evaluations provide information on the toxicokinetics of Cr(III) and Cr(VI) (U.S. EPA 1998a, b; WHO, 1996a, 2000, WHO/IPCS 2009a, 2013; EFSA 2008a, EFSA ANS Panel 2010a,b). The Sections below summarise this information while presenting recent additional data in more detail.

The toxicokinetics of chromium appear to depend on the oxidation state. Hexavalent chromium readily penetrates cell membranes whereas trivalent chromium does not.

7.1.1. Trivalent Chromium

Absorption

Following oral administration, Cr(III) was reported to be very poorly absorbed via the gastrointestinal tract (0.4 to 2.8 %) in both rats and humans (Conn et al., 1932; Visek et al., 1953; Donaldson and Barreras, 1966; Doisy et al., 1971; Henderson et al., 1979; Anderson et al., 1983; Aitio et al., 1984; Anderson and Kozlovsky, 1985; Polansky et al., 1993; Gargas et al., 1994; Olin et al., 1994; Kerger et al., 1996; Gammelgaard et al., 1999; ATSDR, 2012; Febel et al., 2001; Garcia et al., 2001).

The rate of uptake of chromium compounds in the gastrointestinal tract may be governed by the water solubility of the compounds (Langård, 1982; WHO, 2000). WHO indicated that a fractional absorption value of 5 % is considered to be a good estimate for the gastrointestinal absorption of soluble inorganic chromium compounds, but 0.5 % is more appropriate for that of insoluble inorganic chromium compounds such as chromic trioxide pigment (WHO, 1996a).

Some studies have revealed that there can be differences in the bioavailability and tissue levels of chromium resulting from intake of different forms of chromium compounds (U.S. Patent 5.194.615,1993; Olin et al., 1994; Lamson and Plaza, 2002). Differences in bioavailability of Cr(III) have been reported depending on the ionic form and/or the organic or the inorganic forms of the Cr(III). Organic forms of Cr(III) might be better absorbed than inorganic Cr(III) (Mertz, 1969; Vinson and Bose, 1984; Olin et al., 1994; Lamson and Plaza, 2002).

Studies on rats found that the ranking of the relative absorption and retaining of trivalent chromium from different sources was chromium nicotinate > chromium picolinate > chromium chloride (Lamson and Plaza, 2002). In their opinion on chromium picolinate, zinc picolinate and zinc picolinate dihydrate added for nutritional purposes in food supplements the EFSA ANS Panel noted that the bioavailability of inorganic Cr(III) is generally very low (0.1-2 %) and that the bioavailability of chromium from chromium picolinate may be higher because complex formation may influence the chromium bioavailability and that chromium from chromium picolinate is equally or slightly more bioavailable than chromium from other chromium compounds (EFSA, 2009b).

In a former opinion (EFSA, 2008b), the EFSA Scientific Panel on Food Additives Flavourings, Processing Aids and Materials in Contact with Food (AFC Panel) referred to a study provided by a petitioner reporting an animal study, designed to determine the absorption of radioactive chromium from a chromium amino acid chelate (composition not specified by the petitioner in the application) in comparison to the absorption of chromium from inorganic trivalent chromium chloride. In this study two groups of rats were slightly anesthetised and then intragastrically intubated with equal amounts of chromium as either $^{51}CrCl_3$ or the ^{51}Cr-amino acid chelate. Blood was drawn at 1-hour intervals for 3 hours and the radioactivity of equal volumes (100 µL) were measured for corrected disintegration counts per minute. Data show that the absorption of chromium nearly doubled when supplied as chromium amino acid chelate, in comparison to inorganic chromic(III) chloride.

A review article by Lukaski (1999) summarised two articles on the absorption of chromium and stated that amino acids when chelating the dietary chromium prevent precipitation within the alkaline milieu of the small intestine. Similarly, nicotinic acid when administered with trivalent chromium may enhance absorption.

In the intestine of black ducks, administration of saline solutions of chromium potassium sulphate ($KCr(SO_4)_2$) and chromium trioxide (CrO_3) resulted in chromium absorption about 1.5 to 2.0 times greater than observed with solutions of chromium nitrate ($Cr(NO_3)_3$) and the organic salt, 2,4-pentanedione chromium ($Cr(C_5H_7O_3)_3$) (Eastin et al., 1980). Small differences in the absorption of Cr(III) between the inorganic salts chromium chloride and chromium nitrate, and the organic salt chromium picolinate, have been reported, using an *in vitro* model of the rat jejunum, with a more efficient absorption of the organic form in comparison to the inorganic salts (Gammelgaard et al., 1999).

No increase in the absorption of trivalent chromium ($^{51}CrCl_3$) was observed following intraduodenal or intrajejunal administration in comparison to oral administration in humans and rats (Donaldson and Barreras, 1966).

The absorption rate of trivalent chromium from chromium polynicotinate, chromium nicotinate-glycinate and chromium picolinate was several times higher than that from chromium chloride, as indirectly estimated from urinary excretion of chromium in human volunteers (DiSilvestro and Dy, 2007).

Trivalent chromium in the form of propionate or amino acid chelates are also suggested to have a higher absorption rate than inorganic Cr(III) compounds (Ohh and Lee, 2005).

Other studies reported that oral absorption of Cr(III) complexed with an organic ligand was also very low and not higher than the absorption of inorganic forms of Cr(III) **(**Gonzalez-Vergara et al., 1981; Anderson et al., 1996).

Many dietary factors affect the absorption of Cr(III) and the absorption efficiency of trivalent chromium salts depends largely on the nutritional status of the animal as well as the nature of the anion making up the trivalent chromium salt (MacKenzie et al., 1959; O'Flaherty, 1996). Starch, simple sugars, ascorbic acid, oxalate, nicotinic acid and organic acids were shown to increase the absorption rate of Cr(III) (Chen et al., 1973; Kozlovsky et al., 1986; Urberg and Zemel, 1987; Seaborn and Stoecker, 1989; Dowling et al., 1989, 1990; Offenbacher, 1994; Samanta et al., 2008). Carbohydrate intake has been shown to influence chromium urinary excretion and tissue concentrations (Lamson and Plaza, 2002). Some amino acids and histamine were reported to result in a higher chromium absorption rate (Mertz et al., 1965). It has been hypothesized that amino acids act as chromium ligands, resulting in rapid diffusion of chromium complexes of low molecular weight (Dowling et al., 1990).

Habitual consumption of acetylsalicylic acid derivatives enhanced chromium absorption (Davis et al., 1995), while higher phytate, calcium, manganese, titanium, zinc, vanadium and iron inhibited chromium absorption (Mertz, 1970; Chen et al., 1973; Hill, 1975).

In rats co-administration of $^{51}CrCl_3$ with phytate and with oxalate significantly decreased and markedly increased, respectively, chromium absorption (Nelson et al., 1973). Experiments with rats given

$^{51}CrCl_3$ showed that ascorbic acid and a prostaglandin inhibitor, aspirin, enhanced intestinal absorption of chromium, whereas an antacid containing aluminium and magnesium hydroxide reduced it (Davis et al., 1995). In humans, ascorbic acid enhanced chromium chloride absorption (Offenbacher, 1994). Offenbacher (1994) proposed that ascorbate chelated chromium and made it more soluble and more readily absorbed.

Taken together, some studies have revealed that there can be differences in the bioavailability of chromium resulting from intake of different forms of trivalent chromium compounds, with organic complexes being somewhat more bioavailable, but these differences are small and the overall bioavailability of trivalent chromium from all these sources is low.

Distribution

Little Cr(III) appeared to be taken up by RBCs in *in vitro* incubations (Gray and Sterling, 1950; Donaldson and Barreras, 1966; Bentley, 1977; Aaseth et al., 1982). Similarly, in several *in vivo* studies only negligible amounts of Cr(III) were associated with RBC (Doisy et al., 1971; Onkelinx, 1977; Sayato et al., 1980; Suzuki et al., 1984; Wiegand et al., 1984; Minoia and Cavalleri, 1988; Coogan et al., 1991a). Other *in vivo* and *in vitro* studies reported that Cr(III) may be taken up by RBCs, particularly at higher concentrations (Merritt et al., 1984; Suzuki et al., 1984; Venezia and Karol, 1984; Lewalter et al., 1985; Kortenkamp et al., 1987), but the amount of Cr(III) taken up by the RBC resulted substantially lower than that of Cr(VI).

Following absorption, Cr(III) does not enter blood cells, but rather competes for one of the binding sites on the iron-transport plasma protein transferrin, from where it can be transferred to a low molecular-weight chromium binding substance, or chromodulin (Aisen et al., 1969; Frankendal and Stigbrand, 1973; Lim et al., 1983; Ani and Moshtaghie, 1992; Moshtaghie et al., 1992; Yang and Black, 1994; Sun et al., 2000; Vincent, 2000a,b; EVM, 2003; Feng et al., 2003) and transported to the liver, a process partly regulated by insulin (Clodfelder et al., 2001; Clodfelder and Vincent, 2005). Other plasma proteins such as albumin, γ-globulins and lipoproteins can also bind Cr(III) when higher concentrations are present in plasma (Hopkins and Schwarz, 1964; Aisen et al., 1969; Frankendal and Stigbrand, 1973; Yamamoto et al., 1981; Aaseth et al., 1982; Lim et al., 1983; Brock, 1985; Ani and Moshtaghie, 1992; Moshtaghie et al., 1992; Yang and Black, 1994). An apparently non-specific binding of chromium to proteins on the outside of RBCs can also be significant, particularly at higher concentrations (Edel and Sabbioni, 1985, Gao et al., 1993). Once absorbed, Cr(III) may also be complexed with other compounds, such as nicotinic acid (EFSA ANS Panel, 2010b).

Upon oral administration of Cr(III) little tissue uptake occurs (MacKenzie et al., 1958; Sayato et al., 1980; Lindemann et al., 2004; NTP, 2010). Even when administered intravenously, ensuring immediate availability of the metal for tissue and cellular uptake, tissue levels of Cr(III) were low (Visek et al., 1953; Sayato et al., 1980).

Little chromium was detected in liver, spleen, kidney and bone (marrow), tissues known to accumulate Cr(VI), following oral administration of Cr(III) except at the site of its excretion, the kidney (and at much lower levels than when Cr(VI) was administered) (Costa, 1997).

In a radioisotope study, it was found that trivalent ^{51}Cr as nicotinate had significantly greater short-term retention (1-12 hours post-gavage) in muscle, liver, kidney, blood and urine compared to Cr(III) from chromium chloride or chromium picolinate (Olin et al., 1994; Lukaski, 1999). In another study summarised by Lukaski (1999), it was found that Cr(III) nicotinate promoted chromium accumulation in the kidney and that nicotinate, like picolinate and acetate, increased chromium incorporation into the liver (Anderson et al., 1996).

Metabolism

In biological environments, little Cr(III) is converted to the hexavalent form. This is in line with the fact that oxidation of Cr(III) to Cr(VI) requires strong oxidising agents.

Excretion

Given the low intestinal absorption of Cr(III) a relatively large proportion of orally ingested trivalent chromium is excreted in the faeces. Sayato et al. (1980) administered $^{51}CrCl_3$ to rats by stomach tube and reported that 99 % of the oral dose was excreted in faeces and 0.8 % in urine. Similar findings were reported by Donaldson and Barreras (1966) for rats given $^{51}CrCl_3$ by stomach tube with excretion in faeces and urine amounting to 98 % and 1.4 % respectively. These authors observed similar results in humans given $^{51}CrCl_3$ showing a mean recovery in faeces of 99.6 % with the recovery in urine being 0.5 %.

Trivalent chromium is rapidly cleared from the blood and plasma (Onkelinx, 1977; Sayato et al., 1980; Gao et al., 1993) and also rapid declines of urinary chromium levels have been reported (Aitio et al., 1984).

Most Cr(III) that is absorbed is excreted in the urine with small amounts also being lost in perspiration, bile and faeces (Gargas et al., 1994; Anderson et al., 1997; Jeejeebhoy, 1999; ATSDR, 2012; IOM, 2001; Hepburn and Vincent, 2002; Feng, 2007). Trivalent chromium is removed from the tissues at a slower rate (EFSA ANS Panel, 2010b). Following gavage administration of trivalent chromium in rats, the estimated half-time for whole-body elimination was 92 days (Sayato et al., 1980).

Only scarce information is available on renal handling of Cr(III). Some studies suggested that 5 to 40 % of plasma Cr(III) levels were ultrafiltrable, and that the majority of Cr(III) filtered in glomeruli was reabsorbed by renal tubules (Donaldson and Rennert, 1981). However, glomerular filtration was later suggested as the predominant mechanism controlling Cr(III) excretion (Donaldson et al., 1984). In rats administered radiolabelled Cr(III) in a chromium-loaded transferrin transportation study, a single ^{51}Cr-containing species, defined as low-molecular-weight chromium-binding substance (LMWCr), was identified in urine. Trivalent chromium binds to this oligopeptide with a molecular mass of about 1 500 daltons which has been found in the liver and many other organs (Wada et al., 1983; Yamamoto et al., 1989; Vincent, 1999). LMWCr is believed to be central in the endogenous metabolism of chromium.

Wada et al. (1983) investigated the urinary excretion and renal clearance of LMWCr and chromium chloride in Japanese White rabbits following an intravenous injection of 500 µg chromium/kg b.w. of each of the two compounds. LMWCr was excreted more rapidly than chromium chloride: 66 % versus 25 % in 6 hours. Based on this result it was concluded that LMWCr may play an important role in chromium excretion in mammals.

Chromium-transferrin and urinary chromium were not found in direct equilibrium, thus indicating that intermediates are involved in the transport of Cr(III) (Clodfelder and Vincent, 2005). Urinary Cr(III) excretion can be increased in humans by high sugar intake, exercise, physical trauma, pregnancy and lactation (Anderson, 1989; Rubin et al., 1998). Insulin treatment has been also shown to increase urinary excretion of Cr(III) in rats (Clodfelder et al., 2001).

Urinary recovery of administered chromium provides a reasonable estimate of oral absorption of chromium because most of the absorbed chromium is excreted in the urine and little is retained in the carcass (Hopkins, 1965; Yamamoto et al., 1981; Bryson and Goodall, 1983). Two percent or less of a dose of Cr(III)was recovered in the carcass of mice seven days post-administration (Gonzalez-Vergara et al., 1981).

Casey and Hembridge (1984) demonstrated that chromium can be transferred to infants through breast milk. The breast milk of 45 lactating women was reported to have a chromium content of 0.3 µg/L on average. These concentrations were suggested to represent background levels in women whose chromium exposure occurs primarily through the diet.

7.1.2. Hexavalent chromium

Absorption

Following oral administration, Cr(VI) is absorbed to a greater extent than Cr(III) but its absorption from the gut is also poor. Studies report 1 - 6.9 % of the administered dose of Cr(VI) to be recovered in the urine in humans (Kerger et al., 1996; Finley et al., 1996, 1997; Paustenbach et al., 1996) and 2 % in the rat (MacKenzie et al., 1959; Donaldson and Barreras, 1966; Febel et al., 2001).

Both plasma and RBC levels of chromium (peak levels and plasma under the plasma time curve) were much higher in individuals ingesting on 5 mg dose of Cr(VI) than when Cr(III) was ingested (Kerger et al., 1996).

The intestinal absorption of Cr(VI) has been reported to be markedly affected by contact with gastric juices (MacKenzie et al., 1959; Donaldson and Barreras, 1966; De Flora et al., 1987; Kerger et al., 1997; Febel et al., 2001). The infusion of Cr(VI) into the duodenum or jejunum (bypassing the stomach) resulted in a marked increase in absorption in humans (Donaldson and Barreras, 1966; De Flora et al., 1987) and experimental animals (MacKenzie et al., 1959; Donaldson and Barreras, 1966; Febel et al., 2001). Donaldson and Barreras (1966) recovered 11 to 30 % of the administered dose of Cr(VI) in human urine following the infusion of 1 ng/L of $Na_2{}^{51}CrO_4$ into the intestine (under these conditions only 1-2 % of the dose of Cr(III) administered as $Cr(Cl)_3$ was absorbed). Upon intraduodenal administration of $Na_2{}^{51}CrO_4$ to humans (avoiding contact with gastric juices), approximately half of the chromium was absorbed based on faecal excretion (De Flora et al., 1987). Similar results were observed following intrajejunal administration in rats, where 57 % of the dose of Cr(VI) administered into the ligated jejunum of rats was recovered in the jejunum after 60 minutes while approximately 98 % of the dose of Cr(III) was recovered in the jejunum under the same experimental conditions (Febel et al., 2001). Following the oral administration of Cr(VI) to humans, increased recovery of chromium in the urine was observed under conditions of low stomach acidity (pernicious anemia) compared to control (8 % vs. 2 %) (Donaldson and Barreras, 1966).

Incubation of Cr(VI) with gastric juices prior to intraduodenal or intrajejunal administration in humans and rats, respectively, virtually prevented the absorption of chromium (De Flora et al., 1987). The authors concluded that reduction of Cr(VI) to the trivalent form in the stomach significantly reduces absorption by the oral route (De Flora et al., 1987). Absorption of Cr(VI) following intestinal administration of $Na_2{}^{51}CrO_4$ was found to be increased three- to five-fold in comparison with oral administration, consistent with reduction of Cr(VI) during passage through the stomach to Cr(III) which is less well absorbed (MacKenzie et al., 1959).

Both Cr(III) and Cr(VI) are better absorbed from the gastrointestinal tract in the fasted than in the fed state, (MacKenzie et al., 1959; O'Flaherty, 1996). Based on urinary excretion, Cr(VI) absorption was estimated to be 6 % in fasted rats and 3 % in nonfasted rats (MacKenzie et al., 1959).

Kerger et al. (1996) administered Cr(VI) to humans mixed with orange juice to determine to what degree the acidic-organic environment (somewhat analogous to the stomach) reduces oral absorption. Four adult male volunteers ingested a single dose of 5 mg Cr (in 0.5 litres deionized water) in three chromium mixtures: (1) Cr(III) chloride ($CrCl_3$), (2) potassium dichromate reduced with orange juice (Cr(III)-OJ); and (3) potassium dichromate (Cr(VI)). Blood and urine chromium levels were followed for 1–3 days prior to and up to 12 days after ingestion. The three mixtures showed quite different pharmacokinetic patterns. $CrCl_3$ was poorly absorbed (estimated 0.13 % bioavailability) and rapidly eliminated in urine (excretion half-life, about 10 hours), whereas Cr(VI) had the highest bioavailability (6.9 %) and the longest half-life (about 39 hours). Thus, the fraction of the dose of chromium recovered in the urine appeared to be greater for Cr(VI) than when Cr(III) was administered (6.9 % versus 0.13 %). The absorbed fraction was considerably less when Cr(VI) was administered with orange juice (0.6 %) than when Cr(VI) was administered in water (6.9 %).

Kerger et al. (1997) investigated the absorption, distribution and excretion of Cr(VI) after oral exposure of adult male human volunteers to potassium chromate at 5 or 10 mg Cr(VI)/L in drinking water, administered either as a single bolus dose (0.5 L swallowed in 2 minutes) or for 3 days at a dose

of 1 L/day (three doses of 0.33 L at 6-hour intervals). Samples of urine, plasma, and RBCs were collected and analyzed for total chromium. Upon taking the bolus dose urinary chromium excretion with an average half life of about 39 hours was observed. However, total urinary chromium excretion and peak concentrations in urine and blood varied considerably between the five volunteers. Upon the 3 days exposure to repeated low dose levels generally lower chromium uptake/excretion was observed. The authors concluded, based on low or even absent levels of elevated RBC chromium content in the weeks following Cr(VI) ingestion, that the Cr(VI) was reduced rapidly to Cr(III) in the upper gastrointestinal tract or plasma prior to RBC uptake and systemic distribution. Thus they concluded that volunteers ingesting highly soluble chromate (Cr(VI)) at concentrations of 5 – 10 mg Cr(VI)/L in drinking water have a pattern of blood uptake and urinary excretion consistent with uptake and distribution of chromium in the trivalent state. They also concluded that the endogenous reducing agents within the upper gastrointestinal tract and the blood provided sufficient reducing potential to prevent any substantial systemic uptake of Cr(VI) following drinking-water exposures at 5-10 mg Cr(VI)/L.

Finley et al. (1997) reported the urinary recovery in human subjects following dose levels of 0.1, 0.5, 1.0, 5.0 or 10 mg/day of Cr(VI) for four days to amount to respectively 1.7 %, 1.2 %, 1.4 %, 1.7 % and 3.5 %. A dose-related increase in urinary chromium excretion was observed in all volunteers. The authors indicated that the RBC chromium profiles suggested that the ingested Cr(VI) was reduced to Cr(III) before entering the bloodstream, since the chromium concentration in RBCs dropped rapidly post-exposure. The authors concluded that the RBC and plasma chromium profiles are consistent with systemic absorption of Cr(III) not Cr(VI).

Collins et al. (2010) demonstrated that exposure of male F344/N rats and female B6C3F1 mice to Cr(VI) resulted in significantly higher tissue chromium levels compared with Cr(III) following similar oral doses. This indicates that a portion of the Cr(VI) escaped gastric reduction and was distributed systemically. Linear or supralinear dose responses of total chromium in tissues were observed following exposure to Cr(VI), indicating that these exposures did not saturate gastric reduction capacity. The study also reports that *in vitro* experiments demonstrated that Cr(VI) but not Cr(III), is a substrate of the sodium/sulphate cotransporter, providing a partial explanation for the greater absorption of Cr(VI).

Distribution

Hexavalent chromium readily penetrates cell membranes. As a result Cr(VI) is found in both RBC and plasma. When incubated with washed isolated RBCs, almost the entire Cr(VI) dose is taken up by the cells and remains there for the lifetime of the RBC. It is reduced inside the cells to Cr(III), essentially trapping it inside the RBC. Kerger et al. (1997) indicated that sustained elevations in RBC chromium levels provide a specific indication of chromium absorption in the hexavalent state. However, when incubated with whole blood or RBCs plus plasma, only a fraction (depending on conditions) of the Cr(VI) is taken up by the RBC (Lewalter et al., 1985; Wiegand et al., 1985; Coogan et al., 1991a; Corbett et al., 1998). This may be due to the reduction of a portion of the administered Cr(VI) to Cr(III) outside the RBC (Korallus et al., 1984; Richelmi and Baldi, 1984; Capellmann and Bolt, 1992).

Oral administration of Cr(VI) results in increased levels of chromium in a number of tissues including especially the liver, spleen, kidney and bone (marrow) (MacKenzie et al., 1958; Witmer et al., 1989; Witmer and Harris, 1991; Thomann et al., 1994; Sutherland et al., 2000; NTP, 2008). Thompson et al. (2011a, 2012b) reported significant increases in total Cr concentrations in the oral cavity, glandular stomach, duodenum, jejunum, and ileum of rats and mice following 90 days of exposure to sodium dichromate dihydrate (SDD) in drinking water.

Substantial uptake of chromium by the liver is indicated by elevated levels of chromium in the bile following intravenous administration of Cr(VI), (Cikrt and Bencko, 1979; Manzo et al., 1983; Cavalleri et al., 1985).

Increased concentrations of chromium in the blood, kidney and femur were detected in rats, mice and guinea pigs administered 1, 3, 10, 30, 100 or 300 mg/L of Cr(VI) as sodium dichromate in their

drinking water for 21 days (Anderson et al., 2002). Levels of chromium in the tissues increased linearly with dose below 80 ppm. Increased levels of chromium with dose were also observed in the liver and kidney of male and female mice (NTP, 2008).

The WHO (2003) concluded that in animal studies after oral administration of different Cr(VI) compounds, chromium was found to accumulate mainly in liver, kidneys, spleen, and bone marrow, the distribution depending on the speciation. Autopsy data on humans both occupationally and non-occupationally exposed showed the highest concentrations in lungs, followed by spleen, liver, and kidneys (Janus and Kranjc, 1990).

The half-life of chromium in various tissues (other than plasma) of rats administered Cr(VI) exceeds 20 days (Weber, 1983).

Rankov et al. (2010) reported a two generation study in white Wistar male rats exposed to drinking water containing 25, 50 or 75 mg Cr(VI)/L and one control group which received tap water. Results obtained revealed significant accumulation of chromium in genital organs and sexual accessory glands at all doses in comparison to controls, as well as increased chromium levels in genital organs (testis, epididymis) and sexual accessory glands (seminal vesicles, prostate, bulbo-urethral glands), in the F1 generation compared to the F0 generation.

Stern (2010) compared the concentrations of total Cr retained in various tissues after 25 weeks of dosing, with either Cr(III) picolinate or sodium dichromate (NTP, 2008; 2010), and concluded that the concentrations of total Cr were 1.4-16.7 times larger for the rats ingesting Cr(VI), and 2.1-38.6 times larger for mice ingesting Cr(VI) despite a 1.8 and 2.8 times larger dose of Cr(III) in rats and mice, respectively.

Metabolism

Ingested Cr(VI) is efficiently reduced to trivalent chromium by the gastric juices (De Flora et al., 1987, 1997; Kerger et al., 1997; De Flora, 2000). De Flora (2000) estimated that saliva may reduce 0.7 to 2.1 mg of Cr(VI) per day and gastric juices have the capacity to reduce at least 80 to 84 mg of Cr(VI) per day. Saturation or exhaustion of the reducing capacity of saliva and gastric fluids upon high oral doses of Cr(VI) has been suggested to result in increased absorption, elevated blood levels and the appearance of toxicity that may not occur at lower doses. Gammelgaard et al. (1999) using an artificial gastric juice reported a half-life of Cr(VI) of 23 minutes.

Proctor et al. (2012) performed *ex vivo* studies using stomach contents of rats and mice to quantify hexavalent chromium reduction rate and capacity for loading rates amounting to 1-400 mg Cr(VI)/L stomach contents, which are in the range of recent bioassays. Hexavalent chromium reduction followed mixed second-order kinetics, dependent on the concentrations of both Cr(VI) and the native reducing agents. Approximately 16 mg Cr(VI)-equivalents of reducing capacity per litre of fed stomach contents (containing gastric secretions, saliva, water and food) was found for both species. The second-order rate constants were 0.2 and 0.3 L mg /hour for mice and rats, respectively. The authors concluded that these findings support that, at the doses that caused cancer in the mouse small intestine ($\geq$20 mg Cr(VI)/L in drinking water), the reducing capacity of stomach contents was likely exceeded.

In the RBC, Cr(VI) is reduced to Cr(III) by glutathione (Petrilli and De Flora, 1978; Debetto and Luciani, 1988). By fitting the data on radiolabelled Cr(VI) levels in several tissues, such as lung, blood, liver, kidney, gastro intestinal (GI) tract, for the development of a physiologically-based kinetic (PBK) study of Cr kinetics in the rat, O'Flaherty (1996) assumed that hexavalent chromium is reduced to Cr(III) in all tissues.

De Flora and collaborators (Petrilli and De Flora, 1982; De Flora et al., 1987, 1997; De Flora and Wetterhahn, 1989) performed a series of studies to evaluate the ability of various human physiological fluids and tissues to reduce or sequester Cr(VI). Based on these studies the overall Cr(VI) reducing or sequestering capacity of different human body compartment was evaluated. De Flora (2000) proposed that these reducing capacities account for the limited toxicity of Cr(VI) after oral ingestion due to

efficient detoxification by saliva, gastric juice and intestinal bacteria. De Flora (2000) also suggested that the efficient uptake and reduction of Cr(VI) in red blood cells explains the lack of carcinogenicity at sites remote from the portal of entry.

However, Zhitkovich (2005) noted that the analytical methods used to quantify the residual Cr(VI) could have led to an overestimation of the reducing capacity of the biological systems studied by De Flora and co-workers.

Reducing factors that contribute to the reduction of Cr(VI) to Cr(III) have been described in some detail. Especially acidic environments with high organic content promote the reduction of Cr(VI) to Cr(III). Vitamin C-rich products are particularly beneficial for the enhancement of gastric reduction of Cr(VI) (Zhitkovich, 2011). Studies in tissue homogenates and biological fluids reveal that ascorbate is the principal biological reducer of Cr(VI), accounting for 80-95 % of its metabolism (Suzuki and Fukuda, 1990; Standeven and Wetterhahn, 1991, 1992; Zhitkovich, 2011). *In vivo* a combined activity of ascorbate and low molecular weight thiols including especially glutathione (GSH) has been reported to be responsible for > 95 % of Cr(VI) reduction (Zhitkovich, 2011).

Excretion

Upon administration of Cr(VI) by various routes, RBC chromium levels or the ratio of RBC to plasma chromium either did not decline as rapidly or remained elevated for quite some time (Langård et al., 1978; Sayato et al., 1980; Weber, 1983; Suzuki et al., 1984; Coogan et al., 1991a; Gao et al., 1993), although the decrease in RBC chromium levels is apparently more rapid when Cr(VI) is administered by oral route (Coogan et al., 1991a), likely reflecting the conversion to Cr(II) before the GI absorption.

The estimated half-time for whole-body chromium elimination is 22 days following administration of Cr(VI) (WHO, 2000).

7.1.3. Physiologically-based kinetic (PBK) models

Physiologically based kinetic (PBK) models for chromium which incorporate absorption and disposition schemes for Cr(VI) and Cr(III) throughout the body have been presented for rats (O'Flaherty, 1996) and humans (O'Flaherty et al., 2001). The models account for most of the major features of chromium kinetics, including differential absorption of Cr(VI) and Cr(III), rapid reduction of Cr(VI) to Cr(III) in all body fluids and tissues, modest incorporation of chromium into bone, and concentration-dependent urinary clearance. The human model was calibrated against blood and urine chromium levels detected for a group of controlled studies in which adult human volunteers were administered up to 10 mg/day of soluble inorganic salts of either Cr(III) or Cr(VI) (Kerger et al., 1996; Paustenback et al., 1996; Finley et al., 1997). The model outcomes suggest that both Cr(III) and Cr(VI) are poorly absorbed from the gastrointestinal tract. Chromium kinetics were predicted by the model not to be dependent on the oxidation state of the administered chromium except in respect to the amount absorbed. The fraction absorbed was suggested to be strongly dependent on the degree of reduction of Cr(VI) to Cr(III) in the gastrointestinal tract, and thus on the amount and nature of the stomach content at the time of ingestion of the Cr(VI). These human studies are described in more detail in Section 7.4 (Mode of action).

Kirman et al. described a PBK model for rats and mice orally exposed to chromium (Kirman et al., 2012). Data from *ex vivo* Cr(VI) reduction studies were used to characterize reduction of Cr(VI) in fed rodent stomach fluid as a second-order, pH-dependent process. For model development, tissue time-course data for total chromium were collected from rats and mice exposed to Cr(VI) in drinking water for 90 days at six concentrations ranging from 0.1 to 180 mg Cr(VI)/L. Clear species differences were identified for chromium delivery to the target tissue (small intestines), with higher concentrations achieved in mice than in rats, indicated by the authors to be consistent with small intestinal tumor formation, which was observed upon chronic exposures in mice but not in rats. Erythrocyte:plasma chromium ratios suggested that hexavalent chromium entered portal circulation at drinking water concentrations equal to and greater than 60 mg/L in rodents. Species differences were described for

distribution of chromium to the liver and kidney, with liver:kidney ratios being higher in mice than in rats. The tissue data and PBK model predictions indicated a concentration gradient in the small intestines (duodenum > jejunum > ileum).

A PBK model for humans orally exposed to Cr(III) and Cr(VI) (Kirman et al., 2013) was also developed. *Ex vivo* Cr(VI) reduction studies using fasted human gastric fluids were conducted and used to characterize reduction of Cr(VI) in human stomach fluid as a mixed second-order, pH-dependent process. Information from the published literature regarding the toxicokinetics for total chromium in human tissues and excreta was used for model development. The PBPK model was shown to provide a good description of chromium toxicokinetics and to be consistent with the available total chromium data from Cr(III) and Cr(VI) exposures in humans. Additional data for Cr(VI) reduction in both humans and rodents were identified as data needs to further develop key assumptions made in the PBPK models, and allow improved health risk assessment.

7.2. Toxicity in experimental animals

7.2.1. Trivalent chromium

In general Cr(III) salts present low oral toxicity (ATSDR, 2012).

7.2.1.1. Acute toxicity

Table 11 shows the oral LD_{50}s reported in the rat. The lower toxicity of Cr(III) acetate compared with Cr(III) nitrate may be related to solubility; Cr(III) acetate is less soluble in water than is Cr(III) nitrate. Signs of toxicity included hypoactivity, lacrimation, mydriasis, diarrhea, and decrease in body weight.

Table 11: Oral LD_{50}s determined in rats

Compound	LD_{50} (mg Cr(III)/kg b.w.)	Reference
Chromium acetate	2 365	Smyth et al. (1969)
	423	Smyth et al. (1969)
Chromium nitrate	200 (males)	Vernot et al. (1977)
	183 (females)	Vernot et al. (1977)
Cr(III) dinicocysteinate complex	> 2 000	Sreejayan et al. (2010)
Chromium propionate complex	> 2 000	Staniek et al. (2010)
Chromium nicotinate	> 622	Shara et al. (2005)

7.2.1.2. Repeat dose toxicity

Several studies were located in the literature regarding repeated oral exposure (dietary or via drinking water) to Cr(III). Detailed reviews of these studies have been reported by U.S. EPA (1998a), EFSA ANS Panel (2010a, b) and ATSDR (2012). The no-observed-adverse-effect levels (NOAELs) from the relevant studies are reported in Table 12 and the studies are described in details in Appendix H (Table H1). The NOAELs were always the highest dose tested.

Table 12: Repeated dose toxicity studies with Cr(III) compounds. Results for males (M) and females (F) are reported separately when the data allowed.

Study[(a)] Doses in mg Cr(III)/kg b.w. per day	**NOAEL** mg Cr(III)/kg b.w. per day	**Reference**
13-week oral (diet); B6C3F1 mice chromium picolinate monohydrate M/F:0, 2/1.7, 6.2/4.9, 54/44, 273/212, 1419/1090[(b)]	M: 1419 and F: 1090	Rhodes et al. (2005) NTP (2010)
90-day oral (diet); Becton Dickinson rat Cr_2O_3 baked in bread M/F: 0, 570/547, 1368/1217[(b)]	M: 1368 and F: 1217	Ivankovic and Preussman (1975)
14-week oral (diet); F344/N rats chromium picolinate M/F: 0, 0.8/0.7, 2.4/2.4, 19.1/19.1, 95.4/93, 506/507[(b)]	M: 506 and F: 507	Rhodes et al. (2005) NTP (2010)
90-day oral (diet); Sprague-Dawley rats chromium nicotinate M/F: 0, 0.04/0.04, 0.40/0.42, 1.0/1.1[(c)]	M: 1.0 and F: 1.1	Shara et al. (2005)
24-weeks oral (diet); Harlan Sprague Dawley rats chromium chloride or chromium picolinate 0, 0.45, 2.25, 4.5 and 9.0[(d)]	9	Anderson et al. (1997)
52-week oral (diet); Sprague-Dawley rats 0 or 25 mg/kg diet chromium nicotinate (0, M/F: 0, 0.17/0.22[(c)]	M: 0.17[(e)] and F: 0.22	Shara et al. (2007)
2-year oral (diet); Becton Dickinson rat Cr_2O_3 baked in bread 0, 293, 586, 1466[(b)]	M+F: 1466	Ivankovic and Preussman (1975)
2-year oral (diet); F344/N rats chromium picolinate monohydrate M/F: 0, 10.7/12, 55/61, 286/314[(b)]	M: 286 and F: 314	NTP (2010)
2-year oral (diet); B6C3F1 mice chromium picolinate monohydrate M/F: 0, 3029, 143/143, 783/728[(b)]	M: 783 and F: 728	NTP (2010)

b.w.: body weight; NOAEL: no-observed-adverse-effect level; M: male; F: female.

(a): In the conversions from concentration to daily doses, the molecular weight (MW) of the anhydrous salts were used when no information on hydration number was available in the original publication.

(b): Conversion using the data reported in the original publication.

(c): Conversion using drinking water/feed consumption data and average body weight reported in the publication.

(d): Conversion using the default correction factor for subacute/subchronic/chronic exposure via drinking water/feed from EFSA SC (2012).

(e): The only effects observed were statistically significant decreases in body weight gain, between 5.5 and 14.9 %.

In general, Cr(III) displays very little to no toxicity in animals up to the highest dose tested. In the Shara et al. (2007) study, the only effects observed in rats dietary exposed to 25 mg/kg chromium nicotinate were statistically significant decreases in body weight gain, between 5.5 and 14.9 %, which were not considered as adverse by the CONTAM Panel. The lack of toxicity observed at high concentrations of Cr(III) may reflect the poor absorption of Cr(III) by the oral route of exposure. The use of baked bread as a vehicle may have further reduced the absorption of chromium in the Ivankovic and Preussman (1975) study.

The studies conducted by the National Toxicology Program (NTP, 2010) have been performed according to present scientific guidances and reported a comprehensive assessment of toxicological endpoints and adequate reporting for estimation of doses in mg Cr(III)/kg b.w. per day. They were considered by the CONTAM Panel to be the most relevant studies to establish reference doses. No adverse effects have been observed in the sub-chronic or long-term toxicity studies in mice or rats at the highest dose tested of 50000 mg CrO_3 or chromium picolinate/kg diet. The relevant NOAELs corresponded to 506 and 286 mg Cr(III)/kg b.w. per day for the sub-chronic and long-term toxicity in the rat, respectively (NTP, 2010).

7.2.1.3. Developmental and reproductive toxicity

Adverse reproductive effects have been observed in rats and mice exposed orally to Cr(III) compounds, although conflicting results have been reported.

The NOAELs and lowest-observed adverse effect levels (LOAELs) from the relevant studies are reported in Table 13 and the studies are described in details in Appendix H (Table H2).

A series of studies was conducted to determine the effects of chromium chloride on reproduction in rats and mice following 12 weeks of exposure via drinking water (Bataineh et al., 1997; Elbetieha and Al-Hamood, 1997; Al-Hamood et al., 1998). Histopathology was not performed in these studies. Fertility was assessed by mating exposed animals of each sex with unexposed animals of the other sex. Decrease in body weight and absolute testes, seminal vesicles and preputial glands weights as well as inhibitory effect on sexual and aggressive behaviour, without a decrease in fertility, were observed in male Sprague-Dawley rats at exposure concentrations up to 1000 mg/L (30 mg Cr(III)/kg b.w. per day) (Bataineh et al., 1997). Decreased body weights, testes, and preputial gland weights were reported in mice at exposure concentrations of 5000 mg/L (246 mg Cr(III)/kg b.w. per day) and decreased seminal vesicle weight at 1000 mg/L (49 mg Cr(III)/kg b.w. per day). Decrease fertility was also observed in males at 5000 mg/L (Elbetieha and Al-Hamood, 1997). In both studies, increased number of resorptions and dead fetuses were also observed in females mated with treated males. Increases in ovarian weights and reduction in uterine weights were reported in female mice at doses ≥ 2000 mg/L (98 mg Cr(III)/kg b.w. per day). No effect on fertility was noted, however, there was a decrease in the number of implantations and of viable fetuses and an increase in the number of resorptions in treated females (Elbetieha and Al-Hamood, 1997). Impairement of reproductive function and fertility in adulthood was observed in male mice of F1 generation following exposure of dam to 1000 mg/L (79 mg Cr(III)/kg b.w. per day) during gestation and lactation (Al-Hamood et al., 1998). Bataineh et al. (2007) observed a slight decrease in the number of pregnant rats compared to controls after exposure by gavage of mated female rats during gestation days 1-3 to 25 mg chromium chloride/rat (corresponding to 33.6 mg Cr(III)/kg b.w. per day. Results of these studies should be interpreted with caution due to concerns regarding experimental methods.

Mice exposed for 7 week to chromium sulphate (9.2 mg Cr(III)/kg b.w. per day) through the diet had reduced sperm count and degeneration of the outer cellular layer of the seminiferous tubules. Morphologically alterated sperm occurred in diets providing 46 mg Cr(III)/kg b.w. per day (Zahid et al., 1990). Serious questions have been raised regarding the design and conduct of this study (Finley et al., 1993; NTP, 1996a, b, 1997; U.S. EPA,1998a). The methods utilized by Zahid et al. (1990) are considered to be insufficient to identify spermatogonia, likely generated nonreproducible counts of epididymal sperm, and resulted in the biologically implausible conclusion of reduction in spermatogonia numbers concurrent with unchanged spermatocyte and spermatid numbers. Moreover there are inconsistencies regarding the number of mice used in the study, the sizes of the experimental groups do not satisfy minimal requirements for such toxicity study and inappropriate statistical methods were used. Therefore, the CONTAM Panel did not take into account the results of this study in its evaluation of the reproductive toxicity of Cr(III).

Delayed vaginal opening was observed in mice exposed during gestation and lactation to 1000 mg chromium chloride/L (79 mg Cr(III)/kg b.w. per day) (Al-Hamood et al., 1998).

A significant increase in the incidence of bifurcated cervical arches was observed, in the absence of maternal toxicity or an effect on maternal fertility, in the offspring of pregnant CD-1 mice fed diets containing 200 mg chromium picolinate/kg (25 mg Cr(III)/kg b.w. per day) from gestation days 6 to 17 (Bailey et al., 2006). However, this effect was not reproducible in other studies (Bailey et al., 2008a,b).

Other studies do not show show evidence of reproductive or developmental toxicity. There were no changes in testis or epididymis weights in rats following treatment with chromium picolinate or chromium chloride (9 mg Cr(III)/kg b.w. per day) in the diet for 24 weeks (Anderson et al., 1997). There was no evidence of reproductive or developmental toxicity in male or female rats following dietary exposure to chromium oxide (50000 mg/kg diet equivalent to 1806 mg Cr(III)/kg b.w. per day)

for 60 days prior to gestation and during gestation (Ivankovic and Preussmann, 1975). In the 3-month studies on rats and mice following administration of chromium picolinate in the diet up to 50000 mg/kg diet (equivalent to 506 mg Cr(III)/kg b.w. per day in rats and 1090 mg Cr(III)/kg b.w. per day in mice), there were no significant changes in reproductive organ weights in male or female animals, in sperm parameters, or in estrous cyclicity (NTP, 2010).

Developmental effects were not observed following dietary treatment of female mice with 200 mg chromium chloride/kg b.w. (39 mg Cr(III)/kg b.w. per day) (Bailey et al., 2006).

Dietary exposure of male mice to 200 mg chromium picolinate/kg b.w. per day (25 mg Cr(III)/kg b.w. per day) before mating had no effect on fertility, prenatal mortality, fetal weight or skeletal morphology (McAdory et al., 2011).

In summary, conflicting results on reproductive effects of Cr(III) compounds have been reported; some studies do not show show evidence of reproductive or developmental toxicity, whereas in other studies effects on fertility, organ weights, embryo- and fetotoxicity have been observed. The difference in results might be related to experimental methods, including exposure media (drinking water versus feed) or to differences in toxicity of the specific Cr(III) compounds evaluated. The CONTAM Panel noted that a majority of studies have methodological limitations, and were not designed for establishing reference doses (only one dose tested). However, in the absence of adequate data, the results of these studies must be taken into account as they identify potential adverse effects of oral Cr(III) exposure. In the studies where effects on reproduction and development have been observed, the lowest LOAELs were in the order of 30 mg/kg b.w. per day.

Table 13: Developmental and reproductive toxicity studies with Cr(III) compounds.

Study[a] Doses in mg Cr(III)/kg b.w. per day	NOAEL (mg Cr(III)/kg b.w. per day)	LOAEL	Reference
1-generation reproductive toxicity			
1-generation reproductive oral (diet) Cr_2O_3 in rats (90 days) F/M: 0; 547/570; 1217/1368[b]	1217/1368	-	Ivankovic and Preussman (1975)
Fertility studies			
12 weeks oral (drinking water) toxicity study $CrCl_3$ in rats M: 0, 30[c]	-	30	Bataineh et al. (1997)
12 weeks oral (drinking water) toxicity study $CrCl_3$ in mice M :0, 49, 246[c]	-	49	Elbetieha and Al-Hamood (1997)
12 weeks oral (drinking water) toxicity study $CrCl_3$ in mice F: 0, 98, 246[c]	-	98	Elbetieha and Al-Hamood (1997)
4 week oral (diet) toxicity study chromium picolinate in mice M: 0, 25[b]	25	-	McAdory et al. (2011)
Developmental toxicity studies			
GD12– day 20 of lactation oral(drinking water) to $CrCl_3$ mice F: 0, 79[d]	-	79	Al-Hamood et al. (1998)
mice exposed to chromium picolinate via diet on GD 6-17 F: 0, 25[b]	-	25	Bailey et al., 2006
mice exposed to $CrCl_3$ via diet on GD 6-17 F: 0, 39[b]	39	-	Bailey et al. (2006)
mice exposed to Cr(III)picolinate or other sources of Cr(III) via diet on GD 6-17 F: 0, 25[b] (from picolinate) or 3.3 or 26[b] as Cr(III) cation $Cr_3O(O_2CCH_2CH_3)_6(H_2O)_3)^+$	25	-	Bailey et al. (2008a)
mice exposed via diet to chromium picolinate from implantation through weaning F: 0, 25[b]	25	-	Bailey et al. (2008b)
rats exposed to CrCl3 by gavage on GD 1-3 or 4-6 F: 33.6[c]	-	33.6 (GD 1-3)	Bataineh et al., 2007
Toxicity on reproductive organs			
13-week oral (diet), B6C3F1 mice Chromium picolinate monohydrate M/F: 0, 2/1.7, 6.2/4.9, 54/44, 273/212, 1419/1090[b]	M: 1419 F: 1090	-	Rhodes et al. (2005) NTP (2010)
14-week oral (diet); F344/N rats Chromium picolinate monohydrate M: 0, 0.8/0.7, 2.4/2.4, 19.1/19.1, 95.4/93, 506/507[b]	M: 506 F: 507	-	Rhodes et al. (2005) NTP (2010)
24-week oral (diet), Harlan Sprague Dawley rats chromium chloride or chromium picolinate 0, 0.45, 2.25, 4.5 and 9[b]	9	-	Anderson et al. (1997)
35 days oral (diet), mice Chromium sulphate M: 0, 9.2, 19, 46[c]	-	9.2	Zahid et al., 1990

M: male F: female; NOAEL: no-observed-adverse-effect level; LOAEL: lowest-observed-adverse-effect level.

(a): In the conversions from concentration to daily doses, the molecular weight (MW) of the anhydrous salts were used when no information on hydration number was available in the original publication.

(b): Data reported in the original publication;

(c): Conversion using the default correction factor for subacute/subchronic/chronic exposure via drinking water/feed from EFSA SC (2012);

(d): Conversion using drinking water/feed consumption data and average body weight reported in the publication.

7.2.1.4. Genotoxicity

The mutagenic potential of Cr(III) compounds has been studied extensively and recently reviewed (EFSA ANS Panel, 2010a, b; ATSDR, 2012). Although study results vary depending on the test system, experimental conditions and type of Cr(III) compounds tested, the majority of the assay systems used provide evidence of lack of genotoxicity of Cr(III) compounds both *in vitro* and *in vivo*. However, it should be noted that the ultimate mutagen that binds DNA is the trivalent form produced intracellularly from Cr(VI) and therefore the apparent lack of activity of Cr(III) is solely due to its poor cellular uptake (Léonard and Lauwerys, 1980). Here, we will provide a summary of the literature and details only for the most relevant studies.

***In vitro* assays**

Bacteria and yeast

No genotoxic effects have been reported for Cr(III) picolinate in Ames assays using a variety of *Salmonella typhimurium* strains (BDL, 1995; Esber et al., 1997; NTP, 2010; Juturu and Komorowski, 2002; Whittaker et al., 2005) and concentrations up to 10 000 µg Cr(III) picolinate/plate in the presence or absence of metabolic activation (NTP, 2010). Neither Cr(III) chloride nor picolinic acid were mutagenic in the Ames test (Whittaker et al., 2005). Cr picolinate monohydrate was also negative in assays with *Escherichia coli* strain WP2uvr/pKM101, when tested with or without exogenous metabolic activation (S9) (NTP, 2010). Although Cr(III) compounds are largely inactive in bacterial mutagenicity assays it appears that some Cr(III) complexes are mutagenic in bacterial strains that are sensitive to oxidative stress. In the study by Sugden et al. (1992) the Cr(III) complexes that were mutagenic in the *S. typhimurium* strains TA102 and TA2638 (sensitive to oxidative mutagens), i.e. cis-$[Cr(phen)_2Cl_2]^+$ and cis-$[Cr(bipy)_2Cl_2]^+$, presented characteristics of reversibility and positive shifts of the Cr(III)/Cr(II) redox couple, as determined by cyclic voltammetry, consistent with the ability of these Cr(III) complexes to serve as electron donors in Fenton-like reactions. In line with their chemical properties the mutagenic complexes displayed a nicking activity on plasmid DNA presumably by the induction of single-strand breaks. The non-mutagenic compounds did not exhibit these properties. It should be noted that Cr(III) picolinate was negative in *Salmonell*a strains 102 and 104 (Juturu and Komorowski, 2002; NTP, 2010) which are sensitive to oxidative mutagens.

Kirpnick-Sobol et al. (2006) determined the effects of Cr(III) chloride on the frequencies of DNA deletions measured with the deletion assay in *Saccharomyces cerevisiae*. A significant increased in the frequency of DNA deletions was observed and a linear correlation with the intracellular Cr concentrations was reported. The authors concluded that Cr(III) is a potent inducer of DNA deletions (even more potent than Cr(VI) tested in the same study) when it is absorbed.

Mammalian cells

Cr(III) compounds, particularly Cr picolinate, have been tested in numerous bioassays using cultured mammalian cells with mixed, often positive, results.

Cr(III) chloride induced chromosomal aberrations in phytohemagglutinin(PHA)-stimulated human lymphocytes (Friedman et al., 1987) that were suppressed by superoxide dismutase (SOD), catalase and mannitol (specific scavenger of hydroxyl radicals). The authors concluded that the production of oxygen free radicals could contribute to the effects observed.

Stearns et al. (1995) investigated the potential genotoxicity of chelated Cr(III) picolinate in Chinese hamster ovary (CHO) AA8 cells. Cr(III) picolinate was clastogenic in a concentration-dependent manner from 50 µM to 1 mM. Picolinic acid showed dose-dependent chromosome damage up to 2 mM. The data suggest that the picolinic acid rather than the Cr(III) was responsible for the observed effects, because Cr chloride and Cr nicotinate were not clastogenic at equivalent non toxic Cr concentrations.

No induction of micronuclei was observed following exposure of V79 Chinese hamster lung cells to a variety of Cr(III) complexes, except when Cr(III) imine complexes, which could be oxidized to Cr(V)

complexes, were used (Dillon et al., 2000). There was a substantial increase in the permeability of the Cr(V) complex $[Cr(O)_2(phen)_2]^+$, compared to that of its Cr(III) analogue (i.e. *cis*-$[Cr(phen)_2(OH_2)^2]^{3+}$), which was accompanied by a highly genotoxic response. Consequently, any Cr(III) complex that is absorbed by cells and can be oxidized to Cr(V) should be considered as a potential genotoxin.

The ability of Cr(III) compounds to induce mutation and anchorage independence, a marker of cell transformation, was studied in human diploid fibroblasts (HFC) (Biedermann and Landolph, 1990). Mutagenicity at the hypoxanthine phosphoribosyltransferase (HPRT) locus of insoluble Cr(III) occurred only at cytotoxic concentrations (10-100 µM), but induction of anchorage independence occurred at concentrations of 0.1-10 µM, indicating that inductions of mutagenicity and anchorage independence were not coupled for Cr(III) compounds.

Cr chloride was shown to induce micronuclei, that originated from both chromosome breakage and loss of entire chromosomes, in human Medical Research Council (MRC) fibroblasts (Seaone and Dulout, 2001).

Stearns et al. (2002) investigated the mutagenicity of Cr(III) picolinate in CHO AA8 cells, using solutions of Cr(III) picolinate up to 1 mM and of picolinic acid up to 3 mM. Cr(III) picolinate was found positive for HPRT mutations. The HPRT mutations were increased up to 40-fold compared to control. Picolinic acid was more cytotoxic than the corresponding Cr(III) picolinate complex but there was no evidence of mutation induction.

Negative results were obtained by Slesinski et al. (2005) by performing a HPRT assay in CHO cells exposed to concentrations of Cr(III) picolinate up to 1.43 mM for 5- and 48 hour periods.

Juturu et al. (2004) reported also negative results in a chromosomal aberration assay with CHO cells, at concentrations of Cr(III) picolinate up to 1.84 mM for 4 hours in the presence of metabolic activation and 4 and 20 hours in the absence of metabolic activation).

Whittaker et al. (2005) tested the mutagenic potential of Cr(III) picolinate and its component compounds, Cr(III) chloride and picolinic acid in L5178Y mouse lymphoma cells. Cr(III) picolinate induced mutagenic effects with and without the addition of S9. Cr(III) chloride was negative in the absence of metabolic activation but questionable results were observed with metabolic activation and after exposure to picolinic acid due to high cytotoxicity at mutagenic doses.

Cr(III) picolinate was tested in a chromosomal aberration test *in vitro* in CHO K1 cells (Gudi et al., 2005). CHO cells were exposed to a range of cytotoxic and non-cytotoxic concentrations of Cr(III) picolinate up to 770 µg/mL (limit of solubility) for 4 hours or 20 hours in the presence and in the absence of S9 activation. No evidence of clastogenicity was observed at any dose tested.

Coryell and Stearns (2006) evaluated the mutagenic effects of Cr(III) picolinate in the HPRT mutation assay in CHO AA8 cells after 48 h exposure using either acetone or dimethyl sulfoxide (DMSO) as solvents. Cr(III) picolinate increased the mutation frequency under both treatement conditions but it was 3.5-fold more mutagenic when dissolved in acetone. The authors hypothesized that this effect is due to the radical scavenger properties of DMSO suggesting that the free radical production by Cr(III) picolinate contributes to genotoxicity. The molecular analysis of the mutants generated from exposure to Cr(III) picolinate in acetone showed base substitutions, mostly transversions (33 %), deletions (62 %) and 1-4 bp insertions (5 %). It should be noted that the increases in mutation frequency were observed at cytotoxic doses.

The induction of DNA damage by Cr(III) picolinate and other Cr(III) complexes was analysed by the Comet assay with and without hydrogen peroxide-induced stress in human HaCaT keratinocytes (Hininger et al., 2007). Cr(III) picolinate did not induce any DNA damage in the Comet assay at 120 µM (saturated solution, non-cytotoxic dose in the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT)-test) whereas significant induction of DNA breaks was reported after exposure to Cr chloride at 6 mM (LC_{50} in the MTT-test). No DNA damage was also reported after treatment with Cr histidinate at 10 mM (LC_{50} in the MTT-test).

Andersson et al. (2007) measured DNA damage by the Comet assay in cultures of human lymphocytes and L5178Y mouse lymphoma cells exposed *in vitro* to 500 µM Cr(III) picolinate for 3 hours. A slight but statistically significant increase in DNA damage was observed in human lymphocytes, but only when cells were exposed in the absence of serum.

***In vivo* assays**

Four studies were conducted using Cr(III) compounds in *Drosophila melanogaster* as model system. Negative results were obtained in two studies (Amrani et al., 1999; Katz et al., 2001) in which mutagenic or recombinogeneic events were measured in adult flies treated in the larval stage with Cr chloride. In contrast, positive results were reported (Hepburn et al., 2003; Stallings et al., 2006) when Cr(III) picolinate was given in the diet at concentrations up to 2107 µg/kg food (equivalent to 260 µg Cr/kg food). Although no effects on survival, behaviour or fertility of adult Drosophila were reported, developmental delays and decreased pupation success were observed in larvae.

In contrast to the *in vitro* studies in mammalian cells, most of the studies that have been conducted on Cr(III) compounds in animal models have yielded negative results (Table 14) (for detailed information see Appendix H: Table H3, oral route administration and Table H4, non-oral route administration).

In an NTP study (NTP, 2010) the *in vivo* micronucleus assay was performed in male F344/N rats treated with Cr(III) picolinate (anhydrous) (156 to 2500 mg/kg b.w.) by oral gavage three times at 24-hour intervals. Negative results were observed in bone marrow erythrocytes of male rats. In another NTP study (NTP, 2010) the *in vivo* micronucleus assay was performed in male and female B6C3F1 mice administered Cr(III) picolinate monohydrate (80 to 50000 mg/kg diet corresponding to 2-1419 and to 1.7-1090 mg Cr(III)/kg b.w. per day for male and female respectively) in feed for 3 months. Negative results were observed in peripheral blood erythrocytes of the male mice. The weak increases in the micronuclei frequency observed in erythrocytes of female mice were considered equivocal findings as the anhydrous form was inactive.

Itoh and Shimada (1996) analysed the clastogenic properties of $CrCl_3$ in bone marrow cells of Slc:ddY mice following i.p. administration (up to 125 mg/kg b.w.). Cr(III) chloride was negative for micronuclei induction.

Kirpnick-Sobol et al. (2006) determined the effects of Cr(III) chloride administered to dams in drinking water on the frequencies of DNA deletions measured in the *in vivo* p(un) reversion assay in C57BL/6J p(un)/p(un) mice. Cr(III) (Cr(III) chloride salt) was used at either 1875 or 3750 mg/L concentration, which was calculated to yield an average dose of 375 or 750 mg of Cr/kg b.w. per day, respectively. Exposing mice to Cr(III) significantly increased the frequency of DNA deletions in embryos harvested at 17.5 days post-coitum. The authors also quantified tissue Cr concentrations in mice after exposure. The authors concluded that Cr(III) is a potent inducer of DNA deletions (even more potent than Cr(VI) tested in the same study) when it is absorbed.

De Flora et al. (2006) analysed the frequency of micronuclei in bone marrow and peripheral blood cells of BDF1 mice, both males and females, administered Cr(III), as $CrK(SO_4)_2{\cdot}12H_2O$, in the drinking water, at concentrations up to 500 mg Cr/L water (corresponding to 165 and 140 mg Cr(III)/kg b.w. per day for male and female respectively), for 7 months. Cr(III) did not affect the micronuclei frequency at any dose tested.

Andersson et al. (2007) evaluated the potential genotoxicity of Cr(III) picolinate in an *in vivo* assay. Mice were given a single intraperitoneal injection (up to 3 mg/kg b.w.) and the frequency of micronuclei in polychromatic erythrocytes was evaluated in peripheral blood as well as DNA damage in lymphocytes and hepatocytes by the Comet assay. In all other experiments Cr(III) picolinate was found to be inactive, both *in vitro* and *in vivo*.

The *in vivo* cytogenetic effects of Cr(III) picolinate were analysed by Komorowski et al. (2008) in rats in a well conducted study performed according to OECD guideline 475 (1997). The rats received a single oral dose of 33, 250 or 2000 mg/kg b.w. Cr(III) picolinate (corresponding to 4.1, 30.8 and

246 mg Cr(III)/kg b.w. per day) and were sacrificed after 18 or 42 hours. Cr(III) picolinate did not induce chromosomal aberrations in the bone marrow cells (Komorowski et al., 2008).

Table 14: *In vivo* genotoxicity assay with Cr(III) compounds administered by oral route.

Test system/ Endpoint	Compound	Response[(a)] Dose: mg Cr(III)/kg b.w. per day[(b)]	Reference
Rat (F344/N) Micronuclei in bone marrow erythrocytes	Cr picolinate	Negative 310.7	NTP (2010)
Mouse (B6C3F1) Micronuclei in peripheral blood erythrocytes	Cr picolinate monohydrate	Negative - 1419	NTP (2010)
Mouse (BDF1) Micronuclei in Bone marrow and peripheral blood cells	Chromic potassium sulphate dodecahydrate $CrK(SO_4)_2x12H_2O$	Negative 165	De Flora et al. (2006)
Rats (Sprague–Dawley) Micronuclei in bone marrow cells	Cr picolinate	Negative 246	Komorowski et al. (2008)
Mouse (C57BL/6J) DNA deletions (pun reversion assay) in developing embryos	Cr(III) chloride salt	Positive 375	Kirpnick-Sobol et al. (2006)

(a): The lowest effective dose is indicated for positive results and the highest dose tested for negative results.
(b): Doses calculated using data from the original publications.

Genotoxicity studies in humans

A number of biomonitoring studies have been conducted to investigate genetic damage in lymphocytes of tannery workers exposed to Cr(III) compounds but their interpretation is difficult due to the presence of other chemicals (possibly also Cr(VI)) in the work environment.

No significant differences in the frequency of chromosomal aberrations in peripheral lymphocytes were detected between healthy Cr-exposed workers at a tanning plant near Baghdad city and controls matched for age, period of service and social background (Hamamy et al., 1987). However, the average concentrations of Cr in plasma and urine of exposed workers were not different from those of unexposed workers.

An increase in chromosomal aberrations (Sbrana et al., 1990) but not in micronuclei (Migliore et al., 1991) was reported in lymphocytes of tannery workers of a drum workshop with elevated exposures to Cr(III) compounds (and carcinogenic aromatic amine dyes).

Another study (González Cid et al., 1991) reported elevated frequency of chromosomal aberrations in the exposed tannery workers but not statistically different from the frequency seen in the controls. In this study the urinary Cr concentrations did not differ between the exposed and control workers.

Medeiros et al. (2003) reported that the frequency of micronuclei and DNA protein crosslinks were significantly higher (but < 2 fold increase) in the lymphocytes of Cr-exposed tannery workers than controls. A significant correlation was also observed between Cr concentrations in the urine and plasma and frequency of DNA protein cross-links in the lymphocytes.

Zhang et al. (2008) studied DNA damage in peripheral lymphocytes from workers occupationally exposed to Cr(III) by the Comet assay. The study population was divided into three groups: (1) tannery workers highly exposed to Cr from the tanning department; (2) tannery workers with moderate Cr exposure from the finishing department; (3) control individuals without exposure to genotoxic agents. Urinary and blood Cr concentrations and the tail moments (marker of DNA breaks) of lymphocytes as measured by the Comet assay were significantly higher in the two exposed groups as compared to the control group and group 1 presented higher levels than group 2.

7.2.1.5. Oxidative DNA damage and cytotoxicity

***In vitro* studies**

Levis et al. (1978) studied the cytotoxic effects of Cr(III) and Cr(VI) compounds in cultured hamster fibroblasts (BHK line) and human epithelial-like cells (HEp line) by measuring as end-points cell growth and nucleic acid and protein synthesis. The authors concluded that the cytotoxic effects of Cr can be attributed to the action of Cr(VI) at the plasma membrane level on the mechanisms involved in nucleoside uptake, and to the interaction of Cr(III) at the intracellular level with nucleophilic targets on the DNA molecule.

Reactive Oxygen Species (ROS) and DNA fragmentation were measured in murine macrophage cells following exposure to Cr picolinate and Cr polynicotinate. The induction of oxidative damage was attributed to the picolinate moiety (Olin et al., 1994).

Oxidative damage was measured in cultured macrophage cells (J774A.1) following exposure to Cr(III) picolinate and Cr nicotinate (Bagchi et al., 1997). Small dose-dependent increases in lipid peroxidation, superoxide anion and hydroxyl radical production and DNA fragmentation were observed with both Cr salts, compared to control, with greater increases in the case of Cr(III) picolinate in comparison to Cr nicotinate.

Speetjens et al. (1999) reported that in the presence of reductants (ascorbate) and air, Cr(III) picolinate is capable of generating hydroxyl radicals which in turn can cleave supercoiled plasmid DNA. A mechanism is proposed where Cr(III) picolinate is reduced by biological reductants to Cr(II)-containing species that are susceptible to air oxidation, thus resulting in the catalytic generation of hydroxyl radical. They also reported that in the absence of reductants, hydrogen peroxide can interact with Cr(III) picolinate to produce hydroxyl radicals by a second, less efficient mechanism.

Human HaCaT keratinocytes were exposed for 24 hrs to Cr(III) complexes and oxidized bases were measured as 8-hydroxy-2'-deoxyguanosine (Hininger et al., 2007). Concentrations of Cr(III) chloride, Cr(III) histidinate and Cr(III) picolinate that did not result in cytotoxic effects did not produce oxidative DNA damage. Cell exposure at LD_{50} concentrations (as determined by the MTT test) led to a significant increase in oxidized bases with Cr(III) chloride but not with Cr(III) histidinate.

Jana et al. (2009) studied the effect of Cr(III) picolinate uptake in peripheral blood lymphocytes by measuring cytotoxicity and markers of apoptosis. Concentrations of Cr(III) picolinate varying from 5 to 100 μM for different exposure times were tested. The results indicated that Cr(III) picolinate induces apoptosis in a dose-dependent manner. The involvement of ROS in this phenomenon is strongly suggested by the inhibition of apoptosis following pretreatment of the cells with the antioxidant N-acetyl cysteine and by the induction of markers of apoptosis including cytosolic cytochrome c release that indicate mytocondrial alterations.

***In vivo* studies**

Cupo and Wetherhahn (1985) measured the binding of either sodium dichromate or Cr(III) chloride to DNA *in vivo* in rat liver and kidney. Cr was found bound to DNA, nuclear proteins, and RNA protein fraction in liver and kidney tissues, following an i.p. injection of either sodium dichromate or Cr(III) chloride. At early times, there was much less Cr binding to chromatin and DNA after Cr(III) treatment than after Cr(VI) treatment. In addition, after Cr(III) treatment, a large percentage of the Cr bound to chromatin was associated with protein rather than with the DNA. However, 40 hr after injection there was no significant difference in the level of Cr binding to DNA after either Cr(VI) or Cr(III) treatment. In spite of the binding, at this time, DNA damage was found in the kidney only after Cr(VI) but not Cr(III) treatment, suggesting that while Cr(III) was bioavailable it was not particularly bioactive.

The ability of some Cr(III) complexes to undergo Fenton-type reactions could also contribute to their genotoxicity. The generation of oxidative damage by Cr(III) picolinate is suggested by *in vivo* studies

(Hepburn et al., 2003) where rats treated with Cr(III) picolinate showed increased urinary excretion of 8-hydroxy-2'deoxyguanosine and increased lipid peroxidation in liver and kidney.

Tan et al. (2008) investigated the effect of excessive Cr(III) picolinate intake on oxidative damage in pigs. Male pigs were fed a diet withdoses up to 3200 µg of Cr/kg feed as Cr(III) picolinate for 80 days. At the high dose the superoxide dismutase activity was significantly decreased in serum, also the catalase activity in the kidneys. However, the levels of malondialdehyde in tissue and serum, urinary 8-hydroxydeoxyguanosine and DNA strand breaks in liver and kidney were not affected.

7.2.1.6. Conclusions on genotoxicity

The extensive literature on genotoxicity of Cr(III) compounds provides conflicting information regarding their genotoxicity but in general they gave largely negative results in bacterial assays and mixed, often positive, results in mammalian cell assays (although often at cytotoxic doses). *In vivo* tests for genotoxicity were all negative with one exception of a non standard assay (i.e. p(un) reversion assay in mice). Cr(III) compounds have the potential to react with DNA in acellular systems (see also Section on mode of action), however in intact cells restricted access limits or prevents genotoxicity. At high concentrations, multiple studies showed that Cr(III) compounds might cause DNA damage which is potentially mutagenic and clastogenic. The inhibition of these effects by antioxidants as well as the detection of oxidized bases both *in vitro* and *in vivo* indicates that oxidatively-generated DNA damage is involved.

7.2.1.7. Carcinogenicity

Cr(III) carcinogenicity has been recently addressed by the NTP in their study on Cr picolinate monohydrate (Stout et al., 2009; NTP, 2010) which contains Cr(III) chelated with three molecules of picolinic acid in order to increase the absorption of Cr(III). Chromium picolinate is widely used as dietary supplement. Chromium picolinate monohydrate studies included a 3-month toxicity study to select exposure concentrations for the 2-year studies. These studies are described below.

The study was conducted in 50 male and female F344/N rats and B6C3F1 mice exposed in feed to concentrations from 2000 to 50000 mg/kg for 2 years. A maximal concentration of 50000 mg/kg feed of chromium picolinate monohydrate was selected in order to prevent alteration of the nutritional content of the diet. There were no biologically significant changes in survival, body weight, feed consumption or the occurrence of non-neoplastic lesions in rats or mice in the 2-year studies at concentrations up to 50000 mg/kg feed. This corresponds to average daily doses of 286.2 and 313.7 mg Cr(III)/kg b.w. per day for male and female rats, respectively, and of 783.0 and 727.5 mg Cr(III)/kg b.w. per day for male and female mice, respectively. In male rats, a statistically significant increase in the incidence of preputial gland adenomas at 10000 mg/kg feed (corresponding to 54.9 mg Cr(III)/kg b.w. per day) was reported. However, the incidence of preputial gland hyperplasia was not increased at any exposure dose, neither preputial gland carcinomas were observed in exposed males. There were no increases in the incidence of clitoral gland adenomas or hyperplasia in exposed females (the clitoral gland is the female counterpart of the preputial gland). On the basis of these data the CONTAM Panel concluded that Cr(III) is not carcinogenic in experimental animals.

7.2.2. Hexavalent chromium

7.2.2.1. Acute toxicity

In general Cr(VI) salts had greater acute toxicity than Cr(III) salts, and female rats appeared to be more sensitive than males to Cr(VI) salts (ATSDR, 2012). Table 15 shows the oral LD_{50}s reported in the rat.

Table 15: Oral LD_{50}s reported for chromium(VI) in rats.

Compound	LD_{50} (mg Cr(VI)/kg b.w.)	Reference
Sodium chromate	F: 13/M: 28	Gad (1989, erratum 1990)
Sodium dichromate	F: 15.5/M: 23.4	Gad (1989, erratum 1990)
Potassium dichromate	F: 16.9/M: 26.2	Gad (1989, erratum 1990)
Ammonium dichromate	F: 19.5/M: 22.6	Gad (1989, erratum 1990)
Calcium chromate	F: 108/M: 249	Vernot et al. (1977)
Chromium trioxide	F: 25/M: 29	American Chrome and Chemicals (1989)
Strontium chromate	M: 811	Shubochkin and Pokhodzie (1980)

F: female; M: male; b.w.: body weight.

The primary cause of death resulting from acute Cr(VI) exposure is nephrotoxicity. In rats, it has been demonstrated that increased urinary excretion of proteins is the earliest and most sensitive marker of damage (Gumbleton and Nicholls, 1988).

7.2.2.2. Repeat dose toxicity

Several studies were located in the literature regarding repeated oral exposure (dietary or via drinking water) to Cr(VI). Detailed review of these studies has been reported by U.S. EPA (1998b), OEHHA (2011) and ATSDR (2012).

The relevant studies are reported in the text. A more complete overview of the studies performed on Cr(VI) is provided in Table H5 (Appendix H).

Significant decreases in body weight have been reported in several intermediate-duration oral Cr(VI) studies in animals (Chowdhury and Mitra, 1995; NTP, 1996a, b; Elbetieha and Al-Hamood, 1997; Bataineh et al., 1997; De Flora et al., 2006; Yousef et al., 2006; NTP, 2007; Quinteros et al., 2007). However, it should be noted that high concentrations of chromium in drinking water decrease palatability of water, resulting in decreased water consumption; thus, decreased body weight may, in part, be due to decreased water consumption, in addition to other causes.

After repeated oral administration, the major target organs of Cr(VI) compounds in rats and mice are the haematological system (microcytic, hypochromic anemia), the liver (biochemical and histopathological changes: vacuolation, lipid accumulation, chronic inflammation and focal necrosis), the kidney (biochemical and histopathological changes) and the gastrointestinal tract (irritation and histopathological changes to tissues). No adverse effects were reported in oral mucosa, forestomach, glandular stomach or small intestine in the long term rats and mice studies (NTP, 2008); whereas irritation/ulcers were observed in the stomach in the 3-month studies (at 15.7 mg Cr(VI)/kg b.w. per day in mice and at 20.9 mg Cr(VI)/kg b.w. per day in rats (NTP, 2007). However, the high dose in the 2-year studies (5.9 mg Cr(VI)/kg b.w. per day) was substantially lower than the high dose in the 3-month studies.

The studies conducted by the National Toxicology Program (NTP, 2007, 2008) provide dose-response data on the effects of Cr(VI) exposure based on a comprehensive assessment of toxicological endpoints. Several other studies do not provide data suitable for dose-response evaluation, because only one dose was tested and/or comprehensive toxicological endpoints were not evaluated. However, results of these studies are useful for identification of potential adverse effects of oral Cr(VI) exposure.

After 3-month oral exposure (NTP, 2007), the most critical Cr(VI)-induced effects were microcytic, hypochromic anemia (observed at $\geq$ 1.7 mg Cr(VI)/kg b.w. per day), increased serum liver enzyme activities (from 1.7 mg Cr(VI)/kg b.w. per day) and histopathological changes to the duodenum (histiocytic infiltration from 3.1 mg Cr(VI)/kg b.w. per day) and pancreatic lymph nodes (from 1.7 mg Cr(VI)/kg b.w. per day) in rats and microcytic, hypochromic anemia (from 3.1 mg Cr(VI)/kg b.w. per day), histopathological changes in the duodenum (histiocytic infiltration, epithelial hyperplasia from 3.1 mg Cr(VI)/kg b.w. per day) and in mesenteric lymph nodes (histiocytic infiltration from 5.2 mg Cr(VI)/kg b.w. per day) in mice.

The most critical Cr(VI)-induced non-neoplastic effects in the NTP 2-year long term studies (NTP, 2008) were haematological effects (microcytic, hypochromic anemia), histiocytic cellular infiltration in the liver, mesenteric lymph node and duodenum in rats. In mice they were hyperplasia in duodenum, and hystiocytic cellular infiltration in the liver and mesenteric lymph nodes.

In the NTP 2-year studies (NTP, 2008) microcytic, hypochromic anemia characterized by decreased mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), hematocrit and hemoglobin was observed in rats and mice from 22 days to 6 months. The severity of the haematological effects was dose-dependent, with maximum effects observed after 22-23 days of exposure, showing that with time, the animals adapted to exposure. Effects were less severe in mice than those observed in rats. The erythropoietic tissues were able to respond to the anemia, but there was some ineffective erythopoiesis resulting in production of increased number of smaller erythrocytes. The lowest LOAELs for haematological effects were 0.77 mg Cr(VI)/kg b.w. per day in male rats and 0.38 mg Cr(VI)/kg b.w. per day in female mice. The NOAEL in rats was 0.21 mg Cr(VI)/kg b.w. per day and a NOAEL was not established in mice.

The LOAELs for liver toxicity were 0.77 mg Cr(VI)/kg b.w. per day in rats and 0.38 mg Cr(VI)/kg b.w. per day in mice. Chronic inflammation appeared to be more severe than in control animals at 7 and 8.8 mg Cr(VI)/kg b.w. per day in female rats and mice, respectively.

The LOAELs for effects on the mesenteric lymph nodes were 0.77 and 0.38 mg Cr(VI)/kg b.w. per day in rats and mice, respectively.

The LOAEL for histiocytic cellular infiltration in the duodenum in rats was at 0.77 mg Cr(VI)/kg b.w. per day and for hyperplasia in the duodenum in mice was 0.38 mg Cr(VI)/kg b.w. per day.

Effects on the kidney have been reported in a gavage study in rats exposed for 20 days to 50 mg potassium chromate/kg b.w. per day (corresponding to 13.4 mg Cr(VI)/kg b.w. per day). The effects were increased accumulation of lipid, triglycerides and phospholipids in different regions of the kidney compared to controls, and inhibition of kidney membrane enzymes (alkaline phosphatase, acid phosphatase, glucose-6-phosphatase and lipase) (Kumar and Rana, 1982). Toxic effects on the kidney were also reported in male and female Wistar rats exposed for 22 weeks to 25 mg potassium dichromate (corresponding to 0.8 mg Cr(VI)/kg b.w. per day) via drinking water: degeneration of basement membrane in Bowman's capsule and renal tubular degeneration (Chopra et al, 1996; Acharya et al, 2001).

Thompson et al. (2011b) performed a 90-day drinking water study using similar exposure conditions as in a previous cancer bioassay (NTP, 2008) as well as lower concentrations. Female B6C3F1 mice were exposed to 0, 0.3, 4, 14, 60, 170 and 520 mg sodium dichromate dihydrate/L (corresponding to 0, 0.03, 0.3, 1.1, 4.7, 12.2, 31 mg Cr(VI)/kg b.w. per day).

No treatment-related gross lesions were observed after 90 days of exposure. There were no microscopic lesions observed in the oral cavity. Following 90 days of exposure, significant increases in chromium were observed at ≥ 60 mg/L in the oral cavity, glandular stomach, jejunum and ileum. Total chromium concentrations in the duodenum were significantly elevated at ≥ 14 mg/L. Intestinal lesions including villous cytoplasmic vacuolisation were observed at concentrations ≥ 60 mg/L and atrophy, apoptosis and crypt hyperplasia were seen at ≥ 170 mg/L. Multinucleated syncitia (fused cells) in the villous lamina propria were present in mice exposed to 520 mg/L. Similar histopathological lesions were observed in the jejunum. The NOAEL was 14 mg sodium dichromate dihydrate/L (corresponding to 1.1 mg Cr(VI)/kg b.w. per day).

A similar 90-day drinking water study was performed on rats. Female F344 rats were exposed to 0, 0.3, 4, 60, 170 and 520 mg sodium dichromate dihydrate/L (corresponding to 0, 0.02, 0.2, 3.6, 8.7, 24 mg Cr(VI)/kg b.w. per day mg Cr(VI)/kg b.w. per day). Statistically significant increases in total chromium concentrations occured in the oral cavity, duodenum and jejunum at ≥ 60 mg/L. Significant increases in the glandular stomach and ileum occured at ≥ 170 mg/L and 520 mg/L, respectively. No treatment-related gross lesions were observed after 90 days of exposure. There were no microscopic lesions observed in the oral cavity. In the duodenum, apoptosis was observed at ≥ 60 mg/L and crypt

cell hyperplasia at ≥ 170 mg/L. histiocytic infiltration was present in almost all animals at ≥ 60 mg/L. Apoptosis, crypt cell hyperplasia and villous atrophy were observed in the jejunum at concentrations as low as 4 mg/L (incidences not statistically different from control animals and in many instances the lesions were not observed at higher concentrations). There were concentration-dependent increases in histiocytic infiltration beginning at 60 mg/L. The NOAEL was 4 mg sodium dichromate dihydrate/L corresponding to 0.2 mg Cr(VI)/kg b.w. per day (Thompson et al., 2012b).

Table 16 summarised the NOAELs and LOAELs for the critical non-neoplastic toxic effects of Cr(VI) observed in relevant 90 days and 2 year studies for critical endpoints (excluding cancer).

Table 16: NOAELs and LOAELs for the critical non-neoplastic toxic effects of Cr(VI).

Effect	Species	Study duration	NOAEL	LOAEL	Reference
			mg Cr(VI)/kg b.w. per day		
Haematology	Rats	2-year	0.21	0.77	NTP (2008)
Microcytic, hypochromic anemia (maximum effect after 22-23 days)	Mice	2-year	-	0.38	NTP (2008)
Liver	Rats	2-year	0.21	0.77	NTP (2008)
Enzyme induction		90-day	1.7	3.5	NTP (2007)
Histiocytic cellular infiltration or glycogen depletion	Mice	2-year	-	0.38	NTP (2008)
		90-day	3.1	5.2	NTP (2007)
Chronic inflammation	Rats	2-year	0.24 2.4*	0.94 7*	NTP (2008)
		90-day	11.5	21.3	NTP (2007)
	Mice	2-year	3.1	8.7	NTP (2008)
Mesenteric lymph node	Rats	2-year	0.21	0.77	NTP (2008)
Hystiocytic cellular infiltration	Mice	2-year	-	0.38	NTP (2008)
Pancreatic lymph node	Rats	2-year	2.4	7.0	NTP (2008)
Hystiocytic cellular infiltration	Mice	2-year	2.4	3.1	NTP (2008)
Duodenum Hystiocytic cell infiltration	Rats	2-year	0.21	0.77	NTP (2008)
Hyperplasia	Mice	2-year	-	0.38	NTP (2008)
Villous cytoplasmic vacuolisation	Mice	90-day	1.1	4.7	Thompson et al. (2011a)
Apoptosis/crypt cell hyperplasia/ hystiocytic infiltration in the villous lamina propria			4.7	12.2	
Apoptosis	Rats	90-day	0.2	3.6	Thompson et al. (2012b)
Hystiocytic infiltration in the villous crypt cell hyperplasia	Rats	90-day	0.2 3.6	3.6 8.7	Thompson et al. (2012b)
Jejunum	Mice	90-day			Thompson et al. (2011a)
Villous cytoplasmic vacuolation			1.1	4.7	
Crypt cell hyperplasia/hystiocytic infiltration in the villous lamina propria			4.7	12.2	
Hystiocytic infiltration in the villous	Rats	90-day	3.6	8.7	Thompson (2012b)
Kidney degeneration of basement membrane in Bowman's capsule and renal tubular degeneration	Rats	22-week	-	0.8	Chopra et al. (1996)
Stomach	Mice	90-day	9.1	15.7	NTP (2007)
Ulcer, hyperplasia, metaplasia	Rats	90-day	11.1	20.9	NTP (2007)

* increase severity of effect; b.w.: body weight.

7.2.2.3. Developmental and reproductive toxicity

A number of studies have investigated the induction of reproductive effects in animals orally exposed to Cr(VI).

Detailed review of these studies has been reported by EPA (U.S. EPA 1998b; CA EPA, 2009) and ATSDR (2012). NOAELs and LOAELs of the studies are reported in Table 17 and the studies are described in details in Table H6 (see Appendix H).

No treatment-related effects on fertility or reproductive performance have been observed in 2-generation studies on mice exposed via the diet to potassium dichromate up to 400 mg/kg diet (corresponding to 30 mg Cr(VI)/kg b.w. per day) (NTP, 1997). In studies conducted on Sprague-Dawley rats or BALB/c mice exposed up to 400 mg/kg diet potassium dichromate daily (corresponding to 12.7 and 40.7 mg Cr(VI)/kg b.w. per day, respectively) for 9 weeks followed by a recovery period of 8 weeks, no effect on the testis and epididymes or spermatogenesis have been observed (NTP, 1996a, b, 1997). Similarly, exposure to sodium dichromate dihydrate in drinking water did not produce morphological changes to male reproductive organs of B6C3F1 mice exposed to 27.9 or 5.9 mg Cr(VI)/kg b.w. per day or F344 rats exposed to 20.9 or 5.9 mg Cr(VI)/kg b.w. per day for 3 months or 2 years, respectively (NTP, 2007, 2008) or affect sperm count or motility in B6C3F1, BALB/c and C57BL/6N mice exposed to 9.1 mg Cr(VI)/kg b.w. per day for 3 months (NTP, 2007).

Other studies on Cr(VI) showed adverse reproductive effects, with the male reproductive system exhibiting the highest sensitivity. An inhibitory effect on sexual and aggressive behaviour has been observed in male rats treated with potassium dichromate via drinking water, as well as effects on the reproductive organs (decrease testes, seminal vesicles and preputial gland weights) at a dose of 1000 mg/L (corresponding to 32 mg Cr(VI)/kg b.w. per day) (Bataineh et al., 1997). In a study in mice potassium dichromate reduced seminal vesicles and preputial glands weight (Elbetieha and Al-Hamood, 1997). Reduction of epididymal sperm counts, increased frequency of abnormal sperm and decreased diameter of seminiferous tubules have been reported in rats dietary exposed to 10 mg/kg chromium trioxide (5 mg Cr(VI)/kg b.w. per day) (Li et al., 2001) (Li et al., 2001). Low doses of sodium dichromate (≤ 7.9 mg Cr(VI)/kg b.w. per day) caused partial loss of cellular activity in testicular tissues of rats whereas treatment with higher dose of chromium (≥ 15.9 mg/kg b.w. per day) caused deleterious effects both on spermatogenic and steroidogenic activity (Chowdhury and Mitra, 1995).

The effect of Cr(VI) on spermatogenesis was studied in BALB/c mice (Zahid et al., 1990). Mice were given 100, 200 or 400 mg potassium dichromate/kg diet (corresponding to 16, 28 or 63 mg Cr(VI)/kg b.w. per day) for 7 weeks. Histological examination of the testes of treated animals revealed degeneration of the outermost cellular layers of seminiferous tubules with no spermatogenesis present and reported increase in the number of resting spermatocytes. Undegenerated tubules without spermatogenesis were significantly increased in all treated groups. Epididymal sperm count was lower at 200 and 400 mg/kg diet and the percentage of morphologically abnormal sperm was higher. Chromium treatment was reported to cause accumulation of germ cells in resting spermatocyte stage. These data are of questionable value because the methods described by the authors were not sufficient to show that they could identify spermatogonia. The methods used to evaluate the epididymal sperm very probably resulted in clumped sperm and poorly reproducible counts and the reduction in spermatogonia numbers concurrent with unchanged spermatocyte and spermatid numbers is biologically implausible. Moreover there are inconsistencies regarding the number of mice used in the study, the sizes of the experimental groups do not satisfy minimal requirements for such toxicity study and inappropriate statistical methods were used (Finley et al., 1993; NTP, 1996b). Moreover, no effects have been observed in a similar study performed by the NTP (1996b). Therefore, the CONTAM Panel did not take into account the results of the Zahid et al. (1990) study for the evaluation of the reproductive toxicity of Cr(VI).

Effects on testes and epididymal weights as well as on sperm have also been reported in rabbits exposed to potassium dichromate (Yousef et al., 2006).

Decreased testes weight, disorganisation in seminiferous tubules, decreased sperm count and motility, sperm death, disrupted spermatogenesis and histopathological changes to the epididymis (ductal obstruction, development of microcanals, germ cell depletion, hyperplasia of Leydig cells and Sertoli cell fibrosis) have been reported in adult bonnet monkeys exposed to doses ≥ 1.7 mg Cr(VI)/kg b.w. per day as potassium dichromate in drinking water for 180 days (Aruldhas et al., 2004, 2005, 2006 and Subramanian et al., 2006). Effects increased in severity with dose and duration. The studies have also shown that potassium dichromate administered via drinking water caused reversible oxidative stress in the seminal plasma and sperm leading to sperm death and reduced sperm motility and damage to the epididymal luminal spermatozoa, as a consequence of which the principal cells phagocytosed dead sperm from the lumen.

Cr(VI) has also been shown to alter weights of female reproductive organs, to have an effect on lengthening the oestrus cycle, to reduce the number of ovarian follicles and to induce changes in circulating levels of steroid and pituitary hormones (Murthy et al., 1996; Al-Hamood et al., 1998; Banu et al., 2008; Samuel et al., 2011). Atretic follicles and congestion in stromal tissue were observed in female mice receiving 750 mg Cr(VI)/L as potassium dichromate (equivalent to 135 mg Cr(VI)/kg b.w. per day, according to EFSA default values, 2012) for 20 days. Ultrastructural observations revealed disintegrated cell membranes of two layered follicular cells and altered villiform mitochondria in thecal cells of mice exposed to 5 mg Cr(VI)/L (corresponding to 0.8 mg Cr(VI)/kg b.w. per day) for 90 days (Murthy et al., 1996).

A series of studies have examined the effects of Cr(VI) on oxidative stress in rat offsprings.

Samuel et al. (2011) reported that maternal exposure to ≥ 6 mg Cr(VI)/kg b.w. per day as potassium dichromate in the drinking water on lactation days 1-21 resulted in dose-related reductions in antioxidant enzymes activities in uterine tissue from offspring measured on postnatal days 21, 45, and 65. This correlated with significant increases in lipid peroxidation and hydrogen peroxide in uterine tissue. Similar results were reported by Soudani and coworkers (Soudani et al. 2011a, b; Soudani et al., 2013) in the kidney, liver, and bone from 14-day-old pups born to dams exposed to 9.4 mg Cr(VI)/kg/day (only dose level tested) as potassium dichromate in the drinking water on gestation days 14-21 and postnatal natal days 1-14. This dose level also caused histological alterations in the tissues studied. In all of these studies, final body weight of the pups was significantly reduced, 25 % in the Soudani et al. (2011a, b) studies and 10-13 % at 6 mg Cr(VI)/kg per day and 26-33 % at 24 mg Cr(VI)/kg per day in the Samuel et al. (2011) study.

Developmental toxicity (embryotoxicity: increase in pre- and post-implantation loss, and in resorptions, fetotoxicity: decrease fetal weight, number of fetuses, number of live fetuses, and increased frequency of gross, visceral and skeletal malformations) has been observed in rats or mice treated during gestation with potassium dichromate in drinking water. In mice effects were observed at doses of 250 mg/L (corresponding to 45-53 mg Cr(VI)/kg b.w. per day) (Trivedi et al., 1989; Junaid et al., 1995; Junaid et al., 1996a). In rats, effects have been observed at 50 mg/L (corresponding to 1.6 mg Cr(VI)/kg b.w. per day) (Elsaieed and Nada, 2002). No NOAELs have been established. Administration by gavage of 25 mg potassium dichromate/rat (corresponding to 36 mg Cr(VI)/kg b.w. per day during gestation days 1-3 (before implantation) result in absence of implantation and during gestation days 4-6 (during implantation) result in post-implantation loss, decreased number of viable fetuses and increased number of resorptions (Betaineh et al., 2007).

Studies have shown that chromium passed the placental barrier and accumulate in fetal tissues (Trivedi et al., 1989; Junaid et al., 1995, 1996a, b; Kanojia et al., 1996, 1998; Elsaieed and Nada, 2002).

In studies where dams have been exposed prior to mating to potassium dichromate in drinking water, Cr(VI) decreased mating and fertility indices, the number of corpora lutea and the number of implantations as well as it increased the number of resorptions, pre- and post-implantations loss, decreased fetal weight and reduced ossification. Effects were observed at doses of 250 mg/L (equivalent to 45 mg Cr(VI)/kg b.w. per day in rats and 31-52 mg Cr(VI)/kg b.w. per day in mice) (Junaid et al., 1996b; Kanojia et al., 1996, 1998; Elbetieha and Al-Hamood, 1997). No NOAELs have been determined by the authors

Delayed vaginal opening (delayed puberty) was reported in offspring of rats exposed to potassium dichromate (6-24 mg Cr(VI)/kg b.w. per day) during lactation (Banu et al., 2008; Samuel et al., 2011) or mice (76 mg Cr(VI)/kg b.w. per day) exposed from gestation to lactation D20 (Al-Hamood et al., 1998).

Cr(VI) was also reported to alter mandibular growth and tooth eruption in rats (De Lucca et al., 2009). In this study, 4-day-old suckling Wistar pups received gavage doses of 0, 6.25 or 12.5 mg potassium dichromate/kg b.w. per day (corresponding to 0, 2.2 or 4.4 mg Cr(VI)/kg b.w. per day) for 10 consecutive days. Rats dosed at 4.4 mg Cr(VI)/kg b.w. per day showed a statistically significant reduction in mandibular length, base, height and area, and delayed eruption of the first molar. Delayed eruption of the second molar was observed in pups receiving the low-dose. These effects may have been secondary to a delay in body growth, as terminal body weight was reduced by 20 and 40 % in the low- and high-dose groups, respectively, relative to controls. Shorter tail length was also observed in high dose animals.

The CONTAM Panel noted that some of the studies have methodological limitations that were already reported for Cr(III).

The NOAELs and LOAELs from the relevant studies are reported in Table 17 and the studies are described in details in Table H6 (Appendix H).

Table 17: Developmental and reproductive toxicity studies with Cr(VI) compounds.

Study Doses in mg Cr(VI)/kg b.w. per day[(a)]	Species	NOAEL	LOAEL	Reference
		mg Cr(VI)/kg b.w. per day		
Multigeneration reproductive toxicity				
2-generation (diet) potassium dichromate F0/F1: 0, 6.9/7.9, 13.6/16.1, 30.3/37[(b)]	mice	Parental: 13.6 Reproduction: 30.3[(c)]	Parental: 30.3 Reproduction:-	NTP (1997)
Male reproductive toxicity studies				
9-week (diet) + 8-week recovery Potassium dichromate 0, 1.4, 4.6, 9.9, 40.7[(b)]	mice	Reproduction: 40.7[(c)]	-	NTP (1996a, 1997)
9-week (diet) + 8-week recovery Oral (diet) Potassium dichromate 0, 0.5, 1.6, 3.2, 12.7[(b)]	rats	Reproduction: 12.7[(c)]	-	NTP (1996a, 1997)
2-year (drinking water) sodium dichromate dihydrate M: 0, 0.38, 0.91, 2.4 and 5.9[(b)] F: 0, 0.38, 1.4, 3.1 and 8.7[(b)]	mice	Reproduction: 5.9[(c)]	-	NTP (2008)
2-year (drinking water) sodium dichromate dihydrate M: 0, 0.21, 0.77, 2.1 and 5.9[(b)] F: 0, 0.24, 0.94, 2.4 and 7.0[(b)]	rats	Reproduction: 5.9[(c)]	-	NTP (2008)

Table 17: Developmental and reproductive toxicity studies with Cr(VI) compounds (continued).

Study Doses in mg Cr(VI)/kg b.w. per day[(a)]	Species	NOAEL	LOAEL	Reference
		mg Cr(VI)/kg b.w. per day		
12-week (drinking water) potassium dichromate 0 and 32[(d)]	M rats X Untreated F	-	32	Bataineh et al. (1997)
6 days (diet) chromium trioxide Animals sacrificed 6 weeks after treatment 0, 5 and 10[(b)]	M rats	-	5	Li et al. (2001)
90-day (gavage) sodium dichromate 0, 7.9, 15.9, 23.8[(b)]	M rats	-	7.9	Chowdhury and Mitra (1995)
12-week (drinking water) potassium dichromate 0, 53, 106, 212 and 265[(d)] F sacrificed 1-week after mating	M mice X Untreated F	-	53	Elbetieha and Al-Hamood (1997)
Exposure via drinking water to potassium dichromate from GD 12 to lactation D 20 Doses: 0 and 76[(e)]	Pregnant mice	76	-	Al-Hamood et al. (1998)
35 days (diet) potassium dichromate 16, 28 and 63[(e)]	M mice	-	16	Zahid et al. (1990)
10-week (gavage) potassium dichromate 0 and 1.8[(b)]	M rabbits	-	1.8	Yousef et al. (2006)
180 days (drinking water) potassium dichromate 0, 0.8, 1.7, 3.4, and 6.8[(e)]	M monkeys	0.8	1.7	Subramanian et al. (2006)
180 days (drinking water) potassium dichromate 0, 1.7, 3.4, and 6.8[(f)]	M monkeys	-	1.7	Aruldhas et al. (2006)
180 days (drinking water) potassium dichromate 0, 1.7, 3.4, and 6.8[(f)]	M monkeys	-	1.7	Aruldhas et al. (2005)
180 days (drinking water) potassium dichromate 0, 1.7, 3.4, and 6.8[(f)]	M monkeys	-	1.7	Aruldhas et al. (2004)
Female reproductive toxicity studies				
exposure during lactation PND 1-20 (drinking water) potassium dichromate. 0 and 24 [(d)] Sacrifice F offsprings on PND 21 (weaning), PND 45 or PND 65	Rats	-	24	Banu et al. (2008)
exposure during lactation PND 1-21 (drinking water) potassium dichromate 0, 6 and 24 Sacrifice F offsprings on PND 21, 45 or 65[(d)]	rats	-	6	Samuel et al. (2011)

Table 17: Developmental and reproductive toxicity studies with Cr(VI) compounds (continued).

Study Doses in mg Cr(VI)/kg b.w. per day[(a)]	Species	NOAEL	LOAEL	Reference
		mg Cr(VI)/kg b.w. per day		
GD 12 to lactation D 20 (drinking water) potassium dichromate 0 and 76[(e)]	Pregnant mice	-	76	Al-Hamood et al. (1998)
Developmental toxicity				
Gestational exposure				
GD 6-15 (drinking water) potassium chromate 0 and 1.6 mg[(d)]	Pregnant rats	-	1.6	Elsaieed and Nada (2002)
GD 6-14 (drinking water) potassium dichromate 0, 53, 101 and 152[(e)]	Pregnant mice	Maternal toxicity: 53	Maternal toxicity: 101 Developmental toxicity: 53	Junaid et al. (1996a)
GD 14-19 (drinking water) potassium dichromate 0, 45, 90 and 135[(d)]	Pregnant mice	Maternal toxicity: 45	Maternal toxicity: 90 Developmental toxicity: 45	Junaid et al. (1995)
GD 1-19 (drinking water) potassium dichromate 0, 48, 99 and 239[(e)]	Pregnant mice	Maternal toxicity: 48	Maternal toxicity: 99 Developmental toxicity: 48	Trivedi et al. (1989)
GD 1-3 or GD 4-6 (gavage) Potassium dichromate 0, 25 mg/rat[(e)]	Mated F rats	-	Developmental toxicity: 25 mg/rat	Bataineh et al. (2007)
Dams exposed prior to mating				
3-month (drinking water) potassium dichromate Sacrifice F on GD 19 0, 45, 89 and 124[(e)]	Rats (treated F mated with untreated M)	-	45	Kanojia et al. (1998)
20-day (drinking water) potassium dichromate Sacrifice F on GD 19 0, 31, 60 and 75[(e)]	Rats (treated F mated with untreated M)	Maternal toxicity: 31	Maternal Toxicity: 60 Developmental toxicity: 31	Kanojia et al. (1996)
12-week (drinking water) potassium dichromate Sacrifice F 1 wk after mating period 0, 106 and 265[(d)]	Mice (treated F mated with untreated M)	Maternal toxicity: 106	Maternal toxicity 265 Developmental toxicity: 106	Elbetieha and Al-Hamood (1997)

Table 17: Developmental and reproductive toxicity studies with Cr(VI) compounds (continued).

Study Doses in mg Cr(VI)/kg b.w. per day[(a)]	Species	NOAEL	LOAEL	Reference
		mg Cr(VI)/kg b.w. per day		
20-day (drinking water) potassium dichromate, Sacrifice on GD 19 0, 52, 98, and 169[(e)]	Mice (treated F mated with untreated M)	Maternal toxicity: 98	Maternal toxicity 169 Developmental toxicity: 52	Junaid et al. (1996b)

b.w.: body weight; NOAEL: no-observed-adverse-effect level; LOAEL: lowest-observed-adverse-effect level; GD: gestation day; M: male; F: female; PND: post natal day.

(a): In the conversions from concentration to daily doses, the molecular weight (MW) of the anhydrous salts were used when no information on hydration number was available in the original publication.

(b): Data reported in the original publication;

(c): Reproduction means effects on reproductive organs and spermatogenesis. Toxic effects observed in the study are reported in annex H6;

(d): Conversion using the default correction factor for subacute/subchronic/chronic exposure via drinking water/feed from EFSA (2012c);

(e): Conversion using drinking water/feed consumption data and average body weight reported in the publication;

(f): Calculated applying allometric scaling using human data (70 kg b.w. and 2 L daily water consumption) and an exponent of 0.75.

7.2.2.4. Genotoxicity

The mutagenic potential of Cr(VI) has been studied extensively and recently reviewed (ATSDR, 2012). Although study results vary depending on the test system, experimental conditions and type of Cr(VI) compounds tested, results of the assay systems used provide clear evidence for the mutagenic potential of Cr(VI) both *in vitro* and *in vivo*. Here, a brief summary of the literature and details only for the most relevant studies is provided.

In vitro assays

Bacteria and yeast

Cr(VI) compounds have mostly tested positive for gene mutations in bacterial cells.

Reverse mutations were observed after exposure to Cr(VI) compounds in multiple species and strains of *Salmonella typhimurium* and *Escherichia coli* able to detect a wide spectrum of DNA lesions, including oxidative damage and DNA crosslinks, and of mutations such as base pair substitutions and frame-shift mutations (Venitt and Levy, 1974; Nishioka, 1975; Bonatti et al., 1976; Petrilli and De Flora, 1977; Nakamuro et al., 1978; Kanematsu et al., 1980; Matsui, 1980; De Flora, 1981; Gentile et al., 1981; Venier et al., 1982; Bennicelli et al., 1983; Haworth et al., 1983; Singh, 1983; De Flora et al., 1984; Dunkel et al., 1984; Arlauskas et al., 1985; Kharab and Singh, 1985; Marzin and Phi, 1985; La Velle, 1986; Llagostera et al., 1986; Brams et al., 1987; Olivier and Marzin, 1987; Bronzetti and Galli, 1989; Zeiger et al., 1992; Le Curieux et al., 1993; Seo and Lee, 1993; Watanabe et al., 1998; Ryden et al., 2000; Yamamoto et al., 2002; Tagliari et al., 2004; NTP, 2007).

Positive results were also found for forward mutations and mitotic gene conversion in yeast (*Saccharomyces cerevisiae*) (Sora et al., 1986; Vashishat and Vasudeva, 1987).

Mammalian cells

Cr(VI) compounds are also mutagenic in mammalian cell lines. Potassium dichromate was reported to significantly increase mutation frequency at the HPRT locus in Chinese hamster cells AT3-2 and V79 (Paschin et al., 1983), and calcium chromate at the TK locus in mouse lymphoma cells L5178Y (McGregor et al., 1987). Clastogenic activity (micronuclei, chromosomal aberrations and sister chromatid exchanges) of Cr(VI) compounds (i.e. calcium chromate, chromic acid, potassium chromate, potassium dichromate, sodium chromate and sodium dichromate) was reported by several groups in cultured CHO cells (Levis and Majone, 1979; Bianchi et al., 1980; Koshi and Iwasaki, 1983; Seoane and Dulout, 1999), mouse mammary FM3A carcinoma cells (Umeda and Nishimura, 1979), human fibroblasts (MacRae et al., 1979; Seoane and Dulout, 2001; Wise et al., 2002, 2004; Holmes et al., 2006), human epithelial cells (Wise et al., 2006) and human lymphocytes (Nakamuro et al., 1978; Sarto et al., 1980; Gomez-Arroyo et al., 1981; Imreh and Radulescu, 1982; Stella et al., 1982). In general, metabolic activation is not required to detect the mutagenic/clastogenic effects observed in mammalian cells in culture indicating that Cr(VI) is a direct-acting mutagen.

Repair processes have been shown to modulate Cr(VI)-induced mutagenicity. Reynolds et al. (2004) showed that human cells efficiently repair chromium−DNA adducts by nucleotide excision repair (NER) whereas NER-deficient XP-A, XP-C and XP-F cells were severely compromised in their ability to repair chromium-DNA lesions. Intracellular replication of Cr-modified plasmids demonstrated increased mutagenicity in cells with inactive NER (see also Mode of action). Brooks et al. (2008) showed that CHO cells deficient either in NER or base excision repair, grown under the standard ascorbate-deficient conditions, presented a lower HPRT mutation frequency than wild-type cells (Brooks et al., 2008). The CONTAM Panel noted that the results of the study may be opposite to what would be expected, and that the authors postulated that, in the absence of excision repair processes, DNA damage is channeled into an error-free system of DNA repair or damage tolerance.

Morphological cell transformation has also been reported in BALB/3T3, Syrian hamster embryo, and the Rauscher leukemia virus-infected Fischer rat embryo cell lines and rat liver epithelial cells (DiPaolo and Casto, 1979; Dunkel et al., 1981; Briggs and Briggs, 1988).

***In vivo* assays**

Cr(VI) compounds have tested positive for mutations in *Drosophila melanogaster* in several studies (Graf and Wurgler, 1996; Amrani et al., 1999; Spano et al., 2001; Kaya et al., 2002) where larvae were fed the test substance at the lowest effective concentration of 0.1 mM (Amrani et al., 1999).

Positive genotoxicity was observed for Cr(VI) in numerous studies in rats and mice following administration of Cr(VI) compounds via the parenteral, intratracheal or inhalation route (Table H8, Appendix H). Contrasting data have been reported when Cr(VI) was admistered orally (summarised in Table 18 and described in details in Table H7, Appendix H).

The three drinking water exposure positive studies include induction of mutations in a DNA deletion assay using C57BL/6Jpun/pun mice (Kirpnick-Sobol et al., 2006) and induction of chromosomal damage in two mouse strains (NTP, 2007). In the study by Kirpnick-Sobol et al. (2006) chromosome deletions were detected in the offspring of exposed pregnant females (lowest effective dose: 62.5 mg Cr(VI)/L). Statistically significant increases in micronuclei formation with a dose-response were observed in peripheral erythrocytes of am3-C57BL/6 mice (lowest effective dose: 43.6 mg Cr(VI)/L), equivocal results (nearly significant positive trend) in the B6C3F1 strain and no effects in BALB/c mice (NTP, 2007).

Other studies have reported negative results in bone marrow or peripheral blood cells following oral exposure to Cr(VI) compounds (Mirsalis et al., 1996; Shindo et al., 1989; De Flora et al., 2006).

Two studies compared the effects of the parenteral versus oral admistration route within the same experimental setting (Shindo et al., 1989; De Flora et al., 2006). In both cases genotoxicity was detected when the test item was administered i.p. and negative results were observed when administered orally in the drinking water or by gavage. In particular, in the study by Shindo et al (1989) potassium chromate administered to MS/Ae and CD-1 mice by i.p. injection induced the

formation of micronuclei dose-dependently in both strains (lowest effective dose: 17.7 mg Cr(VI)/kg b.w.). In contrast, following p.o. administration, potassium dichromate chemical failed to induce micronuclei (highest dose tested: 113.1 mg Cr(VI)/kg). In the study by De Flora et al. (2006) sodium dichromate dihydrate and potassium dichromate were administered to BDF1 and Swiss mice of both genders either with the drinking water or in a single intragastric dose. No increase of the micronucleus frequency was observed in either bone marrow or peripheral blood erythrocytes following oral admistration (highest dose tested: 500 mg Cr(VI)/L for up to 210 consecutive days) whereas the same compounds induced a clastogenic damage following i.p. injection (lowest effective dose: 50 mg Cr(VI)/kg). In the same study pregnant mice were also treated up to a concentration of 10 mg Cr(VI)/L drinking water. No genotoxic effects were observed either in bone marrow of pregnant mice or in liver and peripheral blood of their fetuses.

The observation of mutagenicity by Cr(VI) is of relevance in light of its tumorigenic effects (see Section 7.2.2.5). Cr(VI) was reported to be mutagenic *in vivo* following oral exposure (Kirpnick-Sobol et al., 2006) however, in this study a non standard assay (i.e. p(un) reversion assay in mice) was used. Cr (VI) was mutagenic in the transgenic lacI mice following intratracheal instillation of potassium dichromate (Chen et al., 2000). Interestingly, in this experimental system mutagenicity was inhibited by tissue GSH depletion by buthionine sulfoximine (BSO) before Cr(VI) treatment suggesting a role for GSH in the generation of mutagenic lesions. GSH-Cr-DNA adducts are mutagenic in mammalian cells *in vitro* (see mechanism of action) and these adducts might also play an important role in mutagenic responses *in vivo.*

DNA damage as measured by the Comet assay has been observed in mice and rats in several tissues including stomach, colon, liver, kidney, bladder, brain and peripheral leukocytes (Devi et al., 2001; Sekihashi et al., 2001; Wang et al., 2006). In particular, a dose-dependent increase in DNA damage was observed in lymphocytes of mice admistered by gavage with potassium dichromate (Devi et al., 2001; Wang et al., 2006). Sekihashi et al. (2001) found DNA damage, in mouse stomach, colon, liver, kidney, bladder, lung and brain following administration by gavage of potassium dichromate.

Table 18: Summary of *in vivo* genotoxicity of Chromium (VI) – oral route.

Test system/ Endpoint	Compound	Response [(a)] Dose[(b)]: mg Cr(VI)/kg b.w. per day	Reference
Female C57BL/ 6J*pun*/*pun* mouse DNA deletions in 20-day-old offspring	Potassium dichromate	Positive 12.5[(c)]	Kirpnick-Sobol et al. (2006)
Pregnant Swiss albino mouse Micronuclei in bone marrow cells from dams and liver and peripheral blood cells from fetuses	Potassium dichromate	Negative	De Flora et al. (2006)
	Sodium dichromate dihydrate	1.8[(d)]	
BDF1 male mouse Micronuclei in bone marrow and peripheral blood cells	Potassium dichromate	Negative 6[(c)]	
BDF1 mouse (male and female)	Sodium dichromate dihydrate	Negative F: 140[(c)] M: 165[(c)]	
Swiss-Webster mouse Micronuclei in bone marrow cells	Potassium dichromate	Negative 3.6[(d)]	Mirsalis et al. (1996)
B6C3F1 BALB/c *am3*-C57BL/6 male mouse Micronuclei in peripheral red blood cells	Sodium dichromate dihydrate	Equivocal 8.7[(c)] (B6C3F1) Negative 8.7 (BALB/c) Positive 5.2[(c)] (*am3*-C57BL/6)	NTP (2007)
B6C3F1 mouse Micronuclei in peripheral red blood cells		Negative 27.9[(c)]	NTP (2007)
BDF1 male mouse Micronuclei in bone marrow cells	Potassium dichromate	Negative 17.7[(c)]	De Flora et al. (2006)
Male MS/Ae and CD-1 mouse Micronuclei in bone marrow cells	Potassium chromate	Negative 85.7[(c)] Negative up to acutely toxic doses	Shindo et al. (1989)
Swiss albino mouse DNA damage Comet assay in leukocytes	Potassium dichromate	Positive 0.21[(c)]	Devi et al. (2001)
Swiss albino mouse DNA damage Comet assay in peripheral lymphocytes	Potassium dichromate	Positive 8.8[(c)]	Wang et al. (2006)
ddY mouse DNA damage Comet assay in cells from stomach, colon, liver, kidney, bladder, lung, brain and bone marrow	Potassium dichromate	Positive 85.7[(c)]	Sekihashi et al. (2001)

(a): The lowest effective dose is indicated for positive results and the highest dose tested for negative results.
(b): In the conversions from concentration to daily doses, the molecular weight (MW) of the anhydrous salts were used when no information on hydration number was available in the original publication.
(c): Doses calculated using data from the original publication.
(d): Doses calculated using the default correction factor for subacute/subchronic/chronic exposure via drinking water/feed from EFSA SC (2012).

Genotoxicity studies in humans

Several studies have evaluated genotoxicity in humans occupationally exposed to Cr(VI). DNA damage (i.e. DNA strand breaks, DNA protein cross-links, oxidative DNA damage) as well as chromosomal damage (i.e. chromosomal aberrations, micronuclei, sister chromatid exchanges) have been detected in circulating lymphocytes and/or buccal and nasal mucosal cells of occupationally exposed workers (mostly chromium plating) (Sarto et al., 1982, 1990; Stella et al., 1982; Deng et al., 1988; Nagaya, 1986; Nagaya et al., 1991; Gao et al., 1994; Kuykendall et al., 1996; Vaglenov et al., 1999; Wu et al., 2000, 2001; Benova et al., 2002; Gambelunghe et al. 2003). Mixed results have been published with some evidence of Cr(VI)-induced genotoxicity in occupationally exposed workers. The uncertainty of the Cr(VI) exposure levels, the small numbers of workers evaluated, and/or potential co-exposure to other compounds with mutagenic activity affect the relevance of these findings.

Conclusions

There is abundant evidence for genotoxicity of Cr(VI) compounds in bacterial and mammalian cells genotoxicity assays. Positive genotoxicity was also observed *in vivo* but with contrasting data depending on the route of administration (parenteral versus oral). These mixed results indicate that the determinant of the genotoxic effects of Cr(VI) *in vivo* is the reductive capacity of the gastrointestinal tract that may significantly limit or fully prevent Cr(VI) uptake in the blood and/or distribution to the target tissues when administered orally. Moreover, it seems that once Cr(VI) uptake occurs the intracellular reduction to Cr(III) that generates intermediate Cr valences (with generation of oxygen radicals) as well as Cr(III) access to DNA (with generation of DNA adducts) are critical factors in the amount of damage induced (see also mode of action Section 7.4).

7.2.2.5. Carcinogenicity

Cr(VI) carcinogenicity has been addressed by the NTP in their study on sodium dichromate dihydrate (NTP, 2007, 2008; Stout et al., 2009), chosen because it is the primary base material for the production of Cr compounds and it is the most water-soluble chromate salt. The NTP sodium dichromate dihydrate carcinogenicity study included a 3-month toxicity study to select exposure concentrations for the 2-year studies (see Section 7.2.2.2).

In the 2-year study groups of 50 male and female F344/N rats and B6C3F1 mice were exposed to sodium dichromate dihydrate in drinking water at concentrations spanning from 14.3 to 516 mg/L (male and female rats and female mice) or from 14.3 to 257.4 mg/L (male mice) of sodium dichromate dihydrate. The average daily ingested doses corresponded to maximal levels of 5.9, 7.0 and 8.7 mg Cr(VI)/kg for male mice and rats, female rats and female mice, respectively.

Statistically significantly increased incidences of neoplasms of the squamous epithelium that lines the oral cavity (oral mucosa and tongue) were reported at 516 mg/L sodium dichromate dihydrate in male and female rats (poly-3 test). Specifically, these increases were observed for squamous cell carcinoma in the oral mucosa and for squamous cell papilloma or carcinoma (combined) of the oral mucosa or tongue (Table 19).

Table 19: Squamous cell neoplastic lesions in the oral cavity of male and female F344/N rats in the 2-year study of sodium dichromate dihydrate (modified from Witt et al., 2013).

	Male					**Female**				
Dose (mg/L)	0	14.3	57.3	172	516	0	14.3	57.3	172	516
Dose as mg Cr(VI)/kg b.w. per day	0	0.21	0.77	2.1	5.9	0.	0.24	0.94	2.4	7.0
N	50	50	49	50	49	50	50	50	50	50
Oral mucosa [(a,b)]										
Papilloma	0	0	0	0	1	0	0	0	0	0
Carcinoma	0***[(c)]	0	0	0	6*	0***	0	0	2+	11***
Tongue [(a,b)]										
Papilloma	0	0	0	0	1	1	1	0	0	0
Carcinoma	0	1	0	0	0	0	0	0	1	0
Oral mucosa or tongue[(a,b)]										
Papilloma or carcinoma (combined)	0***[(c)]	1	0	0	7**	1***	1	0	2++	11**

N: number of animals.

(a): The poly-3 test was applied to compare the incidences of each of the four dose groups with the control. Statistical significance was reported by Witt et al.(2013) as *p < = 0.05, **p < = 0.01, ***p < = 0.001 indicated at the respective dose group;

(b): Incidences exceeding the historical control range was indicated at the respective dose group by (+) for all routes and drinking water or by (++) for drinking water only (historical control data not collected for tongue because it is not a protocol required tissue);

(c): The poly-3 test was applied to test for a trend in the incidences considering the control and the four dose groups. Statistical significance was reported by Witt et al.(2013) as *p < = 0.05, **p < = 0.01, ***p < = 0.001.

Neoplasms in epithelial tissues of the small intestine were observed in mice. In both males and females, there was an exposure concentration–response relationship for adenomas as well as carcinomas in duodenum and jejunum both for males and females, with higher incidences in the duodenum (Table 20). When adenomas and carcinomas were combined at all sites of the small intestine (duodenum, jejunum, or ileum; Table 20) a very clear dose-response relationship was observed (see last line of Table 20) The increases were statistically significant (poly-3 test) at the two highest exposure concentrations in each sex for the adenoma and carcinoma combined ($p < 0.001$), and at the highest concentration for the carcinoma in the duodenum, jejunum or ileum combined ($p < 0.05$), both for males and females. In addition, the incidence in 57.3 mg/L females exceeded the historical control ranges for drinking water studies and for all routes of administration and this increased incidence was also considered to be related to treatment.

Table 20: Epithelial neoplastic lesions in the small intestine of male and female B6C3F1 mice in the 2-year study of sodium dichromate dihydrate (modified from Witt et al., 2013).

	Male					Female				
Dose (mg/L)	0	14.3	57.3	172	516	0	14.3	57.3	172	516
Dose as mg Cr(VI)/kg b.w. per day	0	0.38	0.91	2.4	5.9	0	0.38	1.4	3.1	8.7
N	50	50	50	50	50	50	50	50	50	50
Duodenum[a,b]										
Adenoma	1***[c]	0	1	5	15***	0***	0	2	13***	12***
Carcinoma	0*[c]	0	0	2++	3+	0	0	0	1++	6*
Jejunum[a,b]										
Adenoma	0**[c]	0	0	0	3+	0**	1+	0	2+	5*
Carcinoma	0	2	0	1	2	1	0	2+	2+	1
Duodenum, jejunum or ileum (combined) [a,b]										
Adenoma	1***[c]	1	1	5+	17***	0***	1	2+	15***	16***
Carcinoma	0*[c]	2	1	3++	5*	1***	0	2+	3+	7*
Adenoma or carcinoma (combined)	1***[c]	3	2	7*	20***	1***	1	4+	17***	22***

(a): The poly-3 test was applied to compare the incidences of each of the four dose groups with the control. Statistical significance was reported by Witt et al.(2013) as *p < = 0.05, **p < = 0.01, ***p < = 0.001 indicated at the respective dose group;

(b): Incidences exceeding the historical control range was indicated at the respective dose group by (+) for all routes and drinking water or by (++) for drinking water only (historical control data not collected for tongue because it is not a protocol required tissue);

(c): The poly-3 test was applied to test for a trend in the incidences considering the control and the four dose groups. Statistical significance was reported by Witt et al.(2013) as *p < = 0.05, **p < = 0.01, ***p < = 0.001.

7.3. Observations in humans

Observations on the toxicity of total chromium, Cr(III) and Cr(VI) in humans are available in the published literature only from retrospective observational studies occasionally with partial prospective follow-up and from studies which compare available information on the occurrence of chromium species in polluted enviroments with mortality and incidence figures available from surveys of populations living in polluted and non polluted areas, often in the vicinity of sites of known contamination by chromium. Well designed prospective cohort studies were not identified.

Most study reports on humans stem from occupational surveys. Although those exposures mostly occur by inhalation the CONTAM Panel noted that breathing in chromium could expose tissues in the gastrointestinal (GI) tract due to oral respiration and redistribution of inhaled particulates from the respiratory tract to the GI tract and as such inhalation contributes to oral exposure. However, the amount of chromium ingested can hardly be quantified even for specific exposure scenarios. For all the studies indentified individual exposure information was at best estimated but not available from individual monitoring. As a consequence, human studies do rarely specify the route of exposure (e.g. oral, dermal or inhalation) and the level of exposure is either based on assumptions or characterized using environmental chromium measurements only.

Toxic effects considered in human studies are, in general, overall mortality and disease specific mortality, including cancer, but also the incidence of specific diseases or specific health effects (e.g .gastric tumors) have been reported. A comprehensive overview on the health effects of Cr(III) and Cr(VI) for the three major routes of exposure (inhalation, oral and dermal) was provided by ATSDR (2012) recently. In general, Cr(VI) was found much more toxic in humans than Cr(III).

Much less information is available on the health effects of Cr(III) species than of Cr(VI). Moreover, the interpretation of the study results on Cr(III) is complicated by concomitant exposures to Cr(VI) for which toxicity has been well established. This Section will provide a brief overview of the effects of

chromium in humans which, in some studies, could be related specifically to Cr(III) or Cr(VI) exposure.

Increased chromium (unspecified) concentrations in biological fluids may occur as a consequence of malfunctioning metal-on-metal prostheses of the hip and the knee. Early reports have been reviewed by Sunderman et al. (1989), but the problem became a public health issue only over the last ten years, after the publication of several case reports of severe poisoning in patients with hip implants based on Cobalt-Chromium (sometimes Chromium-Molybdenum) alloys (Delaunay et al., 2010; Sampson and Hart, 2012). After lumbar disc arthroplasty median serum Cr levels before operation or 3, 6, 12, 24, and 36-months post-operation were found as 0.06, 0.49, 0.65, 0.43, 0.52, and 0.50 ng/mL, respectively (Gornet et al., 2013).

7.3.1. Observations in humans related to Cr(III)

Only very limited information from few case studies was not suitable to assess human toxicity after oral exposure to Cr(III) compounds.

Few occupational exposure studies and case reports indicate that respiratory effects can occur from exposure to Cr(III). Workers exposed to high concentrations of chromium trioxide in a chrome plating plant experienced nausea and vomiting and symptoms of dyspnea, dizziness, headache, and weakness (Lieberman, 1941). Anemia and liver damage was reported following swallowing of plating fluid containing Cr trioxide (Fristedt et al., 1965). Musculoskeletal and renal effects were observed in two cases of ingestion of Cr(III) picolinate equivalent to 2.2 µg of Cr(III)/kg b.w. over a 48-hour period (Martin and Fuller, 1998) and 1.1 µg Cr(III)/kg b.w. per day for 6 weeks (Wasser et al., 1997).

The interpretation of studies on Cr(III) in humans is complicated by the proposed beneficial effects of dietary supplements containing high levels of Cr(III), e.g. up to 0.1 - 0.2 mg Cr(III) picolinate/ kg b.w. per day (EFSA ANS Panel, 2010a).

Cr(III) can induce allergic sensitisation in humans after dermal exposure as observed in a few small studies (Fregert and Rorsman, 1964, Estlander et al., 2000, Iyer et al., 2002, Chou et al., 2008). The ATSDR, however, noted that it is unclear if individuals were sensitized to both Cr(VI) and Cr(III) or if cross-sensitivity occurs between Cr(VI) and Cr(III) since positive responses were also observed after challenge with Cr(VI) compounds (ATSDR, 2012). Furthermore, co-exposure to other more significant sources of daily contact, including nickel and cobalt,time of exposure and other factors such as humidity and pH may play a role when assessing the risk of induction and elicitation of an allergic response (Basketter et al., 1993).

Khan et al. (2012) conducted a cross-sectional study on 100 Cr(III) -exposed persons living near tanning industry at Jajmau, Kanpur (India) and 100 unexposed persons living away from tanning industry (reported an increased prevalence of dermal effects, pulmonary tuberculosis, diabetes, asthma and bronchitis and gastrointestinal effects) in the exposed group. Although the authors adjusted for confounding, some confounding cannot be excluded since there were statistically significant differences ($p<0.05$) between the exposed group and the unexposed group with respect to age, marital status, duration of living in exposed area, alcohol habit, smoking habit and tobacco chewing habit.

7.3.2. Observations in humans related to Cr(VI)

Cr(VI) compounds were classified by IARC (IARC, 2012) as carcinogenic to humans (Group 1) with respect to the cancer of the lung and also cancer of the nose and nasal sinuses based on evidence from occupational studies. Oral studies in humans available until 1996 were summarized by U.S. EPA (1998b) by observing that Cr(VI) is considerably more toxic than Cr(III). This included a cross-sectional study of Zhang and Li (1987) where oral ulcer, diarrhea, abdominal pain, indigestion, vomiting, leukocytosis, and immature neutrophils were reported for an estimated exposure of 0.57 mg Cr(IV)/kg b.w. per day (see Section 7.3.2.3 below). Minimal risk levels (MRLs) were derived for acute, intermediate (i.e. essentially subchronic) and chronic studies for oral exposure, but not for cancer by ATSDR (2012).

7.3.2.1. Acute oral toxicity

Most studies identified on acute toxicity are case reports from intentional and accidental poisoning. Those case reports on the consequences of high doses of Cr(VI) allow the identification of the type and nature of possible effects of Cr(VI) in humans when describing a range of effects from mild to serious and life-threatening and lethal effects (see Appendix I1) Clinical effects included haematological, hepatic and renal injury as well as respiratory and gastrointestinal lesions.

Accidental ingestion has been reported for Cr(VI) compounds including chromic acid (Fristedt et al., 1965; Saryan and Reedy, 1988; Loubières et al., 1999), potassium chromate (Goldman and Karotkin, 1935; Partington, 1950; Kaufman et al., 1970; Sharma et al., 1978; Iserson et al., 1983; Clochesy, 1984; Hanston et al., 2005), and ammonium dichromate (Reichelderfer, 1968; Hasan, 2007) resulting in a large variety of clinical presentations such as abdominal pain, nausea, and vomiting; hematemesis and bloody diarrhea; caustic burns of the mouth, pharynx, esophagus, stomach, and duodenum and GI hemorrhage; anemia, decreased blood Hb, abnormal erythrocytes, and intravascular hemolysis; hepatotoxicity (hepatomegaly, jaundice, elevated blood bilirubin, and liver enzymes activities); renal failure (oliguria and anuria); cyanosis; and metabolic acidosis, hypotension, and shock (see also ATSDR, 2012). Fatty degeneration in the liver and tubular degeneration and necrosis in the kidney were observed in biospies (Reichelderfer, 1968; Kaufman et al., 1970; Sharma et al., 1978; Loubières et al., 1999).

Doses of Cr(VI) ranging 4 to 360 mg/kg b.w. were reported to be lethal (Kaufman et al., 1970; Iserson et al., 1983; Clochesy, 1984; Saryan and Reedy, 1988; Loubières et al., 1999).

Paustenbach et al. (1996) investigated the kinetics of Cr(VI) in a male volunteer who ingested 2 L per day of water containing 2 mg/L for 17 consecutive days. Kerger et al. (1996) studied four adult male volunteers ingesting a single dose of 5 mg Cr (in 0.5 liters deionized water) in three choromium mixtures: (1) Cr(III) chloride ($CrCl_3$), (2) potassium dichromate reduced with orange juice (Cr(III)-OJ); and (3) potassium dichromate [Cr(VI)]. Kuykendall et al. (1996) report on four adult male volunteers ingested a bolus dose of 5000 micro chromium in a 0.51 volume of water (10 ppm), Corbett et al. (1997) examined the systemic uptake of chromium in four human volunteers immersed below the shoulders in water at 91 +/- 2.5 degrees F. following three hours of contact with water containing Cr(VI) at a concentration of 22 mg/L. Finley et al., 1997 studied adult male volunteers ingesting a liter (in three volumes of 333 ml, at approximate 6-hr intervals) of deionized water containing Cr(VI) at concentrations ranging from 0.1 to 10.0 mg/L. Kerger et al. (1997) investigated in adult male volunteers the oral exposure to 5 and 10 mg Cr(VI)/L in drinking water administered as a single bolus dose (0.5 L swallowed in 2 min) or for 3 days at a dosage of 1 L per day (3 doses of 0.33 L each day, at 6-h intervals). None of these reports presented indications of clinical effects.

In conclusion, at high doses Cr(VI) exerts acute health effects in the respiratory, haematological, hepatic and renal system and in the gastrointestinal tract where acute effects include abdominal pain, vomiting, ulceration, hemorrhage, necrosis, and bloody diarrhea.

7.3.2.2. Subchronic and chronic toxicity excluding cancer

Haematological effects have been inconsistently reported in the literature such that haemotoxicity after oral exposure cannot be assessed. It was suggested that those effects originate from primary exposure to Cr(VI) and its accumulation in erythrocytes and subsequent reduction to Cr(III) via the reactive intermediates Cr(V) and Cr(IV) and their binding to hemoglobin and other ligands. The Cr-haemoglobin complex is relatively stable and remains sequestered within the cell over the life-span of the erythrocyte (Lewalter et al., 1985). Haematological effects observed in some cases of accidental or intentional ingestion of high doses Cr are detailed in Appendix I2. A cross-sectional study from an alloy plant in the People's Republic of China (summarised below) reports associations between Cr(VI) and the occurrence of leukocytosis and immature neutrophils.

Gastrointestinal effects observed in occupational studies may occur due to exposure via mouth breathing or other means of ingestion of Cr (e.g., mucociliary clearance of inhaled Cr particles to the gastrointestinal tract and/or ingestion secondary to hand-to-mouth activity). In particular, epigastric

pain, irritation, and ulceration have been reported after occupational exposures through inhalation and ingestion of Cr, see ATDSR (2012). Gastrointestinal effects have been reported in some cases of accidental or intentional ingestion of high doses Cr, see Appendix I3.

No respiratory, cardiovascular, hepatic, renal, musculoskeletal effects, except those observed in some cases of accidental or intentional ingestion of high doses (see Appendices I4 - I6), were identified.

7.3.2.3. Cancer

Studies of associations between environmental exposures to Cr and cancer outcomes in humans are limited to several retrospective observational studies where humans were environmentally exposed to total Cr and/or Cr(VI) (Zhang and Li, 1987, 1997; Bednar and Kies, 1991; Bick et al.,1996; Eizaguirre-Garcia et al., 1999, 2000; Fryzek et al., 2001; Beaumont et al., 2008; Kerger et al., 2009; Linos et al., 2011).

These studies investigated possible associations between incidence of diseases (including cancer) and mortality within a geographic area and exposure to Cr. Actual exposures of individuals were not determined and therefore, exposure misclassification may bias the reported results. Eizaguirre-Garcia et al. (1999, 2000) examined the risk of leukemia and birth defects in people residing stratified by distance (up to 9–10 km) from the site of a former Cr processing facility in Glasgow (UK) where soil was contaminated with chromium at a radius of approximately 2–3 km. The study examined a total number of 1205 leukemia cases in a population of 873 643 inhabitants. There was no statistically significant association between the occurrence of leukemias nor of birth defects when considering the distance to the source of contamination as surrogate of the extent of exposure. In contrast, persons living 4-9 km from the plant were at higher risk than those living directly near the plant.

A study of an area of Greece (the Oinofita region) investigated the effects of elevated Cr(VI) levels in the public drinking water supply ranging from 8.3 to 51 µg/L (Linos et al., 2011). Using the greater prefecture of Voiotia as the standard population, the authors found significantly higher Standardised Mortality Ratio (SMR) for primary liver cancer (SMR = 11.0; 95 % CI: 4.0-24.0) in total and for males and females separately (SMR = 8.1; 95 % CI: 2.2-20.8 and SMR = 39.5; 95 % CI: 4.8-142.8), lung cancer (trachea and bronchus) (SMR = 1.4; 95 % CI: 1.0-2.0) in total and for males (SMR = 1.7; 95 % CI: 0.5-3.9), and cancer of the kidney and other genitourinary organs for women (SMR = 3.67; 95 % CI: 1.2-8.6),

A study evaluated the cancer mortality rate in Kings County and San Bernardino County, California, where Cr(VI) compounds had been used as anti-corrosion additives in cooling tower water at natural gas compressor plants from 1950 to approximately 1980 and waste material was released to surface ponds and groundwater and may have also contaminated soil, crops, and surface water (Fryzek et al., 2001). Thus, exposure may have occurred by several routes (i.e., inhalation, ingestion, and dermal contact). Age-adjusted cancer mortality rate ratios (comparing areas near the plants with those at a distance using ZIP code) were equal to 1.03 (95 % CI: 0.90-1.17) for lung cancer, 0.93 (95 % CI: 0.87-1.00) for all cancer deaths, and 0.98 (95 % CI: 0.95-1.02) for all deaths.

A mortality study in 453 communities in Nebraska counties associated levels of Cr (and other chemicals) in drinking water (mean total Cr = 0.002 with range < 0.001-0.01 mg Cr/L) between the period of 1986-1987 (Bednar and Kies, 1991). There was no statistically significant correlation between exposure at county level and health outcome such as cancer, cerebrovascular disease, heart disease, pneumonia, and chronic lung disease.

A retrospective mortality study of an area near a ferrochromium production plant (starting smelting chromium in 1965) in the Liaoning Province, China, compared mortality in general and cancer mortality (lung and stomach) in locations that had relatively high or low chromium concentrations in well water (Zhang and Li, 1987), see also ADTSR (2012). The population was followed from 1970 to 1978. The main sources of chromium in well water were from discharges from the plant to surface water and groundwater. Chromium levels in well water from samples collected in the contaminated areas in 1965 (by this time, full-scale production was occurring) ranged from 0.6 to 20 mg/L with 15 % of wells having concentrations > 2 mg/L. A more detailed mortality analysis was published in

1997 but retracted in 2006 by the editors because 'financial and intellectual input to the paper by outside parties was not disclosed' (Brandt-Rauf, 2006). Thereafter, Beaumont et al. (2008) and Kerger et al. (2009) published two independent re-analyses of these data. Presence of Cr(VI) (in 75 of 265 wells) was confirmed in both studies but these authors disagreed as to what exposure in later years can be assessed in the drinking water in five villages along a path of the groundwater contamination from the alloy plant from 1965-1979. All cancer mortality and stomach and lung cancer mortality rates (crude and age adjusted) were calculated for the five areas/villages in the contamination zone per 100 000 person years and compared with the rates of four non-contaminated areas which included the industrial town surrounding the ferrochromium alloy plant. The association between Cr exposure and cancer mortality, based on the five villages in the contamination zone and the various comparison groups was quantified using risk ratios (using a Poisson distribution for calculation of 95 % confidence intervals). Beaumont et al. (2008) found a statistically significant ratio of 1.82 (95 % CI: 1.11-2.91) or 1.69 (95 % CI: 1.12-2.44) for stomach cancer when comparing to controls either four or only three areas (excluding the town TangHeZi), respectively. However, Kerger et al. (2009) could not confirm such an increase and calculated a non significant risk ratio of 1.22 (CI: 0.74-2.01) when excluding the town. For other than stomach and lung cancer none of the two investigations reported statistically significant risk ratios; also not for all cancer combined. For lung cancer, Beaumont et al. (2009) obtained a statistically significant risk ratio of 1.78 (CI: 1.03-2.87) when comparing the contaminated areas with the control areas but only when excluding the town. For a discussion of the limitations of the Zhang and Li (1987) study see also Smith and Steinmaus (2009).

In summary, the results of few observational studies on the effects of Cr after oral exposure are inconclusive and do not support a possible association between cancer mortality and exposures to Cr.

A meta-analysis of 49 epidemiological studies published since 1950 by Cole and Rodu (2005), found statistically significant SMRs for the association between exposure to Cr(VI) (mostly in occupational environment) and cancer mortality (all cancer and 8 organ specific cancer types such as lung, stomach, prostate gland, kidney, central nervous system (CNS), leukemia, Hodgkin, and other lymphatohematopoietic). Statistically significant SMRs were identified for: all cancer = 1.1 (95 % CI: 1.1-1.2); lung 1.4 (95 % CI: 1.4-1.5) (higher for smokers than non-smokers); stomach: 1.1 (95 % CI: 1.0-1.2), and prostate: 1.1 (95 % CI: 1.0-1.3), when performing multiple statistical analyses. Except for lung cancer, the authors identified confounding and heterogenity among the studies which weakened the observed association and concluded that chromium is only weakly carcinogenic for the lung and not at all for other organs.

More recently, Gatto et al. (2010) performed a meta-analysis of 32 studies based on a systematic literature review using pubmed referenced studies from 1950-2009 motivated by the findings of the NTP in animals and the public concerns on cancer risk of Cr (including the controversial discussion of the study in Liaoning Province, China). The study aimed to examine the question of whether cancers observed in rodents are relevant to humans, and whether epidemiologic findings for GI cancers among Cr(VI)-exposed workers can contribute to a weight of evidence analysis for cancer risk assessment. The study was undertaken under the premise that 'although occupational exposures mostly occur by inhalation, breathing in Cr(VI) could expose tissues in the GI tract due to oral respiration and redistribution of inhaled particulates from the respiratory tract to the GI tract'. Therefore, six types of GI tract tumors (oral cavity, esophagus, stomach, colon, rectum, and small intestine) were examined in detail but no statistically significant association between occupational exposure to Cr(VI) and any of those cancers were found and the authors concluded that this work indicates that Cr(VI) workers are not at greater risk of GI cancers than the general population.

In conclusion, the data from the limited number of human studies do not show convincing evidence of an association between oral exposure to total Cr or Cr(VI) and adverse health effects including cancer. The data cannot be used for a dose-response analysis since the data on exposure are too limited or inadequate.

7.3.2.4. Allergenic response

Oral doses of potassium dichromate exacerbated the dermatitis of sensitized individuals.

Worsening of dermatitis was observed in a randomized double-blind cross-over study in 11 of 31 Cr-sensitive individuals after ingestion of 0.036 mg Cr(VI)/kg b.w. as potassium dichromate (Kaaber and Veien, 1977).

Goitre et al. (1982) carried out an oral tolerance test using 7 mg $K_2Cr_2O_7$ equivalent to 2.5 mg Cr in an 52 year old worker with a 20 year history of chromuium contact dermatitis with mild potassium dichromate sensitivity. At 2.5 mg Cr an increased local itching after 2 days was observed. Applying 5 mg Cr led to appearance of dysdrotic lesions on the hands 12 h after intake, microbial invasion with slight lymphangitis, axillary lymphadenitis and fever.

Insufficient data are available to assess the allergenic potential of Cr(VI) by oral exposure.

7.3.2.5. Developmental and reproductive toxicity

The Reproductive and Cancer Assessment Branch of the Office of Environmental Health Hazard Assessment of the CA EPA evaluated in 2009 Cr(VI) for developmental and reproductive toxicity including human data.

Two studies on developmental toxicity were identified by CA EPA: The matched case-control study of Aschengrau et al. (1993) that associated late adverse pregnancy outcomes (congenital abnormlity, stillbirth, neonatal death) in the period 1977-1980) with drinking water quality in Boston (MA) in USA and the study of Eizaguirre-Garcia et al. (2000) on birth defects (congenital anomalies) in a population near Glasgow (UK) which has been reported in Section 7.3.2.3, in particular, for the investigation of leukemia risks. Both studies geo-linked exposure to Cr including Cr in drinking water to effects and were unable to identify statistically significant associations between estimated exposure and developmental effects although the odds ratio for all stillbirth in the first study was elevated (adjusted OR = 1.2). CA EPA noted several limitations of both studies regarding the definition of the exposure, time-delay between conception and exposure determination, co-exposure, selection of the endpoint.

For female reproductive toxicity with direct exposure to Cr(VI) (i.e. not mediated via male exposure) only studies from Russia (Shmitova, 1978, 1980) were available which had been assessed by ATSDR (2012). The cited rates of birth complications were larger than 70 % in exposed women reflecting possibly both exposure and working conditions when that of controls were larger than 40 %. Because the publication were in Russian and the ATSDR judgement of poor study quality and reporting no conclusions were made.

Male reproductive toxicity studies on Cr(VI) has been studied extensively for welding occupations in stainless steel production regarding semen quality, infertility, fecundability and male-mediated spontaneous abortion, in particular, in Danish populations but also in India. Since the studies were based on exposure measurements on ambient air of the occupational site or on urine or blood (whole blood, erythrocytes) concentrations of workers the CONTAM Panel could not use their results to assess developmental and reproductive toxicity of Cr(VI) in food and water.

7.3.3. Other observations in humans

The Chinese Public Health Epidemiological Study investigated the association between oral cancer and Cr concentrations in blood and in farm soil in 79 patients from Changhua County in Taiwan recruited from 2008 to 2009 in one single hospital in Changhua (Chiang et al., 2010). Using n = 641 controls identified as non-cancer residents log(Cr) blood levels were regressed, using piecewise linear and rank regression on log(Cr) farm soil concentrations adjusted for covariates (using a propensity type balancing score) and a statistically significant association ($p < 0.02$) was found. A case-control study on the association of oral cancer with Cr and Ni exposure concentrations in blood in patients from the same hospital in Changhua County was reported later by Yuan et al. (2011). Blood levels of nickel and Cr in oral cancer cases were 1.6 and 1.4 times higher, respectively, than

those of controls (patients treated for allergy and rheuma). After adjusting for potential confounders, those with high blood-Cr levels had 7-fold greater odds of having oral cancer than those with low blood-Cr levels. The study population may overlap with the cohort of Chiang et al. (2010) and it has some limitations since a steady state of Cr levels is assumed for both, cases and controls.

7.3.4. Biomonitoring

Biological monitoring of exposure to Cr(VI) compounds is a common practice in occupational settings, where exposure generally occurs through inhalation and dermal contact and contaminants are usually characterized from both a physical (e.g., welding fumes, plating mist, chromate dust) and a chemical point of view (oxidation state, solubility). Sampling strategies, particularly timing with respect to exposure patterns, can be defined taking into account kinetics and therefore it is possible to interpret observed data, particularly in blood, urine and even exhaled breath condensate (Mutti et al., 1984; Goldoni et al., 2006). In principle, an accurate assessment of systemic exposure to Cr(VI) escaping reduction by the bronchial lining fluid and plasma upon inhalation or by the gastro-intestinal tract and plasma upon oral exposure, can be obtained measuring RBC-Cr, though the procedure is delicate and requires skilled personnel (Lewalter et al., 1985). As compared to other biomarkers of exposure, RBC-Cr has two main advantages: (i) it is species specific since only Cr(VI) is able to cross RBC membranes; (ii) it is long-lived as compared to plasma Cr(III), once inside the RBCs Cr(VI) remains trapped and is very slowly released from RBCs.

The general population is exposed most often by ingestion of chromium contaminated soil, food, and water. Human biomonitoring data following oral ingestion of Cr(VI) usually come from individuals accidentally or intentionally ingesting hexavalent chromium compounds. After accidental poisoning (Goullé et al., 2012), Cr concentrations in plasma, RBC and urine were monitored for 49 days. Over this period, Cr decreased respectively from 2088 µg/L to 5 µg/L, 631 µg/L to 129 µg/L and 3512 µg/g to 10 µg/g. The half-life was much shorter in plasma than in RBC as the Cr was more quickly cleared from the plasma than from the RBC, suggesting a cellular trapping of the metal within RBCs. Thus, in principle, RBC-Cr could be used to assess absorption of Cr(VI) escaping reduction by gastric juice and plasma, and accumulating in RBC.

Unfortunately, no data are available on chromium concentration in RBCs from the general population. If available, such data would provide a straightforward way to demonstrate that indeed ingested water soluble Cr(VI) can escape reduction in the gastro-intestinal tract, giving rise to systemic exposure. Indeed, several factors preclude back calculation of ingested Cr(VI) from urinary and blood concentrations: (i) varying rates in GI absorption depending on solubility and oxidation state of different Cr species; (ii) odd distribution of blood Cr (in RBC and plasma) depending on absorption processes and the fact that only soluble Cr(VI) enters RBC, whereas both Cr(III) and Cr(VI)-derived Cr(III) compounds contribute to measured plasma concentrations; (iii) differences in excretion kinetics, much faster from plasma than from RBC, and hence varying RBC:plasma ratio depending on time elapsed since ingestion.

7.4. Modes of action

A key issue in the risk assessment of chromium is how the oxidation state of chromium influences bioavailability, cellular uptake and genotoxicity and thus the mode of action. The following Sections give an overview of the mode of action of chromium and how this is influenced by the oxidation state.

The relevance of gastrointestinal reduction of Cr(VI) for the mode of action

An important matter to be evaluated with respect to the mode of action and toxicity of Cr(VI) appears to be the level of reduction of Cr(VI) to Cr(III) in the gastrointestinal tract. Given the lower absorption of Cr(III) than of Cr(VI), this reduction is considered to reflect a detoxification and some authors proposed that reduction of Cr(VI) to Cr(III) accounts for the limited toxicity of Cr(VI) after oral ingestion due to efficient detoxification to Cr(III) by saliva, gastric juice and intestinal bacteria (De Flora, 2000).

In contrast, once inside the cells reduction of Cr(VI) to Cr(III) may reflect its bioactivation to a DNA reactive form.

Reduction of Cr(VI) to Cr(III) upon oral intake has been well described (see Section 7.1.2). The question remaining, however is whether this reduction of Cr(VI) to Cr(III) is efficient and fast enough to prevent hexavalent chromium from reaching and being taken up by tissues and cells.

Arguments in favour of this fast reduction, especially at low dose levels when no saturation of reducing capacity occurs, are mainly based on kinetics studies comparing uptake and distribution of different forms of chromium using red blood cell (RBC) chromium concentrations as a biomarker for systemic absorption of unreduced Cr(VI) (Kerger et al., 1996, 1997; Finley et al., 1997). This approach is based on the fact that upon systemic availability of Cr(VI), Cr(VI) would be taken up in the RBC and upon its reduction to Cr(III) be withheld in the RBC resulting in kinetics for the decrease of RBC chromium being different (slower) than those for the decrease of chromium in plasma. Studies reporting on this fast and complete reduction of Cr(VI) upon oral exposure are the following:

De Flora et al. (1987) reported that incubation of Cr(VI) with gastric juices prior to intraduodenal or intrajejunal administration in humans and rats, respectively, virtually eliminated absorption of chromium. Absorption of trivalent chromium ($^{51}CrCl_3$) was not increased by intraduodenal or intrajejunal administration. The authors concluded that reduction of Cr(VI) to Cr(III) in the stomach significantly reduces absorption by the oral route.

Kerger et al. (1996) studied the absorption of Cr(III) and Cr(VI) alone or mixed with orange juice in four adult male volunteers to investigate the effects of the acidic-organic environment on oral absorption. Cr(III) was poorly absorbed (estimated 0.13 % bioavailability) and rapidly eliminated in urine (excretion half-life, about 10 hr) whereas Cr(VI) had the highest bioavailability (6.9 %) and the longest half-life (about 39 hr). The absorbed fraction was considerably less when Cr(VI) was administered in orange juice (0.6 %) than in water (6.9 %). The authors concluded that the data suggested that nearly all the ingested Cr(VI) was reduced to Cr(III) before entering the bloodstream based on comparison to RBC and plasma chromium patterns in animals exposed to high doses of Cr(VI) and that their findings supported their other work (Kerger et al., 1997), which suggested that water-soluble organic complexes of Cr(III) formed during the reduction of Cr(VI) *in vivo* explain the patterns of blood uptake and urinary excretion in humans at drinking water concentrations of 10 mg/L or less. Zhitkovich (2011) argued however that the approximately 10-fold higher bioavailability of ingested Cr(VI) compared to that of Cr(VI) reduced with orange juice prior to ingestion suggests that the bulk of absorbed Cr from Cr(VI) was likely a cell-permeable chromate.

In a following study in human volunteers, Kerger et al. (1997) treated adult male subjects with potassium chromate at 5 or 10 mg Cr(VI)/L in drinking water, administered either as a single bolus dose (0.5 L swallowed in 2 minutes) or for 3 days at a dose of 1 L/day (3 doses of 0.33 L at 6-h intervals). The authors reported a low or no increase in Cr concentration in RBC following the exposure period, suggesting a rapid reduction of Cr(VI) to Cr(III) in the upper gastrointestinal tract or plasma prior to RBC uptake and systemic distribution. The author concluded that volunteers ingesting 5-10 mg Cr(VI)/L in drinking water showed a pattern of blood uptake and urinary excretion consistent with Cr(III) uptake and distribution, and thus that the endogenous reduction to the less absorbable species within the upper gastrointestinal tract and the blood prevent any substantial systemic uptake of Cr(VI) under the experimental conditions described.

Paustenbach et al. (1996) studied uptake and elimination of Cr(VI) in a male volunteer who ingested 2 L/day of water containing 2 mg/L for 17 consecutive days. Steady state chromium concentrations in urine and blood were achieved after 7 days. From the fact that both plasma and red blood cell (RBC) chromium concentrations returned rapidly to background levels within a few days after cessation of dosing the authors concluded that concentrations of 10 mg Cr(VI)/L or less in drinking water of exposed humans appear to be completely reduced to Cr(III) prior to systemic distribution. The authors indicated that their data added to an increasing weight of evidence that relatively low concentrations of Cr(VI) in drinking water (less than 10 mg/L) do not produce adverse effects in humans.

Finley et al. (1997) reported a study in which five healthy male volunteers ingested a liter of deionized water containing Cr(VI) concentrations ranging from 0.1 to 10.0 mg/L. A dose-related increase of chromium was observed in urine, plasma and RBC in all volunteers. The authors indicated that the RBC chromium profiles suggest that the ingested Cr(VI) was reduced to Cr(III) before entering the bloodstream, since the chromium concentration in RBCs dropped rapidly post-exposure. The authors concluded that the RBC and plasma chromium profiles are consistent with systemic absorption of Cr(III) not Cr(VI). They also indicated that their findings suggest that the human gastrointestinal tract has the capacity to reduce ingested Cr(VI) following ingestion of up to 1 liter of water containing 10.0 mg/L of Cr(VI), and that this is consistent with U.S. EPA position that the Cr(VI) drinking water standard of 0.10 mg Cr(VI)/L is below the reductive capacity of the stomach.

Coogan et al. (1991a) dosed rats intravenously or orally with Cr(VI). Upon intravenous administration RBC chromium levels were increased significantly 1 hr post dosing and these levels had not decreased 7 days later. When the animals were dosed orally with Cr(VI), RBC chromium levels were increased at the 1 hr time point but returned almost to background levels after 7 days. Thus the toxicokinetics have the appearance as if Cr(III) had been administered and may reflect the predominance of Cr(III). De Flora (2000) estimated that saliva may reduce 0.7 to 2.1 mg of Cr(VI) per day and gastric juices have the capacity to reduce at least 80 to 84 mg of Cr(VI) per day.

O'Flaherty et al. (2001) presented a PBK model for the ingestion of Cr(III) and Cr(VI) by humans. The model was calibrated against blood and urine chromium concentration data from a group of controlled studies in which adult human volunteers drank solutions generally containing up to 10 mg/day of soluble inorganic salts of either Cr(III) or Cr(VI) (Kerger et al., 1996; Paustenbach et al., 1996; Finley et al., 1997). Chromium kinetics were shown not to be dependent on the oxidation state of the administered chromium except in respect to the amount absorbed. The fraction absorbed from administered Cr(VI) compounds was highly variable and was presumable strongly dependent on the degree of reduction in the gastrointestinal tract, that is, on the amount and nature of the stomach contents at the time of Cr(VI) ingestion.

Kirman et al. (2012) reported a PBK model for rats and mice orally exposed to chromium. The results on erythrocyte to plasma chromium ratios suggested that Cr(VI) entered portal circulation at drinking water concentrations equal to and greater than 60 mg/L in rodents. The authors also indicated that the cancer bioassays of NTP were collected at Cr(VI) doses where saturable toxicokinetics may be expected. They pointed out that at doses above 1 mg Cr(VI)/kg per day (corresponding to drinking water concentrations of approximately 5-6 mg Cr(VI)/L in rodents), the reductive capacity of the GI lumen begins to become depleted resulting in a greater fraction of Cr(VI) remaining for uptake. They also indicated the fraction of total chromium remaining as Cr(VI) in the GI lumen was predicted to be higher in mice than in rats, which can be ascribed to higher transition rates in mice (i.e. less time for reduction to occur in the stomach lumen), combined with fairly similar rates and capacities for Cr(VI) reduction.

Arguments against complete reduction of Cr(VI) to Cr(III) upon oral administration can be found in the following studies/evaluations:

Collins et al. (2010) reported that exposure of male F344/N rats and female B6C3F1 mice to Cr(VI) resulted in significantly higher tissue chromium levels compared with Cr(III) following similar oral doses. The authors stated that this indicates that a portion of the Cr(VI) escaped gastric reduction and was distributed systemically.

Stern (2010) compared the concentrations of total Cr retained in various tissues after 25 weeks of dosing, with either Cr(III) picolinate (NTP, 2010) or sodium dichromate, and concluded that the concentrations of total Cr were 1.4-16.7 times larger for the rats ingesting Cr(VI), and 2.1-38.6 times larger for mice ingesting Cr(VI) despite 1.8 and 2.8 times larger doses of Cr(III) in rats and mice, respectively. From this the authors concluded that despite the assumed capacity of the gastrointestinal tract to reduce Cr(VI) Cr was absorbed as Cr(VI) rather than as Cr(III). The authors also argued that if the reduction capacity of the mice was exceeded at the higher Cr(VI) water concentrations that were associated with intestinal tumors, there would be a threshold concentration at which Cr(VI) would become available for absorption resulting in an increased rate of accumulation of total Cr in the

various tissues. In such a situation below the threshold, reduction would be efficient and allow only low level systemic absorption of Cr(III). Exceedance of the threshold would be expected to appear as a positive change in the slope of the tissue Cr concentration versus drinking water concentration. Stern et al. (2010) reported that analysis of available experimental data (NTP, 2007; NTP 2010) indicated that the dose-reponse data were inconsistent with the existence of such a reduction threshold since the curves were supra-linear across all doses. The authors concluded that their findings do not support the hypothesis that the reduction capacity of the mouse gastroitestinal tract was exceeded within the dose range of the NTP study, where hyperplasia was seen as all doses. Thus at least some Cr(VI) seems to escape gastric reduction. The authors further corroborated this conclusion by comparing the estimated Cr(VI) intake rate to the estimated reducing capacity of the mouse gastric fluid, domstrating that only the estimated intake rate for female mice at the highest Cr(VI) water concentration in the NTP study exceeds the estimated reduction rate. Furthermore, the authors added the arguments that the half-time for gastric emptying of liquids in the mouse has been reported to amount to < 5-9 minutes and that Cr(VI) can be absorbed directly through the stomach membranes. Thus, they argued that even when the hourly rate of Cr(VI) reduction would greatly exceed the hourly rate of Cr(VI) intake, a substantial fraction of the ingested Cr(VI) can be expected to escape reduction by being transported from the stomach to the small intestine. Finally, the authors concluded that, based on pharmacokinetic data in both mice and humans, even low, environmentally relevant doses of Cr(VI) are likely to escape reduction in the stomach, due to the ability of absorption and gastric emptying to successfully compete with reduction.

Zhitkovich (2011) concluded that a review of the literature showed that hexavalent chromium was not completely converted to trivalent chromium in animal or human stomachs and that bioavailability results and kinetic considerations suggest that 10-20 % of ingested low dose Cr(VI) would not be reduced in the GI system of humans. Zhitkovich argued that on the basis of the reported high reduction capacity of the stomach (> 80 mg/day), the rate of reduction by gastric juice under fasting conditions could exhibit pseudo first-order kinetics in a broad range of low to moderate Cr(VI) concentrations. Since a fundamental property of first-order reactions is independence of the reaction half-time on concentration it is argued that the extent of gastric reduction should be the same for both very small and very large amounts of Cr(VI). It was pointed out that in line with first-order kinetics, the initial rates of reduction by human gastric juice were found to be independent of Cr(VI) concentrations and that the reduction of 0.1 mg/L Cr(VI) (the current EPA standard for total chromium) by artificial gastric juice was a first-order reaction. Furthermore, it was pointed out that a similar bioavailability of Cr(VI) for small and large doses further supports the first-order reaction kinetics of gastric reduction. In addition the review analyses literature data to estimate the percentage of Cr(VI) that would escape the stomach detoxification and concluded that overall bioavailability and gastric reduction rate-based estimations suggest that 10-20 % Cr(VI) ingested with water escapes the gastric inactivation and reaches the small intestine. For example the fact that 10.6 % and 2.1 % of an equal dose of Cr(VI) was excreted in urine upon dosing directly into the duodenum or upon oral ingestion, respectively, was taken to calculate that upon oral intake 2.1/10.6 x 100 % = 19.8 % of the oral dose of Cr(VI) reached the duodenum and escapes reduction in the stomach. The author notes that these estimates do not apply to the consumption of water with food, which is expected to promote Cr(VI) reduction through increased stomach residence time and delivery of additional reducers. The Panel noted that these calculations assumed that Cr(III) would not be absorbed at all which is not fully correct.

The author also compared estimated reduction rates for Cr(VI) by human gastric juice at physiological temperature ($t_{1/2}$=7 min) and the time for human stomach emptying (t1/2 = 15.2 min) to calculate that 22.2 % of Cr(VI) will reach the duodenum. Taking all together Zhitkovic concluded that the bioavailability results and kinetic considerations indicate incomplete gastric detoxification of Cr(VI) at environmental levels of exposure.

Proctor et al. (2012) performed *ex vivo* studies using stomach contents of rats and mice to quantify Cr(VI) reduction rate and capacity for loading rates amounting to 1-400 mg Cr(VI)/L stomach contents, which are in the range of recent bioassays. Cr(VI) reduction followed mixed second-order kinetics, dependent on the concentrations of both Cr(VI) and the native reducing agents.

Approximately 16 mg Cr(VI)-equivalents of reducing capacity per L of fed stomach contents (containing gastric secretions, saliva, water and food) was found for both species. The authors concluded that these findings support that, at the doses that caused cancer in the mouse small intestine (≥ 20 mg Cr(VI)/L in drinking water), the reducing capacity of stomach contents was likely exceeded.

Taking all together the CONTAM Panel concluded that the absorption and tissue distribution of Cr(VI) depend strongly on the rate and extent of its reduction in the gastrointestinal tract but also on the ligands bound to Cr(VI) or the Cr(III) formed upon reduction of Cr(VI). The data available so far support that reduction along the gastrointestinal tract is efficient but that it cannot be excluded that even at low dose levels a small percentage of Cr(VI) escapes gastrointestinal reduction to Cr(III). Such a low fraction of Cr(VI) that would not be reduced may not be adequately detected in subsequent toxicokinetic studies if the majority of Cr(VI) would be reduced to Cr(III).

The relevance of metabolism of Cr(VI) for the mode of action and interpretation of genotoxicity and carcinogenicity data

Although the final product of Cr(VI) reduction is always Cr(III) the formation of specific intermediates and ternary Cr-DNA adducts is dependent on the nature of the reducing agent. The main intracellular biological reducers of Cr(VI) are low molecular weight thiols (glutathione and cysteine) and ascorbate. Studies on the reduction of Cr(VI) by extracts from rat lung, liver, or kidney have found that ascorbate accounted for at least 80 % of Cr(VI) metabolism in these target tissues (Standeven et al., 1991, 1992). Ascorbate is also the fastest reducer of Cr(VI) in the *in vitro* reactions (Quievryn et al., 2003). It should be noted that outside the cell ascorbate plays a protective-antioxidant role which contrasts with the pro-oxidative role inside the cells.

Depending on the nature of the reducing agent and its concentration, this process can generate various amounts of unstable Cr(V) and Cr(IV) intermediates. Reductive reactions with ascorbate yield Cr(IV) as the first reaction intermediate when ascorbate is present in molar excess over Cr(VI) (Goodgame et al., 1987; Stearns et al., 1994; Dillon et al., 1997). The presence of Cr(V) was only detectable in reactions of Cr(VI) at nonphysiological conditions under conditions of limited ascorbate concentrations. It is of interest to note that there is approximately a 20-fold difference in the levels of ascorbic acid when comparing the in vivo cellular levels (about 1 mM) with those in cells in culture (about 50 μM) where the only source of ascorbic acid is the supplemented foetal bovine serum (Costa and Klein, 2006).

Reduction of Cr(VI) can also be accomplished through non enzymatic reactions with cysteine and glutathione (O'Brien et al., 1992; Quievryn et al., 2003). However, in the target tissues of chromium toxicity such as lung, ascorbate is the primary reducer of Cr(VI). In mitochondria, the primary reductant of Cr(VI) appears to be NADPH leading to the formation of stable Cr(III) that effectively binds DNA (De Flora and Wetterhahn, 1989). In cell cultures, reduction of Cr(VI) is mainly facilitated by glutathione, which has been shown to produce a much higher concentration of oxidants than ascorbate (Wong et al., 2012).

This difference in reduction processes may underlie the different types and amounts of DNA damage seen with Cr(VI) *in vivo* compared with *in vitro* exposure situations. The relative concentrations of Cr species and available reductants determine the rate and pathways involved in the reduction process, and, hence, the type and extent of DNA damage that may be produced. In the course of the Cr(VI) reduction many reactive oxygen species, including free radicals, such as the hydroxyl radical, singlet oxygen, superoxide anion, are formed. The final product of Cr(VI) reduction, Cr(III), forms stable adducts with macromolecules and other cellular constituents.

The efficiency of the reduction processes as well as species-specific differences in metabolism should also be considered when interpreting carcinogenicity data. Stout et al. (2009) concluded that the induction of tumors in the small intestine of mice occurred at dose levels that did not exceed the estimated Cr(VI) reducing capacity for gastric juices in mice, based on the assumption of similar reduction capacity of humans versus rodents. Since the reduction capacity of human gastric juice has been estimated to be of 84-88 mg Cr(VI)/day (De Flora et al., 1997), Stout et al. (2009) extrapolated

this figure to rodents to conclude that the reduction capacity of a 50 g mouse would be approximately 0.4 mg/day (approximately 8 mg/kg/day). This value is greater than all of the male mouse doses and equivalent to the average daily dose of Cr(VI) in the high dose group of female mice in the 2-year carcinogenicity study by NTP. However, it should be noted that several lines of evidence suggest that Cr(VI) reduction is less efficient in rodents than in humans. Cr(VI) reduction is attenuated by raising the pH (see Section 1.1) and the pH of the gastric environment is higher in rodents than in humans (Kararli, 1995). Moreover, no post-meal peaks of gastric juice secretion occur in rodents, whereas this phenomenon provides the bulk of Cr(VI) reduction in humans. Unfortunately, experimental data are not available for Cr(VI) reduction by mouse gastric juice. The differential anatomy and functional properties of the stomach in rodents and in humans adds uncertainty to the use of tumor data in mice to estimate risk for humans.

The relevance of oxidative damage for the mode of action and interpretation of genotoxicity data.

Cr(VI) has been postulated to exert its genotoxic effects, at least in part, through the generation of oxygen radicals. *In vitro* studies indicate that in the reduction of Cr(VI) by cellular reductants, Cr(V) complexes are produced that react with hydrogen peroxide to generate hydroxyl radicals (reviewed in Bagchi D et al., Toxicology, 2002). This mechanism is consistent with results of in vitro mammalian cell studies showing a decrease in the Cr(VI)- induced DNA damage in the presence of a variety of oxygen radical scavengers, reducing agents, and metal chelators (Pattison et al., 2001; Cemeli et al., 2003; O'Brien et al., 2003) and dose-dependent increases in intracellular levels of reactive oxygen species such as hydrogen peroxide and superoxide anion radicals, as detected by electron spin resonance, in mouse epidermal cells exposed *in vitro* to Cr(VI) (Son et al., 2010). Similarly, *in vivo* studies showed reduction of the clastogenic potency when administration of radical scavengers occurred simultaneously with or prior to administration of Cr(VI) salts to rodents (Chorvatovičová et al., 1991, 1993; Sarkar et al., 1993). In the study by Wang et al. (2006) the increase in DNA damage as measured by the Comet assay in lymphocytes of mice administered by gavage with potassium dichromate was accompanied by increased ROS formation and apoptosis, but no lipid peroxidation, in the liver. No induction of oxidative DNA damage was reported in forestomach, glandular stomach and duodenum of SKH-1 mice administered Cr(VI) in drinking water (highest dose tested 20 mg Cr(VI)/L equivalent to 4.82 mg Cr(VI)/kg b.w. per day) (De Flora et al., 2008). Similarly, no significant increases in 8-hydroxy-2'-deoxyguanosine (8-OHdG), a biomarker of oxidative DNA damage, were detected in the oral mucosa or duodenum of female rats and mice dosed with Cr(VI) in the drinking water (0.3-520 mg sodium dichromate dihydrate/L) for 90 days (Thompson et al., 2011a, 2012b). However, in this study significant decreases in the ratio of reduced/oxidized glutathione were reported in various tissues (oral mucosa, jejunum and duodenum) in both species. Whole genome microarray analysis (Kopec et al., 2012a, b) of duodenal epithelial samples identified changes in genes involved in oxidative stress response, cell cycle regulation, or lipid metabolism and species-specific in the number and functionality of upregulated genes (Kopec et al., 2012b).

The relevance of Cr-DNA adducts for the mode of action and interpretation of genotoxicity data

The ability to form stable complexes with many ligands and the presence of six coordination sites gives Cr(III) the opportunity to generate various DNA cross-links with other molecules. Ternary DNA cross-links formed by Cr(III)-mediated bridging of DNA with glutathione, cysteine, histidine or ascorbate represent the major form (approximately 50 %) of Cr-DNA adducts in Cr(VI)-exposed mammalian cells at non-toxic levels of exposure (Zhitkovich et al., 1995; Quievryn et al., 2002). All ternary DNA adducts are formed through the attachment of Cr(III) to DNA phosphates **(**Zhitkovich et al., 1996, Quievryn et al., 2002**)**.

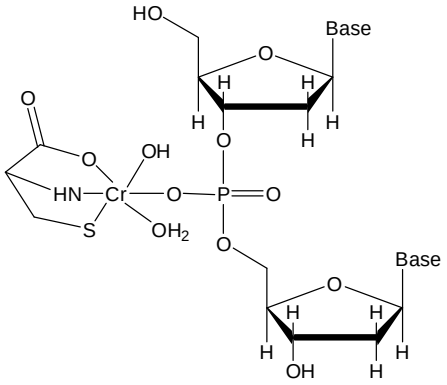

Figure 15: Cysteine-Cr(III)-DNA cross-link structure as determined by analysis of crystal structure (de Meester et al., 1977; Madafiglio K et al., 1990)

The information from several studies indicate that all cellular Cr-DNA adducts are ternary cross-links. Reductive metabolism of Cr(VI) *in vitro* usually generates a large number of binary Cr(III)-DNA adducts (Zhitkovich et al., 1996, 2000; Quievryn et al., 2002), but the presence *of these* DNA modifications in cells has not yet been established and is expected to be strongly inhibited due to the abundance of intracellular ligands capable of rapid coordination to Cr(III) prior to its binding to DNA. In cells in culture (human A549 cells) the restoration of physiological concentrations of ascorbic acid is required to detect ascorbate-Cr(III)-DNA adducts (Quievryn et al., 2002). The authors concluded that the availability of intracellular ascorbate for Cr(VI) reduction may be key to the amount of Cr-induced DNA damage observed.

DNA-protein cross-links (DPC) have also been detected *in vitro* during Cr(VI) reduction (Salnikow et al., 1992) as well as in various Cr(VI)-treated cells (Costa et al., 1996) and tissues in vivo (Hamilton, 1986; Coogan et al., 1991b; Zhitkovich and Costa, 1992) as well as in vitro during Cr(VI) reduction (Salnikow et al., 1992). In particular, Coogan et al. (1991b) reported the induction of DPC in male Fischer 344 rat liver following 3-6 weeks of exposure via drinking water to potassium chromate at the lowest effective dose of 100 mg Cr(VI)/L. In contrast, no DPC were reported by De Flora et al. (2008) in forestomach, glandular stomach and duodenum cells of female SKH-1 hairless mice administerd with sodium dichromate dihydrate in drinking water at concentrations up to 20 mg Cr(VI)/L for 9 months.

Although DPC represent only a very small fraction of initially formed DNA adducts in cultured cells (about 0.1 % according to calculations by Zhitkovich group), DPC have been broadly utilized as a biomarker of Cr-exposure in human populations (Costa et al., 1993). However, it is important to note that the currently used methodologies do not allow differentiating between Cr(VI)-induced and other forms of DPC. Macfie et al. (2010) have recently proposed a three-step mechanism for Cr(VI)-induced DPC involving (i) reduction of Cr(VI) to Cr(III), (ii) Cr(III)-DNA binding and (iii) protein capture by DNA bound Cr(III).

In vitro reduction of Cr(VI) by ascorbate (O'Brien et al., 2002; Bridgewater et al., 1994) or cysteine (Zhitkovich et al., 2000) also produces a small number of Cr(III)-mediated interstrand DNA cross-links. The most extensive DNA cross-linking was always observed under conditions of limited reducer concentrations. On the basis of the steric considerations and the fact that the yield of interstrand cross-links had the exponential dose dependence, Zhitkovich et al (2000) proposed that Cr(III) oligomers, not monomeric Cr(III), are the cross-linking species.

Mutagenic and cytotoxic properties of Cr adducts

The fact that chromium binds preferentially to the N7 position of guanine on DNA was originally suggested by in vitro studies where DNA polymerases of different origin produced guanine-specific

arrests of DNA replication on DNA templates exposed to trivalent or hexavalent chromium in the presence of ascorbate (Bridgewater et al., 1994, 1998). Cr(VI) ascorbate-generated DNA adducts were later shown to be mutagenic and replication blocking by using adduct-carrying shuttle vectors transfected into human cells (Quievryn et al., 2003). Replication of plasmids containing either Cr(III)-DNA or Asc-Cr(III)-DNA adducts revealed that the ternary adducts have a much greater mutagenic potential than the binary adducts. It was estimated that Asc-Cr(III)-DNA adducts accounted for > 90 % mutagenicity induced by ascorbate-dependent reduction of Cr(VI) under these experimental conditions. An approximately equal number of deletions and G/C targeted point mutations characterized the Cr(VI) induced mutational spectrum in human cells. The occurrence of deletion is consistent with the strong replication-blocking potential of these adducts. Voitkun et al. (1998) in their analysis of ternary DNA adducts [Cr(III)-mediated crosslinks of DNA with cysteine, histidine, or glutathione (GSH)] found that these adducts were also mutagenic after replication of adducted plasmids in human fibroblasts. The GSH-Cr(III)-DNA adducts was the most potent pro-mutagenic lesions while binary adducts were only weakly mutagenic. Single base substitutions at the G:C base pairs were the predominant type of mutations for all Cr(III) adducts. Cr(III), Cr(III)-Cys and Cr(III)-His adducts induced G:C--> A:T transitions and G:C--> T:A transversions with almost equal frequency, whereas the Cr(III)-GSH mutational spectrum was dominated by G:C--> T:A transversions. Sequence-specificity for adduct-induced mutations was also reported with mutations occurring preferentially at G:C pairs where a 3' purine was adjacent to the mutated guanine. The formation of mutagenic adducts was confirmed by Zhitkovich et al. (2002) using a similar approach. In this study they also showed that the cysteine-dependent metabolism of Cr (VI) caused the formation of mutagenic and replication-blocking DNA lesions. These adducts, which are mutagenic in human fibroblasts, are formed in the absence of oxidative damage to DNA (Zhitkovich, 2000). The Asc-Cr(III)-DNA adducts appears to be more mutagenic and replication-blocking than His/Cys adducts and possibly even the GSH adducts (Quievryn et al., 2003). Intracellular replication of Cr-modified plasmids demonstrated increased mutagenicity of binary Cr(III)-DNA and ternary cysteine-Cr(III)-DNA adducts in cells with inactive nucleotide excision repair (Reynolds et al., 2004).

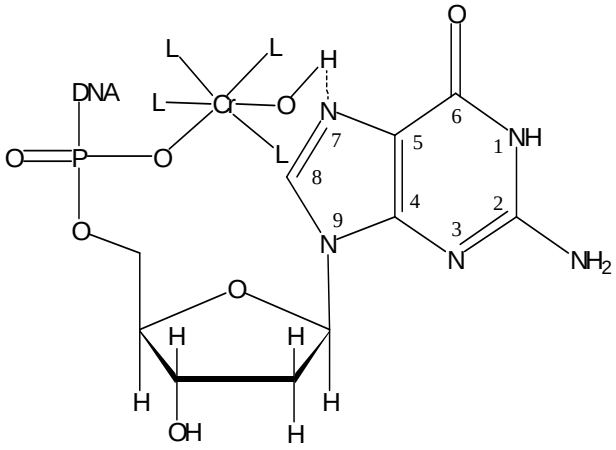

Figure 16: Direct coordination of Cr(III) to 5'-phosphate and hydrogen bonding to N-7 of dG. This binding mode can occur for both binary and ternary Cr(III)-DNA adducts. It has been proposed to explain the G selective mutagenesis.

To gain insights into the mutagenic properties of chromium induced DNA lesions mutational spectra have been also analysed in mammalian cells exposed to chromate (Yang et al., 1992; Chen and Thilly, 1994). In the first report where hprt induced mutational spectrum was analysed in CHO cells (Yang et al, 1992) mutations occurred predominantly at A:T base pairs whereas in the second study in human lymphoblastoid cells (Chen and Thilly, 1994) G:C base pairs were the mostly frequently mutated with both GC > AT and GC > TA changes. This last mutational spectrum is consistent with the mutagenicity of Cr(III)-derived DNA adducts as detected in single-lesion plasmids replicated in human cells (see above) and differs significantly from the spectra induced by known oxygen radical-producing agents (H_2O_2, Fe^{2+} and X-ray) analysed in the same study (Chen and Thilly, 1994).

Although Cr(VI) is generally believed to induce dG-DNA adducts, both bulky DNA adducts and oxidative damage at adenines and guanines were recently (Arakawa et al., 2012) detected in the p53 gene in Cr(VI) treated human lung cells. The analysis of the binding sites for the three major cellular Cr forms, namely Cr(III), Cr(VI) and Cr(V), suggested that Cr(VI) induction of lesions at dA and dG residues is likely to be through Cr(V) intermediates. Cr(III) binding sites were preferentially at dG sites whereas Cr(V) binding sites included both Cr(III) and Cr(VI) binding sites. The authors speculated that it is probable that Cr(VI) once reduced to Cr(V) is transferred to N7 of the dA to form a Cr(V)-dA adduct which is eventually converted to stable Cr(III)-dA. These Cr(VI) induced lesions could contribute to mutagenesis of the p53 gene that leads to lung carcinogenesis.

Finally, Cr-DNA adducts have been also directly associated with the cytoxic effects of Cr (VI). NER deficient mammalian cells that are characterized by persistence of Cr-DNA adducts (see also Section on genotoxicity) showed increased apoptosis and clonogenic death by Cr(VI) (Reynolds et al., 2004). Another repair pathway, mismatch repair (MMR), is also involved in the toxicity of Cr(VI) adducts. Following exposure to Cr(VI), mouse and human cell lines defective in MMR showed higher survival and lower apoptosis when compared to MMR-proficient cells lines (Peterson-Roth et al., 2005). A significant induction of double-strand breaks (as detected by gamma-H2AX foci) was detected before apoptosis in MMR-proficient cells suggesting that the repair by MMR of bulky adducts formed by chromium leads to the formation of double-strand breaks (Salnikov and Zhitkovich, 2008).

Mechanistic studies showed that Cr-DNA adducts lost their ability to block replication of Cr-modified plasmids in human colon cells lacking the MMR protein MLH1 (Peterson-Roth E et al, 2005). Reynolds et al. (2009) later showed that MMR complex MSH2-MSH6 (MutSalpha) effectively bound DNA containing ascorbate-Cr-DNA and cysteine-Cr-DNA cross-links. Conversely, binary Cr-DNA adducts were poor substrate for MSH2-MSH6 and their toxicity in cells was weak and MMR independent. The MMR complex MSH2 and MSH3 (MusSbeta) was shown to cooperate with MutSalpha in processing of Cr-DNA cross-links being essential for the induction of double-strand breaks, micronuclei and apoptosis in human cells by chromate.

Conclusions

It is clear that a key determinant for the genotoxic action of Cr(VI) is its intracellular reduction via Cr(V) to Cr(III). This reduction of Cr(VI) to Cr(III) is also important in an earlier phase of the mode of action since it is an important factor in the bioavailability of Cr(VI) upon oral intake, especially given the fact that bioavailability of Cr(III) may be more limited than that of Cr(VI) since Cr(III) can not easily pass cell membranes and enter cells. Only once absorbed, Cr(VI) is reduced to Cr(III) with formation of Cr-DNA adducts and other DNA damage resulting in mutagenesis, (MOA I in Figure 17) (McCarroll et al, 2010, OEHHA, 2011; Zhitkovich, 2011;). An additional MOA contribution to the DNA damage induced by Cr(VI) is the reduction of Cr(VI) resulting in production of Cr(V) that can result in formation of ROS upon reaction with hydrogen peroxide to generate hydroxyl radicals, ROS and oxidative stress (Bagchi D et al., 2002; Thompson et al., 2011 b), resulting in damage to DNA, and mutation (MOA II in Figure 17). Both modes of action can occur and contribute to the genotoxic effects of Cr(VI).

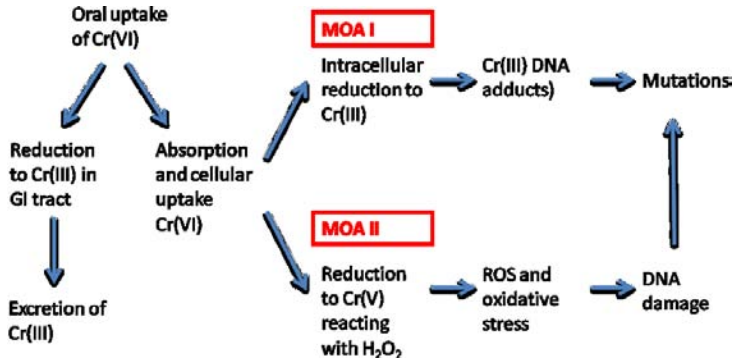

Figure 17: Proposed mode of action for carcinogenicity of Cr(VI).

7.5. Dose-response assessment

No human data could be identified to perform a dose-response assessment of chromium or any chromium species for oral exposure.

Therefore, the CONTAM Panel considered the available data on neoplastic and non-neoplastic health effects in experimental animals for the evaluation of dose-response relationships separately for Cr(III) and Cr(VI) species.

For Cr(III) no dose-response modelling was possible for the most reliable studies in experimental animals, since no effects were observed even at the highest dose tested, see Section 7.2.1. Although dose-response data were available on developmental and reproductive toxicity regarding the fertility of male and female mice, the CONTAM Panel noted their limitations (using only two dose groups) and since concerns were raised regarding the design, conduct and reporting of the data the CONTAM Panel concluded that these data could not be analysed by dose-response modelling.

The data base for Cr(VI) allowed dose-response assessment of both, neoplastic and non-neoplastic effects in experimental animals.

7.5.1. Assessment of neoplastic effects of Cr(VI)

The CONTAM Panel identified the neoplastic effects of Cr(VI) as the critical effects and identfied the data available from the 2-year studies on the carcinogenicity of sodium dichromate dihydrate in male and female F344/N rats and in male and female B6C3F1 mice (see Section 7.2.2.5) as suitable for dose-response evaluation. To this end, the CONTAM Panel applied the BMD approach to analyse the data on the incidence of neoplastic effects according to the guidance given in EFSA (2009c). Using the default BMR of 10 % extra risk for the incidence, the BMD_{10} and its 95 % lower confidence limit $BMDL_{10}$ were calculated. For details see Appendix J.

The dose-response data on squamous cell neoplastic lesions in the epithelium of the oral cavity in male and female rats were suitable for a BMD analysis. For each sex, the incidences of papilloma and of carcinoma were reported by the NTP separately both for oral mucosa and tongue. For the two sites of oral mucosa and tongue combined the joint incidence of papilloma or carcinoma combined) was reported (see Table 19) and the CONTAM Panel decided to perform a dose-response evaluation of the neoplastic activity of sodium dichromate dihydrate in the oral cavity in rats, a) for the incidence of papilloma or carcinoma in the oral cavity (oral mucosa or tongue) and b) for carcinoma in the oral mucosa only since the incidence in tongue only was very low. The incidences of the two endpoints exhibited a statistically significant dose-response relationship (poly-3 test for trend: $p < 0.001$), separately for both sexes. Since the dose ranges, the range of the observed carcinoma incidences and the shape of the dose-response were comparable in both sexes the CONTAM Panel investigated the possibility of a dose-response evaluation of males and females combined using the PROAST software (RIVM) which allows testing for differences between two dose-response curves of males and female.

Table 21(A) presents the BMD/L_{10} values for male and female rats, separately as well as combined, a) for the incidence of papilloma or carcinoma in oral cavity (mucosa or tongue) and b) for carcinoma in the oral mucosa, using the BMDS software BMDS 2.4 of US-EPA and PROAST (RIVM). Since there were no statistically significant differences between males and female, the CONTAM Panel derived from these data a $BMDL_{10}$ of 3.4 mg/kg b.w. per day for the incidence of papilloma or carcinoma in the oral cavity and a $BMDL_{10}$ of 3.6 mg/kg b.w. per day for carcinoma in oral mucosa only.

The dose-response data on epithelial neoplastic lesions of the small intestine in male and female mice were also suitable for a BMD analysis. For each sex, the incidences of adenoma and of carcinoma were reported by the NTP separately for two sites, namely duodenum and jejunum, whereas the incidence of adenoma or carcinoma (combined) was reported for all three sites (i.e. duodenum, jejunum and ileum) combined (see Table 21(B)). Since the adenoma-carcinoma sequence is a well recognised pathway of carcinogenesis in different sections of the GI tract (e.g. Höhn, 1979;

McConnell et al, 1986; Vogelstein et al., 1988; Spigelmann et al., 1994; and Dr. M. Iezzi[17] and Dr. M. Piantelli[17], 2013, personal communication), the CONTAM Panel performed a dose-response evaluation for the neoplastic activity of sodium dichromate dihydrate in the small intestine in mice by considering both, a) the incidence of adenoma or carcinoma) and b) the incidence of carcinoma only at the three sites of duodenum, jejunum and ileum (combined). The incidence of adenoma or carcinoma and the incidence of carcinoma only exhibited a statistically significant dose-response relationship in both sexes (poly-3 test for trend for adenoma or carcinoma ($p < 0.001$) and for carcinoma in females ($p < 0.001$) and for carcinoma in males ($p < 0.05$) Since the dose ranges, the range of the observed carcinoma incidences and the shape of the dose-response were comparable in both sexes the CONTAM Panel performed also for the data of the small intestine a dose-response evaluation of males and females combined in the same way as described above for the oral cavity. Table 20(B) presents the BMD/L_{10} values for male and female mice a) for the incidence of adenoma or carcinoma and b) for carcinoma only at all three sites of the small intestine investigated BMDS software BMDS 2.4 of US-EPA and PROAST (RIVM). Since there were no statistically significant differences between males and females, the CONTAM Panel derived for the incidence of adenoma or carcinoma combined a $BMDL_{10}$ of 1.0 mg/kg b.w. per day and for the incidence of carcinoma only at all sites a $BMDL_{10}$ of 3.8 mg/kg b.w. per day.

Table 21: BMD analysis of the data on neoplastic effects observed in the 2-year-studies of the NTP (2007, 2008) on sodium dichromate dihydrate in male and female F344/N rats (A) and in male and female B6C3F1 mice (B).

	BMD_{10} (mg/kg b.w. per day)	**$BMDL_{10}$ (mg/kg b.w. per day)**
(A) Dose-response analysis of the neoplastic changes in rat oral cavity		
Papilloma or carcinoma of the oral mucosa or tongue		
Male rat [1)]	5.87	3.30
Female rat [1)]	4.11	2.61
Male and female rats[2)]	4.85	3.36
Carcinoma of the oral mucosa		
Male rat [1)]	7.45	4.07
Female rat [1)]	3.95	2.58
Male and female rats[2)]	5.09	3.57
(B) Dose-response analysis of the neoplastic changes in mouse small intestine		
Adenoma or carcinoma in duodenum, jejunum and/or ileum		
Male mouse [1)]	1.48	1.08
Female mouse[1)]	1.15	0.61
Male and female mice[2)]	1.53	1.00
Carcinoma in duodenum, jejunum and/or ileum		
Male mouse [1)]	7.54	2.53
Female mouse[1)]	6.63	3.12
Male and Female mice[2)]	6.38	3.81

1): using BMDS software for the analysis of single data sets
2): using PROAST software for the analysis of combined data. No statistical differences were observed in dose response relationship between the two sexes.

7.5.2. Assessment of non-neoplastic effects of Cr(VI)

In order to assess the risk of non-neoplastic effect the CONTAM Panel considered dose-response data available from the 2-year NTP study on non-neoplastic lesions in liver, duodenum, mesenteric lymph nodes and pancreas and on haematological effects (NTP, 2008) (see Section 7.2.2.2 and Table 16).

For the non-neoplastic lesions, considering the available data, the CONTAM Panel identified the occurrence of chronic inflammation of the liver in female rats, diffuse epithelial hyperplasia in the duodenum in male and female mice, histiocytic cellular infiltration in mesenteric lymph nodes in male

[17] Immuno-Oncology Laboratory, Aging Research Center (CeSI), G.d'Annunzio University Foundation of Chieti-Pescara (Italy),

and female mice, histiocytic cellular infiltration in the liver in female mice and acinus, cytoplasmic alteration in pancreas in female mice as the most relevant endpoints for the risk assessment of Cr(VI) see also ADTSR (2012). A dose response analysis was therefore performed using the default BMR of 10 % extra risk for the incidence of the aforementioned non-neoplastic lesions, and the BMD_{10} and its 95 % lower confidence limit $BMDL_{10}$ were calculated (see Appendix J.2 for details). The CONTAM Panel noted that several dose-response data were not suitable for a BMD analysis since the BMD/BMDL ratios and the range of the BMDL values of the acceptable models were larger than one order of magnitude such that a $BMDL_{10}$ value would either extrapolate orders of magnitude below the observed dose range or it would depend highly on the model chosen. Therefore no $BMDL_{10}$ values for some of these endpoints could be identified from these data according to guidance given by EFSA (2009). The $BMDL_{10}$ values for endpoints which could be evaluated varied from 0.27 mg Cr(VI)/kg b.w. per day for acinus, cytoplasmic alterations in pancreas to 0.011 mg Cr(VI)/kg b.w. per day for histiocytic cellular infiltration in liver in female mice. For male mice the BMD approach was only applicable to the data of diffuse epithelial hyperplasia in duodenum and resulted in a $BMDL_{10}$ of 0.11 mg Cr(VI)/kg b.w. per day (see Table 22).

Table 22: BMD analysis of the data on non-neoplastic effects in the 2-year-studies of the NTP (2007, 2008) on sodium dichromate dihydrate in male and female F344/N rats and in male and female B6C3F1 mice. Presence or absence of lesions (i.e. a quantal effect) had been reported in the publications. For details see Appendix J2.

Effect/ species/sex	**BMD_{10} (mg/kg b.w. per day)**	**$BMDL_{10}$ (mg/kg b.w. per day)**
liver chronic inflammation		
female rats	No BMDL could be determined [(a)]	
histiocytic cellular infiltration in liver		
female mice	0.067	0.011
diffuse epithelial hyperplasia in duodenum		
male mice	0.14	0.11
female mice	No BMDL could be determined [(a)]	
histiocytic cellular infiltration in mesenteric lymph node		
male mice	No BMDL could be determined [(a)]	
female mice	No BMDL could be determined [(a)]	
acinus, cytoplasmic alteration in pancreas		
female mice	0.61	0.26

(a): No BMDL could be determined since the BMD/BMDL ratios and the range of the BMDL values of the acceptable models were larger than one order of magnitude such that a $BMDL_{10}$ value would either extrapolate orders of magnitude below the observed dose range or it would depend highly on the model chosen.

Regarding haematological effects the CONTAM Panel noted that several parameters measured in the 2-year NTP study on male rats at day 4, 22, and months 3, 6 and 12 exhibited a statistically significant change compared to controls and identified the effects on haematocrit, haemoglobin, MCV and MVH at day 22 after start of treatment with sodium dichromate dihydrate as critical effects, describing the haematotoxcity of Cr(VI), see also ADTSR (2012). The CONTAM Panel noted that the four data sets of means and standard errors available for the controls and each of the four dose groups can be modelled as continuous data. Using the default BMR of 5 %, in the absence of statistical or toxicological considerations supporting a deviation, the PROAST software was applied and the best fitting models of the nested Exponential and the Hill family was identified, respectively. The BMD/L values for the four haematological endpoints are listed in Table 23. The lowest $BMDL_{05}$ of 0.2 mg Cr(VI)/kg b.w. per day was calculated for decreased haematocrit in male rats.

Table 23: Result of the BMD analysis of haematological effects in male F/334 rat exposed to sodium dichromate dihydrate in drinking water for 22 days.

	BMD_{05} (mg/kg b.w. per day)	$BMDL_{05}$ (mg/kg b.w. per day)
Haematocrit		
PROAST Exponential	0.64	0.21
PROAST Hill	0.85	0.74
Haemoglobin		
PROAST Exponential	0.34	0.27
PROAST Hill	0.31	0.23
MCV		
PROAST Exponential	0.55	0.41
PROAST Hill	0.61	0.50
MCH		
PROAST Exponential	0.53	0.33
PROAST Hill	0.62	0.49

BMD: benchmark dose; MCV: mean corpuscular volume; MCH: mean corpuscular haemoglobin; BMD: Benchmark dose; $BMDL_{05}$: 95 % lower confidence limit of BMD.

7.6. Derivation of health-based guidance value(s)/margin of exposure

The CONTAM Panel considered the critical effects of Cr(III) and Cr(VI) in order to derive health-based guidance values (HBGV).

Trivalent Chromium

No carcinogenic or other adverse effects have been observed in the sub-chronic or long-term oral toxicity studies of Cr(III) in mice or rats. The relevant NOAELs derived from these studies corresponded to 506 and 286 mg Cr(III)/kg b.w. per day for the sub-chronic and long-term toxicity in the rat, respectively (NTP, 2010). No significant changes in reproductive organ weights in male or female animals, in sperm parameters, or in estrous cyclicity were reported in the sub-chronic oral toxicity studies on rats and mice at the highest doses tested (506 mg/kg b.w. per day and 1090 mg/kg b.w. per day, respectively) (NTP, 2010). However, the CONTAM Panel noted that in some other studies in rats or mice, reproductive or developmental toxicity by oral exposure to Cr(III) was reported. The lowest LOAELs for these effects were in the order of 30 mg/kg b.w. per day. The Panel noted that these studies have methodological limitations and were not designed for establishing reference doses. Taking together these observations the CONTAM Panel decided to use the relevant NOAEL in the long-term rat NTP study of 286 mg/kg b.w. per day as a RP for risk characterisation of Cr(III) and to apply, besides the standard uncertainty factor of 100, an additional factor of 10 to account for the absence of adequate data on reproductive and developmental toxicity. Therefore, the CONTAM Panel derived a tolerable daily intake (TDI) of 300 µg Cr(III)/ kg b.w per day.

Hexavalent chromium

Cr(VI) compounds are genotoxic. Cr(VI) is a human carcinogen by inhalation, and oral exposure via drinking water is associated with gastrointestinal system cancers in experimental animals. $BMDL_{10}$ values were derived from the animal carcinogenicity data (NTP, 2010). In this study increased incidence of tumours of the squamous epithelium of the oral cavity and of epithelial tissues of the small intestine were reported in male and female rats and mice, respectively. Since the adenoma-carcinoma sequence is a well recognised pathway of carcinogenesis in the GI tract, in a conservative approach, the CONTAM Panel selected the $BMDL_{10}$ of 1.0 mg Cr(VI)/kg b.w. per day for combined adenomas or carcinomas of the small intestine in male and female mice as RP for the estimation of the margin of exposure (MOE) for neoplastic changes.

After repeated oral administration of Cr(VI), in addition to the cancer effects, several toxic effects were identified in rats and mice including microcytic, hypochromic anaemia, and non-neoplastic lesions of the liver, duodenum, mesenteric and pancreatic lymph nodes and pancreas. The lowest

NOAEL for haematological effects from long-term toxicity studies in rats was 0.21 mg Cr(VI)/kg b.w. per day, whereas a NOAEL of 0.77 mg Cr(VI)/kg b.w. per day was identified in this species for liver toxicity, histiocytic cellular infiltration in mesenteric lymph nodes and in the duodenum. No NOAEL was established in the long-term toxicity mouse study for haematological effects, liver toxicity, hystiocytic cellular infiltration in mesenteric lymph nodes and hyperplasia in the duodenum observed at the lowest tested dose of 0.38 mg Cr(VI)/kg b.w. per day. The other toxic effects reported in repeated toxicity studies, including effects on fertility and development, appeared at higher doses. BMD analysis was performed on the suitable dose-response data for non-neoplastic effects. The $BMDL_{10}$ values of 0.27, 0.11 and 0.011 mg Cr(VI)/kg b.w. per day were calculated for non-neoplastic lesions in pancreas (acinus, cytoplasmic alteration), duodenum (diffuse epithelial hyperplasia) and liver (histiocytic infiltration), respectively. The CONTAM Panel noted that the biological significance and cause of histiocytic cellular infiltration are unknown and therefore it was not considered as a critical adverse effect. The $BMDL_{10}$ value of 0.11 mg Cr(VI)/kg b.w. per day for diffuse epithelial hyperplasia of the duodenum in female mice was selected as the RP for the estimation of the MOE for non-neoplastic lesions in the small intestine. In the case of haematological effects a $BMDL_{05}$ of 0.2 mg Cr(VI)/kg b.w. per day was calculated for decrease of haematocrit in male rats. The CONTAM Panel selected this value to be used as reference point for MOE estimation of hematotoxic effects of Cr(VI).

8. Risk characterisation

Trivalent chromium

The CONTAM Panel established a TDI of 300 µg /kg b.w. per day for Cr(III). Under the assumption that all chromium in food is Cr(III) (see Section 4.1) the mean dietary exposure across all age groups and surveys (minimum LB of 0.6 µg/kg b.w. per day and maximum UB of 5.9 µg/kg b.w. per day) as well as the 95th percentile exposure (minimum LB of 1.1 µg/kg b.w. per day and maximum UB of 9.0 µg/kg b.w. per day) are well below the TDI. Therefore, the CONTAM Panel concluded that the current dietary exposure to Cr(III) does not raise concern from a public health point of view.

Regarding the vegetarian population, although based on limited consumption data, the dietary exposure to Cr(III) seems to be similar to that estimated for the general population. Therefore, the dietary exposure of vegetarians is well below the TDI of 300 µg Cr(III)/ kg b.w. per day.

A significant exposure to Cr(III) may occur via dietary supplemental intake. The combined exposure from supplemental intake in adults (i.e. from fortified foods, PARNUTS and food supplements) was estimated to be between 910 µg /day for a typical intake and 1540 µg /day for upper intake. Assuming a default value of 70 kg b.w. per adults, the exposure to Cr(III) from the upper supplemental intake would be 22 µg/kg b.w. per day. Considering this exposure and the maximum estimated contribution coming from the diet for adults (95th percentile of 2.6 µg/kg b.w. per day), the total exposure remains well below the TDI of 300 µg Cr(III)/ kg b.w. per day.

Hexavalent chromium

Neoplastic effects

As recommended for substances which are both genotoxic and carcinogenic (EFSA, 2005), the CONTAM Panel decided to adopt the MOE approach for the risk characterisation of neoplastic effects of Cr(VI), by using the $BMDL_{10}$ of 1.0 mg Cr(VI)/kg b.w. per day for the combined incidence of adenomas and carcinomas in the mouse small intestine as RP. The EFSA Scientific Committee concluded that, for substances that are both genotoxic and carcinogenic, an MOE of 10 000 or higher, based on a $BMDL_{10}$ from an animal study, is of low concern from a public health point of view (EFSA, 2005). In a conservative approach, the CONTAM Panel decided to consider all chromium in water intended for human consumption and natural mineral waters as Cr(VI) (see Section 4.1). The chronic exposure levels calculated across the different dietary surveys and age classes, ranged from 0.7 to 159.1 ng/kg b.w. per day (minimum LB - maximum UB) for mean consumption and from 2.8 to

320.2 ng/kg b.w. per day (minimum LB - maximum UB) for 95th percentile consumption, with the highest exposure estimated for infants. The MOEs for the different age groups across the different European dietary surveys calculated on the basis of the selected RP vary for the different ages groups as shown in Table 24 for mean and 95th percentile exposure when calculated for both LB and UB exposure estimates.

The MOEs indicate low concern regarding Cr(VI) intake via the consumption of water intended for human consumption and mineral waters for all age groups when considering the mean chronic exposure values with the exception of infants at UB exposure estimates. However, the exposure assessment for infants should be cautiously taken because only two surveys were available for this age group. The MOEs calculated taking into account the 95th percentile exposures to Cr(VI) indicate a potential concern, but only at UB exposure estimates and particularly for 'Infants', 'Toddlers' and 'Other children' age groups. When interpreting these MOEs, it should be considered that there is a remarkable influence of left-censored data (91.3 % of the total data) on the UB estimates since UB occurrence values were 10-fold higher than LB for the most consumed water, i.e. tap water. Moreover, these MOEs were calculated by using as RP the $BMDL_{10}$ derived from dose-response analysis of incidence of tumours (combined incidence of adenomas and carcinomas) in the small intestine of mice. There is evidence of differences in anatomy and functional properties of the stomach in rodents and in humans that are expected to impact significantly on the efficiency of Cr(VI) reduction in the GI tract. Efficient Cr(VI) reduction in the GI tract would reduce chances of cellular uptake and subsequent induction of genotoxicity/carcinogenicity. In particular, the reduction capacity of rodents is expected to be significantly lower than that of humans, which makes rodents a worst case model for human carcinogenicity. When interpreting the numerical value of the MOE it should be considered that there is a significant uncertainty associated with the use of tumour data in mice to estimate risk at doses of Cr(VI) relevant for human exposure.

Based on the MOE values for neoplastic effects, the CONTAM Panel concluded that the current levels of exposure to Cr(VI) via the consumption of water intended for human consumption and mineral waters are of low concern from a public health point of view for average consumers but there might be a potential concern for high consumers particularly for 'Infants', 'Toddlers' and 'Other children'.

Table 24: Margin of exposure (MOE) calculated across the different European dietary surveys for Cr(VI) through the consumption of drinking water (water intended for human consumption and mineral waters) as such. MOEs are rounded to two significant digits.

	Mean exposure[(a)]		**95th percentile exposure[(a)]**	
	MOE (min LB-max UB)	Dietary surveys with MOE below 10 000/Total surveys[(b)]	MOE (min LB-max UB)	Dietary surveys with MOE below 10 000/Total surveys[(b)]
Infants[(c)]	71 000 - 6300	2/2	21 000 - 3100	1/1
Toddlers	130 000 - 11 000	0/9	62 000 - 4200	6/6
Other children	1 400 000 - 16 000	0/15	360 000 - 6600	9/15
Adolescents	1 200 000 - 23 000	0/10	350 000 - 9100	1/10
Adults	710 000 - 23 000	0/13	230 000 - 9200	1/13
Elderly	540 000 - 29 000	0/6	210 000 - 11 000	0/6
Very elderly	740 000 - 29 000	0/4	95 000 - 11 000	0/3

(a): Dietary surveys with less than 50 % consumers were not considered (surveys from Greece (age class 'Other children'), Cyprus (age class 'Adolescents'), Latvia (age classes 'Other children', 'Adolescents' and 'Adults') and Hungary (age classes 'Adults', 'Elderly' and 'Very elderly', see Table G2 in appendix);
(b): Number of surveys with a MOE lower than 10000 at the UB;
(c): Estimate only available from two dietary surveys for the mean and only one for the 95th percentiles;

The highest chronic exposure to Cr(VI) through the consumption of bottled water was estimated in the youngest population ('Infants' and 'Toddlers') (Table 10). Due to the lack of consumption data on bottled water, in several dietary surveys no exposure to Cr(VI) through the consumption of bottled

water could be estimated. The maximum estimates of chronic exposure to Cr(VI) in mean consumers were 149.8 ng/kg b.w. per day (UB) for infants, and 148.7 ng/kg b.w. per day (UB) for 'Toddlers' at the 95th percentile exposure. In general, the exposure to Cr(VI) was lower than that estimated through the consumption of all types of water due to the small amount of consumption data reported for bottled water (27.7 % of the total). However, considering the estimates of exposure in several dietary surveys, the CONTAM Panel concluded that regarding the exposure to Cr(VI) through the consumption of bottled water there is a low concern from a public health point of view for average consumers but there might be a potential concern for high consumers particularly for 'Infants', 'Toddlers' and 'Other children' (see Table 25).

Table 25: Margin of exposure (MOE) calculated across the different European dietary surveys for Cr(VI) through the consumption of bottled water. Dietary surveys with no exposure to Cr(VI) (no reported consumption on bottled water) were not considered when calculating the MOEs. MOEs are rounded to two significant digits.

	Mean exposure[(a)]		**95th percentile exposure[(a)]**	
	MOE (min LB-max UB)	Dietary surveys with MOE below 10000/Total surveys[(a)]	MOE (min LB-max UB)	Dietary surveys with MOE below 10000/Total surveys[(a)]
Infants[(b)]	140 000-6700	½	26 000-6900	1/1
Toddlers[(c)]	520 000 000-16 000	0/9	38 000-6700	4/6
Other children[(c)]	77 000 000-22 000	0/16	1 600 000-7900	5/16
Adolescents[(c)]	8 900 000-28 000	0/11	1 200 000-9300	1/11
Adults[(c)]	840 000 000-26 000	0/15	940 000-9400	1/15
Elderly[(c)]	8 900 000-35 000	0/7	1 700 000-11 000	0/7
Very elderly[(c)]	18 000 000-41 000	0/6	190 000-14 000	0/5

(a): Number of surveys with a MOE lower than 10000 at the UB;
(b): Estimate only available from two dietary surveys for the mean and only one for the 95th percentiles;
(c): Those dietary surveys with 95th percentile exposure equal to zero were not included in the MOE calculation (see Table 10).

The inclusion of the water used in the preparation of specific foods (coffee, tea infusions, and dry infant and follow-on food mainly, but also some others such as instant soup, evaporated and dried milk, and dehydrated fruit juice) led to an increase up to two-fold of the exposure to Cr(VI). However, the CONTAM Panel was not able to consider this additional contribution to the exposure to Cr(VI) when deriving MOEs since no reliable data to quantify Cr(VI) in food exist.

Non-neoplastic effects

The $BMDL_{10}$ value of 0.11 mg Cr(VI)/kg b.w. per day for diffuse epithelial hyperplasia of the duodenum in male mice was selected as RP to estimate the MOE for non-neoplastic lesions. The comparison of this RP with estimated daily intakes of Cr(VI) via drinking water ranging up to 159.1 and 320.2 ng/kg b.w. per day (maximum UB for mean and 95th percentile exposure) for the different age groups resulted in an MOE of 690 and 340, respectively.

The $BMDL_{05}$ of 0.2 mg Cr(VI)/kg bw per day calculated for decreased haematocrit was selected as RP to estimate MOEs for haematological effects. The comparison of this reference point with estimated daily intakes of Cr(VI) via drinking water ranging up to 159.1 and 320.2 ng/kg b.w. per day (maximum UB for mean and 95th percentile exposure) for the different age groups resulted in an MOE of 1300 and 630, respectively.

The CONTAM Panel considered that for the critical thresholded effects, MOEs larger than 100 would indicate a low concern for human health and therefore concluded that for non-neoplastic lesions and

haematological effects the current exposure levels to Cr(VI) via drinking water are of no concern from a public health point of view.

9. Uncertainty analysis

The evaluation of the inherent uncertainties in the assessment of exposure to chromium, in particular to Cr(III) in food and to Cr(VI) in drinking water, has been performed following the guidance of the Opinion of the Scientific Committee related to Uncertainties in Dietary Exposure Assessment (EFSA, 2006). In addition, the report on 'Characterizing and Communicating Uncertainty in Exposure Assessment' has been considered (WHO-IPCS, 2008). According to the guidance provided by the EFSA opinion (2006), the following sources of uncertainties have been considered: assessment objectives, exposure scenario, exposure model, and model input (parameters).

9.1. Assessment objectives

The objectives of the assessment were clearly specified in the terms of reference.

9.2. Exposure scenario/Exposure model

In response to EFSAs request to submit occurrence data on chromium in food and water intended for human consumption and natural mineral waters, 79 809 analytical results were available in the EFSA data base among them about 65 % for drinking water. The samples were collected mostly (80 %) by one Member State. Around 50 % of the analytical results for food and 90 % for water were left-censored. All food groups were well represented with around 17 % belonging to the group of 'Vegetables and vegetable products (including fungi)'. The majority of the water samples belonged to the type of tap water (60.6 %). There is an uncertainty in possible regional differences in the presence of chromium in food commodities and types of waters and it is evident that the dataset is not fully representative for all Member States.

Highest chromium concentrations in food (assumed to be all Cr (III)) were reported for specific foods such as 'Products for special nutritional use', 'Herbs, spices and condiments' and 'Sugar and confectionary'. The concentration data in water ranged within one order of magnitude. However, the CONTAM Panel noted different reported consumption data for water intended for human consumption and natural mineral waters across Europe, such that the variation of the exposure to chromium (all assumed to be Cr(VI)) through the consumption of water was considerably high.

The majority (99.9 %) of the analytical results were reported to EFSA as total chromium or as chromium without specification (only 88 analytical results were received on Cr(VI), all in bottled water). No data on speciation of Cr in food were provided in the occurrence dataset and this adds to the uncertainty of exposure assessment of both Cr(III) and Cr(VI) in food. The CONTAM Panel's assumption that all reported analytical results in food related to Cr(III) was based on information on the reducing capacity of the organic food components, and the fact that Cr(III) is the most stable oxidation state. This assumption adds to uncertainty in particular with respect to the exposure assessment of Cr(VI), since if even a small proportion of total chromium in food was in the form of Cr(VI), it could contribute substantially to Cr(VI) exposure levels.

The CONTAM Panel noted that in the analysed water samples the average ratio Cr(VI)/total Cr was almost equal to one, and that drinking water is usually treated with oxidizing agents to make it potable, which would promote the presence of Cr(VI) instead of Cr(III). Therefore, the CONTAM Panel decided to consider all Cr present in drinking water as Cr(VI). This approach adds to the uncertainty of exposure assessment since the chemical analyses of Cr(VI) was performed in a very limited number of samples. Despite the assumption that the presence of Cr(VI) in food is unlikely, exposure scenarios considering the additional contribution of the Cr(VI) present in the water used to prepare certain foods (tea infusions, coffee, and infant and follow-on food, mainly, but also some others such as instant soup, evaporated and dried milk, and dehydrated fruit juice) were evaluated. These scenarios are highly conservative since it is assumed that all Cr(VI) remains oxidized before the ingestion of the foods.

Food preparation using stainless steel containers, processors and utensils may add Cr(III) to the presence of chromium in food. As data on food as consumed are practically not present in the dataset applied, this could have led to a potential underestimation of the exposure to Cr(III) in food.

A large proportion of samples with left-censored data introduced considerable uncertainties to the overall dietary exposure estimate, particularly for drinking water. Therefore the LB values reported in this opinion tend to underestimate, while UB tends to overestimate the dietary exposure.

The limited data on both consumption and occurrence data on human milk led to use a simulated scenario to estimate the exposure to Cr(III) in infants exclusively fed with human milk. This adds uncertainty to the estimated contribution of human milk to the exposure to Cr(III). There is uncertainty associated to the dietary exposure calculated for the vegetarian population since very limited consumption data are available.

The lack of appropriate consumption data on fortified foods, foodstuffs for particular nutritional use (PARNUTS) and food supplements obliged to the use of a simulated scenario that adds uncertainty to the contribution of these products to the exposure to Cr(III).

There are also insufficient data on consumption for children younger than one year (infants), which adds uncertainty to the exposure calculations in this age group.

Overall, there is considerable uncertainty regarding the total dietary exposure to chromium from food and water intended for human consumption and mineral waters.

9.3. Model input (parameters)

Standardized methods exist for the determination of total chromium in food and in water. For Cr(VI) in water, standardised methods exist, however, no validated or standardised method for speciation of chromium in food is available. Limited standard or certified reference materials are available for chromium species. Regular proficiency testing is organised for total chromium in foodstuffs and water, and only proficiency testing for Cr(VI) in water exists. The analytical results used for exposure assessment were performed by different laboratories at largely varying LOQ/LODs. Those limitations may have added to the overall uncertainty of the analytical results.

9.4. Other uncertainties

Toxicity of trivalent chromium

The CONTAM Panel considered it appropriate to establish a TDI Cr(III) based on the NOAEL of a 2-year NTP study in rats (NTP, 2010) where no adverse effects were observed even at the highest dose tested. Due to the uncertainty in the available data on developmental and reproduction toxicity, the CONTAM applied an uncertainty factor of 10 in addition to the default uncertainty factor of 100 for the extrapolations from rodents to humans and for human variability.

Toxicity of hexavalent chromium

Cr (VI) has been classified by IARC as being carcinogenic to humans (group 1) and was identified by the CONTAM Panel as genotoxic and carcinogenic. An MOE approach was applied, based on the combined incidence of adenomas and carcinomas in the small intestine from a 2-year study in mice (NTP, 2008). The CONTAM Panel noted that the $BMDL_{10}$ and resulting MOEs would be 3.8 times higher if based on carcinoma incidence only.

Observations in humans showed toxicity of chromium at very high doses resulting after accidental and intended intoxications. Epidemiological data on dietary exposure were negative or inconclusive.

Given that the CONTAM Panel used the rodent tumour data and the MOE approach for the risk assessment of Cr(VI), uncertainty exists on whether the MOE of 10 000 adequately accounts for possible differences in the level of reduction of Cr(VI) in GI tract in humans as compared to rodents. Uncertainty exists on the impact of the competing processes of reduction and absorption of ingested

hexavalent chromium, the transit of chromium through the GI tract prior to absorption, and the efficiency of Cr(VI) reduction at the low human exposure levels as compared to the high dose levels used in the rodent bioassay. This adds considerably to the overall uncertainty of the risk assessment of ingested hexavalent chromium

9.5. Summary of uncertainties

Summaries of the uncertainty evaluations for Cr(III) and Cr(VI) highlighting the main sources of uncertainty and indicating an estimate of whether the respective source of uncertainty might have led to an over- or underestimation of the exposure or the resulting risk are presented in Table 26 and Table 27, respectively.

Trivalent chromium in food

Table 26: Summary of qualitative evaluation of the impact of uncertainties on the risk assessment of the dietary exposure of Cr(III) in food.

Sources of uncertainty	Direction
Measurement uncertainty of analytical results	+/-[(a)]
Extrapolation of occurrence data from mainly one Member States to the whole of Europe	+/-
Use of lower bound and upper bound occurrence data in the dietary exposure estimations	+/-
Possible use of occurrence data from targeted sampling	+
Use of different dilution factors on the occurrence data to calculate exposure	+/-
Limited data on exposure for specific groups (vegetarians, consumers of supplements)	+/-
Limited information on exposure of infants	+/-
Influence of food preparation with stainless steel on Cr(III) concentration	-
Exposure from human milk based on limited data	+/-
Insufficient data on developmental and reproductive toxicity	+/-

(a): +: uncertainty with potential to cause over-estimation of exposure/risk; -: uncertainty with potential to cause under-estimation of exposure/risk

The CONTAM Panel concluded that the impact of the uncertainties on the risk assessment of exposure to Cr(III) in food is large.

Hexavalent chromium in drinking water

Table 27: Summary of qualitative evaluation of the impact of uncertainties on the risk assessment of the exposure of Cr(VI) in water intended for human consumption and mineral waters.

Sources of uncertainty	Direction
Measurement uncertainty of analytical results	+/-[(a)]
Extrapolation of occurrence data from mainly one Member State to the whole of Europe	+/-
Use of lower bound and upper bound occurrence data in the exposure estimations	+/-
Possible use of occurrence data from targeted sampling	+
Cr(VI) levels obtained from the analysis of a very limited number samples and covering only bottled water	+/-
Limited information on exposure of infants	+/-
Assuming that all chromium in water is Cr(VI)	+
Assuming that no Cr(VI) is present in food, including beverages	-
Insufficient data on the impact of exposure from smoking to the dietary exposure	-
Uncertainty on the level of reduction and absorption of Cr(VI) in GI tract in humans as compared to rodents	+/-
Uncertainty on the efficiency of Cr(VI) reduction at the low dose human exposure levels as compared to the high dose levels used in the rodent bioassay.	+
Combined incidence of adenoma and carcinoma in the small intestine for the MOE calculations	+

(a): +: uncertainty with potential to cause over-estimation of exposure/risk; -: uncertainty with potential to cause under-estimation of exposure/risk

The CONTAM Panel concluded that the impact of the uncertainties on the risk assessment of exposure to Cr(VI) in drinking water is very large.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

General

- Chromium can exist in different oxidation states, of which the trivalent form (Cr(III)) and the hexavalent form (Cr(VI)) are the major forms in food and drinking water, respectively.
- Chromium can be present in food and drinking water arising from both natural and anthropogenic sources.

Sampling and methods of analysis

- Two European standardised methods for the determination of total chromium in food are available while four standardised methods are available for water.
- For Cr(VI) analysis, two standardised methods exist for various types of water, based on colorimetric reactions with 1,5-diphenylcarbazide, by UV-Vis and spectrometric detection.
- Modern analytical techniques, such as liquid chromatography (LC) coupled to inductively coupled plasma mass spectrometry (ICP-MS), and the use of speciated isotope dilution (SID) are a suitable tool for speciation of chromium in both food and water.
- Several standard or certified reference materials are available for total chromium.
- Regular proficiency testing schemes are organised by a number of providers for total chromium in foodstuffs and water, and for Cr(VI) in water.

Occurrence

- A total of 27 074 analytical results were reported for food and 52 735 for drinking water, mainly from one Member State, although 11 other European countries were represented.
- Information on oxidation state was not available for occurrence data in food. For water, only 88 analytical results were received on Cr(VI), all in bottled water.
- In the final dataset, left-censored data represented 50 % of the analytical results in food and 91 % of the data on drinking water. Concerning the data on bottled water reported as Cr(VI) and total chromium, 11 % of the samples reported no quantified values for both parameters.
- At FoodEx level 1, all food groups were well represented, with a maximum of 4 647 samples in the food group 'Vegetables and vegetable products (including fungi)'.
- The food groups at FoodEx Level 1 with the highest mean Cr occurrence values were 'Products for special nutritional use' (12129 µg/kg, LB = UB), 'Herbs, spices and condiments' (1627-1665 µg/kg, LB-UB), and 'Sugar and confectionery' (625-639 µg/kg, LB-UB).
- Among the data on water, tap water samples were the most reported (60.6 %) with mean Cr occurrence values of 0.2 µg/L and 1.9 µg/L at the LB and the UB, respectively. In bottled water, the mean occurrence values were similar, ranging between 0.3 µg/L for carbonated mineral water (LB) and 3.4 µg/L at the UB reported for unspecified bottled water.
- There is a lack of data on the presence of Cr(VI) in food. The CONTAM Panel decided to consider all the reported analytical results in food as Cr(III). This assumption was based on

the outcome of recent speciation work, the fact that food is by-and-large a reducing medium, and that oxidation of Cr(III) to Cr(VI) would not be favoured in such a medium.

- However, the CONTAM Panel noted that if even a small proportion of total chromium in food was in the form of Cr(VI), it could contribute substantially to Cr(VI) exposure.
- The CONTAM Panel decided to consider all the chromium present in drinking water as Cr(VI). This assumption was based on the evidence that those water samples where both Cr(VI) and total Cr were quantified showed an average ratio Cr(VI)/total Cr of almost one. In addition the water intended for human consumption is usually treated with oxidizing agents to make it potable, which could favour the presence of Cr(VI) over that of Cr(III).

Exposure to trivalent chromium via food excluding drinking water

- Mean chronic dietary exposure to Cr(III), across the different dietary surveys and age classes, ranged from 0.6 (minimum LB) to 5.9 µg/kg b.w. per day (maximum UB). The 95th percentile dietary exposure ranged from 1.1 (minimum LB) to 9.0 µg/kg b.w. per day (maximum UB).
- Among the different age classes, toddlers showed the highest mean chronic dietary exposure to Cr(III) with values ranging from 2.3 (minimum LB) to 5.9 (maximum UB) µg/kg b.w. per day.
- In 'Infants' and 'Toddlers' the main contributor to the exposure to Cr(III) were 'Foods for infants and small children', followed by 'Milk and dairy products' and 'Bread and rolls'.
- In the other age classes, the main contributors to the exposure to Cr(III) were the food categories 'Milk and dairy products, 'Bread and rolls', 'Chocolate (cocoa) products' (except for 'Elderly' and 'Very elderly' population) and 'Non-alcoholic beverages'. The food group 'Vegetables and vegetable products (including fungi)' contributed to the exposure to Cr(III) with median values that ranged between 4 % in 'Adolescents' and 'Other children', and 8 % in the 'Elderly' population.
- The assessment of the chronic dietary exposure to Cr(III) in vegetarians was based on very limited data. The results indicated virtually the same mean and 95th percentile dietary exposure in the vegetarian population as for the general population.
- Overall, the Comprehensive Database contains limited information on the consumption of fortified foods, foodstuffs for particular nutritional use (PARNUTS) and food supplements. Based on previous EFSA opinions, the combined exposure from supplemental intake in adults (i.e. from fortified foods, PARNUTS and food supplements) would be between 910 µg/day for a typical intake and 1540 µg/day for upper intake (13 µg/kg b.w. per day and 22 µg/kg b.w. per day, respectively for an adult of 70 kg b.w.).

Exposure to hexavalent chromium (via drinking water and water used for food preparation)

- The mean chronic exposure to Cr(VI) from drinking water consumption ranged from 0.7 (minimum LB) to 159.1 ng/kg b.w. per day (maximum UB). The 95th percentile exposure ranged from 2.8 (minimum LB) to 320.2 ng/kg b.w. per day (maximum UB).
- The highest exposure to Cr(VI) through the consumption of drinking water was estimated in the youngest populations ('Infants' and 'Toddlers').
- In those dietary surveys with reported data on consumption of bottled water, the mean chronic exposure to Cr(VI) from bottled water consumption ranged from < 0.1 (minimum LB) to 149.8 ng/kg b.w. per day (maximum UB, infants). The 95th percentile exposure ranged from 0.0 (minimum LB) to 148.7 ng/kg b.w. per day (maximum UB, 'Toddlers').
- The highest exposure to Cr(VI) through the consumption of bottled water was also estimated in the youngest populations ('Infants' and 'Toddlers').

- An additional contribution to the exposure to Cr(VI) was considered from the water used to prepare certain foods (coffee, tea infusions, and dry infant and follow-on food mainly, but also some others such as instant soup, evaporated and dried milk, and dehydrated fruit juice). A worst-case scenario, which assumed there was no reduction of the Cr(VI) present in water into Cr(III) when these foods are ingested immediately after their preparation. This scenario led to an increase up to two-fold in the exposure levels to Cr(VI), in comparison to those estimated via the consumption only of drinking water.

Non dietary exposure to trivalent and hexavalent chromium

- The CONTAM Panel could not quantify the contribution of non-dietary exposure to Cr(III) or Cr(VI) due to the existing uncertainties on the levels of exposure via inhalation, the absorption rates of different chromium compounds via the respiratory system and the relevance of different chromium species for non-dietary exposure.
- The CONTAM Panel concluded that the exposure via the diet likely represents the most important contribution to the overall exposure to Cr in the general population. Inhalation of Cr compounds present in particular in cigarette smoke may contribute to the overall exposure levels but the currently available information does not allow quantification of its relative contribution.

Hazard identification and characterisation

Toxicokinetics

- There can be differences in the bioavailability of chromium resulting from intake of different forms of Cr(III) compounds, with organic complexes being somewhat more bioavailable, but these differences are small and the overall bioavailability of trivalent chromium from all these sources is low.
- In contrast to Cr(III), Cr(VI) is able to cross cellular membranes.
- The absorption and tissue distribution of Cr(VI) depend strongly on the rate and extent of its reduction in the gastrointestinal tract but also on the ligands bound to Cr(VI) or the Cr(III) formed upon reduction of Cr(VI). The data available so far support that reduction along the gastrointestinal tract is efficient but that it cannot be excluded that even at low dose levels a small percentage of Cr(VI) escapes gastrointestinal reduction to Cr(III).

Trivalent chromium

Repeated dose toxicity

- Cr(III) displays very little (small decrease in body weight or body weight gain) to no toxicity in experimental animals.
- The relevant NOAELs were 506 and 286 mg Cr(III)/kg b.w. per day (the highest doses tested) for the sub-chronic and long-term toxicity in the rat, respectively.

Developmental and reproductive toxicity

- Conflicting results on reproductive effects of Cr(III) compounds have been reported. In the studies where effects on reproduction or development were reported, the lowest LOAELs were in the order of 30 mg/kg b.w. per day. The CONTAM Panel noted that a majority of the studies have methodological limitations and were not designed for establishing reference doses.

Genotoxicity and carcinogenicity

- Cr(III) compounds have the potential to react with DNA in acellular systems, however restricted cellular access limits or prevents genotoxicity.
- Cr(III) compounds did not induce genotoxicity in the majority of bacterial assays; mixed results were reported in mammalian cells and results in standard *in vivo* assays by oral route of exposure were negative.
- Several *in vitro* and *in vivo* studies showed that Cr(III) compounds at high concentrations cause oxidatively-generated DNA damage.
- Cr(III) is not carcinogenic in experimental animals after oral intake.

Hexavalent chromium

Repeat dose toxicity (non neoplastic effects)

- After repeated oral administration, the major target organs of Cr(VI) compounds in rats and mice are the haematological system, liver, kidney and the gastrointestinal tract.
- The lowest NOAEL in a 2-year rat study was 0.21 mg Cr(VI)/kg b.w. per day based on haematological effects, liver toxicity, hystiocytic cellular infiltration in mesenteric lymph nodes and the duodenum observed at 0.77 mg Cr(VI)/kg b.w. per day.
- No NOAEL was established in a 2-year mouse study, based on haematological effects, liver toxicity, hystiocytic cellular infiltration in mesenteric lymph nodes and hyperplasia in the duodenum observed at the lowest tested dose of 0.38 mg Cr(VI)/kg b.w. per day.

Developmental and reproductive toxicity

- Studies in animals show that acute- and intermediate-duration exposure to Cr(VI) produce adverse reproductive effects, with the male reproductive system exhibiting the highest sensitivity.
- Developmental effects (embryotoxicity, fetotoxicity and increased frequency of gross, visceral and skeletal malformations) have been observed in rats or mice treated with Cr(VI) during gestation.
- Cr(VI) has been shown to cross the placental barrier and accumulate in fetal tissues.
- Effects on reproduction and development occur at higher doses than the effects on the haematological system, liver and duodenum.

Genotoxicity and carcinogenicity

- Cr(VI) compounds are genotoxic in bacterial and mammalian cell assays.
- Genotoxicity was also observed in some but not all *in vivo* studies upon oral administration.
- Cr(VI) was clearly genotoxic following intraperitoneal administration, indicating that the reductive capacity of the GI tract influences the genotoxic effects of Cr(VI) *in vivo*.
- Cr(VI) is carcinogenic in experimental animals after oral administration. Increased incidences of tumors of the squamous epithelium of the oral cavity were reported in male and female rats and of epithelial tissues of the small intestine in male and female mice.
- Intracellular reduction of Cr(VI) generates lower Cr valences, facilitating the production of reactive oxygen species, and ultimately Cr(III), which generates DNA adducts, representing the two possible modes of action for induction of carcinogenicity.

Human observations

- No well-designed prospective human studies were identified for oral exposure to total chromium, Cr(III) or Cr(VI).
- The very limited information from few case studies was not suitable to assess human toxicity after oral exposure to Cr(III) compounds.
- At very high doses Cr(VI) after accidental or intentional intoxication exerted acute health effects in the respiratory, haematological, hepatic and renal system and in the gastrointestinal tract where acute effects include abdominal pain, vomiting, ulceration, haemorrhage, necrosis, and bloody diarrhea.
- Cr(VI) was classified by IARC as carcinogenic for humans with respect to the cancer of the lung and also cancer of the nose and nasal sinuses based on evidence from occupational studies. The data on oral exposure are limited and provide no convincing evidence of an association with adverse health effects including cancer.
- Available data were insufficient to assess developmental and reproductive toxicity and the allergenic potential of Cr(VI) after oral exposure from food or water.

Biomonitoring

- Biological monitoring of exposure to Cr(VI) compounds is a common practice in occupational settings. In principle, an accurate assessment of systemic exposure to Cr(VI) escaping reduction, can be obtained measuring chromium in red blood cells (RBC), since only Cr(VI) is able to cross RBC membranes and is very slowly released from these cells.
- No biomonitoring data are available on chromium concentrations in RBCs from the general population.

Dose response assessment

- The available human data did not provide information on dose-response relationships for Cr(III) or Cr(VI) upon oral exposure.
- For Cr(III) no dose-response modelling was possible for data in experimental animals since no effects were observed even at the highest dose in the relevant studies.
- For Cr(VI) dose-responses could be assessed for neoplastic effects and for non-neoplastic lesions in male and female rats and mice, and for haematotoxic effects in male rats.
- Dose-response data on squamous neoplastic lesions on the epithelium of the oral cavity in rats and on epithelial cell neoplastic lesions in the small intestine in mice were suitable for applying the BMD approach and calculating the $BMDL_{10}$ for neoplastic effects of Cr(VI).
- Since there were no statistically significant differences between males and females, the CONTAM Panel derived for the incidence of adenoma or carcinoma combined a $BMDL_{10}$ of 1.0 mg/kg b.w. per day and for the incidence of carcinoma only at all sites a $BMDL_{10}$ of 3.8 mg/kg b.w. per day.
- From the dose-response data for effects in the liver, pancreas and small intestine in mice, the CONTAM Panel identified incidences of chronic inflammation of the liver in female rats, diffuse epithelial hyperplasia in the duodenum in male and female mice, and histiocytic cellular infiltration in mesenteric lymph nodes in male and female mice as relevant non-neoplastic endpoints suitable for applying the BMD approach.
- When applying the BMD approach most dose response data did not allow identification of a $BMDL_{10}$ value, since the BMD/BMDL ratios and the range of the acceptable BMDL values were larger than one order of magnitude.

- For the incidence of diffuse epithelial hyperplasia in the duodenum in male mice the $BMDL_{10}$ value of 0.11 mg Cr(VI)/kg b.w. per day was calculated.
- The CONTAM Panel identified haematocrit, haemoglobin, MCV and MVH values measured in male rats at day 22 after start of treatment as critical endpoints for haematological effects of Cr(VI) and suitable for a BMD analysis. The lowest $BMDL_{05}$ of 0.2 mg Cr(VI)/kg b.w. per day was calculated for decreased hematocrit in male rats.

Derivation of Health-based Guidance Values/Margin of exposure approach

Trivalent chromium

- The Panel derived a TDI of 300 µg Cr(III)/kg b.w. per day from the relevant NOAEL of 286 mg/kg b.w. per day identified in a long-term rat study, applying the default uncertainty factor of 100 to account for species differences and human variability, and an additional uncertainty factor 10 to account for the absence of adequate data on reproductive and developmental toxicity.

Hexavalent chromium

- Since the adenoma-carcinoma sequence is a well recognised pathway of carcinogenesis in the GI tract, in a conservative approach, the CONTAM Panel selected the $BMDL_{10}$ of 1.0 mg Cr(VI)/kg b.w. per day for combined adenomas or carcinomas of the small intestine in male and female mice as the reference point for the estimation of the MOE for neoplastic changes.
- From the analysis of non-neoplastic lesions in experimental animals, the CONTAM Panel selected the lowest $BMDL_{10}$ value of 0.11 mg Cr(VI)/kg b.w. per day for diffuse epithelial hyperplasia of the duodenum in female mice as the reference point for the estimation of the MOE for non-neoplastic lesions.
- From the analysis of haematological effects in rats, the CONTAM Panel selected the lowest $BMDL_{05}$ of 0.2 mg/kg b.w. per day for decrease of haematocrit in male rats. This value was used as the reference point for the MOE estimation of haematotoxic effects.

Risk characterisation

Trivalent chromium

- The mean dietary exposure across all age groups (minimum LB of 0.6 µg/kg b.w. per day and maximum UB of 5.9 µg/kg b.w. per day) as well as the 95th percentile exposure (minimum LB of 1.1 µg/kg b.w. per day and maximum UB of 9.0 µg/kg b.w. per day) are well below the TDI of 300 µg Cr(III)/ kg b.w. per day.
- Although based on limited consumption data, the dietary exposure to Cr(III) of the vegetarian population seems to be similar to that estimated for the general population. Therefore, the dietary exposure of vegeterians is well below the TDI of 300 µg Cr(III)/kg b.w. per day.
- The combined exposure from supplemental intake in adults (i.e. from fortified foods, PARNUTS and food supplements) would be between 910 µg/day for a typical intake and 1540 µg/day for upper intake (13 µg/kg b.w. per day and 22 µg/kg b.w. per day, respectively for an adult of 70 kg b.w.). Considering this exposure and the maximum estimated contribution coming from the diet for adults (95th percentile of 2.6 µg/kg b.w. per day), the total exposure is well below the TDI of 300 µg Cr(III)/kg b.w. per day.

- The current dietary exposure to Cr(III) does not raise concerns from a public health point of view.

Hexavalent chromium

- As recommended for substances which are both genotoxic and carcinogenic, the CONTAM Panel adopted the MOE approach for the risk characterisation of neoplastic effects of Cr(VI), by using the $BMDL_{10}$ of 1.0 mg Cr(VI)/kg b.w. per day for the combined incidence of adenomas and carcinomas in the mouse small intestine as RP.
- The EFSA Scientific Committee has concluded that for substances that are both genotoxic and carcinogenic, an MOE of 10 000 or higher, based on a $BMDL_{10}$ from an animal study, is of low concern from a public health point of view.
- The MOEs calculated for all age groups on the basis of the mean chronic exposure to Cr(VI) via consumption of drinking water indicate low concern (MOE values > 10 000) for all age groups with the exception of infants at UB exposure estimates (maximum UB - minimum LB, 6 300 - 71 000).
- When considering the 95th percentile exposure, MOE values below 10 000 were found at UB exposure estimates, particularly for 'Infants' (maximum UB - minimum LB, 3 100 - 21 000), 'Toddlers' (maximum UB - minimum LB, 4200 - 62 000), and 'Other children' (maximum UB - minimum LB, 6 600 - 360 000).
- Similarly to the risk characterisation carried out for all drinking water, in the case of exposure to Cr(VI) through the consumption of bottled water MOEs values below 10 000 were mainly found at UB estimates when considering the 95th percentile exposure in the youngest populations ('Infants', 'Toddlers' and 'Other children').
- The CONTAM Panel noted that the MOE values calculated for exposure to Cr(VI) via consumption of all types of drinking water, as well as only bottled water were highly influenced by the high proportion of left-censored data.
- In addition, when interpreting the numerical values of the MOEs, it should be considered that they were calculated by using as RP the $BMDL_{10}$ for the combined incidence of adenomas and carcinomas in the mouse small intestine. Because of lack of *in vivo* data on the capacity and rate of reduction of Cr(VI) in the rodent and human gastrointestinal tract, there is a significant uncertainty associated with the use of tumour data in mice to estimate risk at doses of Cr(VI) relevant for human exposure.
- Based on the MOE values for neoplastic effects, the CONTAM Panel concluded that the current levels of exposure to Cr(VI) via the consumption of all types of water or of bottled water only are of low concern from a public health point of view for the average consumers but there might be a potential concern for high consumers particularly in 'Infants', 'Toddlers' and 'Other children'.
- The inclusion of the water used in the preparation of specific foods (coffee, tea infusions, and infant dry and follow-on food mainly, but also some others such as instant soup, evaporated and dried milk, and dehydrated fruit juice) led to an increase up to two-fold of the exposure to Cr(VI). However, the CONTAM Panel was not able to consider this additional contribution to the exposure to Cr(VI) when deriving MOEs since no reliable data to quantify Cr(VI) in food exist
- The MOEs calculated for non-neoplastic lesions, based on the $BMDL_{10}$ of 0.11 mg Cr(VI)/kg b.w. per day selected as RP, were 690 and 340 when considering the maximum UB for mean and 95th percentile chronic exposure, respectively. The MOEs calculated for haematotoxic effects, based on the $BMDL_{05}$ of 0.2 mg/kg b.w. per day selected as RP, were 1 300 and 630 when considering the maximum UB for mean and 95th percentile chronic exposure, respectively.

- The CONTAM Panel considered that for the critical thresholded effects, MOEs larger than 100 would indicate a low concern for human health and therefore concluded that for non-neoplastic lesions and haematological effects the current exposure levels to Cr(VI) via drinking water are of no concern from a public health point of view.

RECOMMENDATIONS

- Data should be generated using sensitive analytical methodologies which specifically measure the content of Cr(III) and Cr(VI) in food and drinking water in different EU Member States.
- Further data are needed to characterise the percentage of Cr(VI) reduction in the GI tract at doses relevant for human exposure and at the doses used in the rodent bioassays.

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APPENDICES

APPENDIX A: LIMITS OF DETECTION (LOD) FOR CR(III) AND/OR CR(VI) IN WATERS ACCORDING TO THE ANALYTICAL METHODS REPORTED IN THE LITERATURE (IN µG/L)

Analytical technique	LOD of Cr(III)	LOD of Cr(VI)	Reference
Off-line separation			
UV-Vis	-	1	Jamaluddin and Reazul (2011)
IC-UV-Vis	-	0.004 - 0.015	EPA 218-7 (2011)
IC-UV-Vis	0.008	-	Amin and Kassem (2012)
IC-UV-Vis	-	0.001 - 0.3	Water Research Foundation (2012)
Chemiluminescence	-	0.2	Li et al. (2006)
Chemiluminescence	-	0.0002	Kanwal et al. (2012)
DPAdSV	< 0.053[(a)]	< 0.071	Dominguez and Arcos (2002)
DPAdSV	2		Zhu et al. (2007)
CAdSV	-	0.004	Bobrowski et al. (2004)
CAdSV	-	0.016	Lin et al. (2005)
CAdSV	-	0.002	Abbasi and Bahiraei (2012)
FAAS	6.1	-	Aydin and Soylak (2007)
FAAS	0.7	-	Bulut et al. (2009)
FAAS	0.7	-	Matos et al. (2009)
FAAS	1.33	-	Uluozlu et al. (2009)
FAAS	-	0.7	Zeng et al. (2012)
IC-FAAS	0.2	-	Cespon-Romero et al. (1996)
SPE-FAAS	0.75	-	Tuzen and Soylak (2006)
SPE-FAAS	-	0.6	Duran et al. (2007)
SPE-FAAS	-	1.94	Saygi et al. (2008)
GFAAS	0.021	-	Liang and Sang (2008)
On-line separation			
HPLC-UV-Vis	0.005	0.007	Kaur and Malik (2009)
HPLC-Chemunilescence	0.05	0.1	Beere and Jones (1994)
HPLC-FAAS	30	0.5 - 20	Posta et al. (1993)
HPLC-ICP-AES	1000	2000	Byrdy et al. (1995)
HPLC-ICP-MS	0.4	1.0	Byrdy et al. (1995)
HPLC-ICP-MS	0.1	0.2	Barnowski et al. (1997)
HPLC-ICP-MS	0.6	0.6	Seby et al. (2003)
HPLC-ICP-CCT-MS	0.013	0.016	Sakai and McCurdy (2007)
HPLC-ICP-CCT-MS	0.017	0.009	McSheehy et al. (2006)
HPLC-ICP-CCT-MS	ni[(b)]	ni	Agilent (2011)
HPLC-ICP-CCT-MS	0.05	0.05	Wolf et al. (2011)
HPLC-ICP-SID-MS	0.4	0.04	Ma and Tanner (2008)

ni: not indicated; LOD: Limit of detection; UV-Vis: Ultraviolet-visible; DPAdSV: Differential pulse adsorptive stripping voltammetry; CAdSV: Catalytic adsorptive stripping voltammetry; FAAS: Flame atomic absorption spectrometry; IC: Ion chromatography; SPE: Solid-phase extraction; GFAAS: Graphite furnace atomic absorption spectrometry; HPLC – High performance liquid chromatography; ICP: Inductively coupled plasma; AES: atomic emission spectrometry; MS: Mass spectrometry; CCT: Collision/reaction cell technology; SID: Speciated isotope-dilution.
(a): no LOD indicated, estimation based of quantified values given;
(b): not indicated, just indication of low levels (ng/L) and background equivalent concentration (BEC) < 5 ng/L for Cr(VI).

APPENDIX B: STANDARD OR CERTIFIED REFERENCE MATERIALS

Table B1: Standards or certified reference materials relevant to total chromium analysis in food and water (in mg/kg dry mass or µg/L).

Food Type	Descriptor (supplier)[a]	Total chromium[b]
Food		
Dogfish muscle	DORM-2 (NRCC)	34.7 ± 5.5
Fish protein	DORM-3 (NRCC)	1.89 ± 0.17
Lobster hepatopancreas	TORT-2 (NRCC)	0.77 ± 0.15
Lobster hepatopancreas (non defatted)	LUTS-1 (NRCC)	0.53 ± 0.08
Fish muscle	IAEA 407 (IAEA)	0.73 ± 0.06
Tuna fish	IAEA 436 (IAEA)	0.194 ± 0.025
Whey Powder	IAEA 155 (IAEA)	0.59 ± 0.07
Milk powder (non-fat)	SRM 1549 (NIST)	0.0026 ± 0.0007
Tomato leaves	SRM 1573a (NIST)	1.99 ± 0.06
Bovine liver	SRM 1577c (NIST)	0.053 ± 0.014
Mussel tissue	ERM-CE278k (IRMM)	0.73 ± 0.22
Crab	LGC 7160 (LGC)	0.29± 0.14
Mixed polish herbs	INCT-MPH-2 (INCT)	1.69 ± 0.13
Tea Leaves	INCT-TL-1 (INCT)	1.91 ± 0.22
Wheat	GBW 10011 (IGGE)	0.096 ± 0.014
Soybean	GBW 10013 (IGGE)	0.28 ± 0.04
Cabbage	GBW 10014 (IGGE)	1.8 ± 0.3
Spinach	GBW 10015 (IGGE)	1.4 ± 0.2
Tea	GBW 10016 (IGGE)	0.45 ± 0.10
Milk powder	GBW 10017 (IGGE)	0.39 ± 0.04
Chicken	GBW 10018 (IGGE)	0.59 ± 0.11
Apple	GBW 10019 (IGGE)	0.30 ± 0.06
Cod fish tissue	7402-a (NMIJ)	0.72 ± 0.09
White rice flour	7502-a (NMIJ)	0.075 ± 0.013
Water		
Hard drinking water	ERM-CA011b (IRMM)	48.2 ± 1.6
Soft drinking water	ERM-CA022a (IRMM)	50.8 ± 2.7
Lyophilised solution	CRM 544 (IRMM)	49.4 ± 0.9
Drinking water	TMDW-500 (HPS)	20.0 ± 0.1
Simulated freshwater	SRM 1643e (NIST)	19.90 ± 0.23
Natural water	SRM 1640a (NIST)	40.54 ± 0.30
Spiked/fortified water	NWTM-15.2 (LGC)	16.4 ± 1.4
Spiked/fortified water	NWTM-23.4 (LGC)	6.8
Spiked/fortified water	NWTM-24.3 (LGC)	5.01
Spiked/fortified water	NWTM-27.3 (LGC)	1.74
Spiked/fortified water	NWTMDA-61.2 (LGC)	67.2
Spiked/fortified water	NWTMDA-64.2 (LGC)	290
Spiked/fortified water	NWTMDA-51.4 (LGC)	66
Spiked/fortified water	NWTMDA-53.3 (LGC)	340
Spiked/fortified water	NWTM-DWS.2 (LGC)	44.4
Water	NIM-GBW08608 (LGC)	33
Simulated rain water	NWTRAIN-04 (LGC)	0.861
River water	LGC6019 (LGC)	0.78
Surface water	SPS-SW1 (LGC)	2.00 ± 0.02
Surface water	SPS-SW2 (LGC)	10.0 ± 0.05
Water	NCS ZC76308 (LGC)	30 ± 2

(a): HPS: High Purity Standard (USA); IAEA: International Atomic Energy Agency (Austria); IGGE: Institute of Geophysical Exploration (China); INCT: Institute of Nuclear Chemistry and Technology (Poland); IRMM: Institute for Reference Materials and Measurements (Belgium); LGC: LGC (UK); NIST: National Institute of Standards and Technology (USA); NMIJ: National Metrology Institute of Japan (Japan); NRCC: National Research Council of Canada (Canada).

(b): The uncertainty usually given as 95 % confidence interval.

APPENDIX C: COMMONLY CONSUMED FOODS IN UNITED STATES AND THEIR CORRESPONDING ANALYTICAL CHROMIUM VALUES (ADAPTED FROM THOR ET AL., 2011)

Commonly consumed foods (descending order)	Mean mg/kg	Median mg/kg	Range mg/kg	SD
Protein sources, include meat, poultry, fish, eggs, nuts				
Beef: meat, beef, ground beef	1.68	0.09	0.013–4.95	2.83
Chicken: chicken breast	0.083	-	0.006-0.16	0.11
Pork: ham	0.021	0.022	0.00003-0.042	0.021
Eggs: egg, whole, cooked	0.023	0.005	0.00001-0.10	0.039
Peanuts: peanut butter	0.028	-	0.0018-0.038	0.014
Fish and shellfish: shrimp	0.158	0.210	0.004-0.26	0.136
Fruits and fruit juices				
Orange juice	0.005	0.004	0.001-0.009	0.004
Apple	0.082	0.033	0.00002-0.397	0.142
Banana	0.049	0.008	0.00001-0.164	0.068
Apple juice	0.002	-	0.0001-0.003	0.002
Strawberries	0.017	0.010	0.008-0.032	0.013
Orange	0.049	0.017	0.00001-0.255	0.092
Peach	0.062	-	0.050-0.074	0.017
Cantaloupe	0.043	0.050	0.00001-0.080	0.040
Vegetables				
Potato: potato, peeled, raw	0.011	0.006	0.003-0.030	0.013
Head lettuce	0.005	0.001	0.001-0.013	0.007
Dry edible beans: pinto beans	0.580	-	0.28-0.88	0.424
Romaine and leaf lettuce	0.110	0.057	0.00001-0.327	0.147
Onion, fresh	0.510	0.342	0.017-1.34	0.593
Tomato, fresh	0.082	0.007	0.00003-0.461	0.170
Cabbage	0.166	0.079	0.006-0.50	0.229
Carrot	0.032	0.017	0.004-0.090	0.035
Celery	0.051	0.070	0.003-0.080	0.042
Milk and dairy products				
Whole milk (fluid)	0.011	0.002	0.001-0.029	0.016
Skim milk (fluid)	0.009	0.009	0.00001-0.020	0.011
Yogurt	0.015	0.016	0.00001-0.030	0.017
American cheese	0.021	0.020	0.014-0.030	0.008
Grains				
Ready-to-eat cereals: Kellogg's Raisin Bran	0.116	0.132	0.080-0.135	0.031
Nonwhole grain yeast bread: white bread	0.091	0.047	0.00003-0.305	0.116
Whole grain yeast bread: whole wheat bread	0.149	0.105	0.00008-0.382	0.157
Hot cereals: Nabisco quick prepared cream of wheat	0.072	0.086	0.039-0.090	0.028
Fats				
Butter	0.027	0.007	0.003-0.130	0.050
Margarine	0.019	0.003	0.0004-0.070	0.034

SD: Standard deviation

APPENDIX D: OCCURRENCE DATA OF TOTAL CHROMIUM IN BREAST MILK[18]

Country	n (number of samples)	Total maternal intake (µg/day) Mean (range)	Stage of lactation	Chromium concentration (µg/L)			Reference
				mean ± SD	median ± SD	range	
United Arab Emirates	209 (205)	Not reported	< 1 week-80 weeks	0.689 ± 0.517	0.591	0.00-2.53	Abdulrazzaq et al. (2008)
USA	17	41.08 ± 0.416 [(a)]	60 days	0.178 ± 0.021 [(a, b)]			Anderson et al. (1993)
Italy	8	Not reported	2-6 days 12-16 days 21 days	1.1 ± 0.4 1.1 ± 0.2 1.2 ± 0.5			Aquilio et al. (1996)
France	(8)	Not reported	1-88 days	1.2 ± 0.4 [(c)]			Bougle et al. (1992)
Egypt		Not reported	3 weeks 6 weeks	53 80			Carter et al. (1968)
USA	17 6 26 23 9 (overall 255)	Not reported	0-14 days 15-28 days 1-3 months 4-6 months 7+ months overall	0.29 ± 0.09 0.27 ± 0.13 0.28 ± 0.11 0.26 ± 0.12 0.46 ± 0.41 0.30 ± 0.17		0.06-1.56	Casey and Hambidge (1984)

[18] This table was prepared by the Standing Working group on Dietary Reference Values for minerals 2012-2015 (DRV MIN) of the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA Panel). The table will be published in the Scientific Opinion on Dietary Reference Values for chromium (EFSA NDA Panel, in preparation).

Appendix D: Occurrence data of total chromium in breast milk (continued)

Country	n (number of samples)	Total maternal intake (µg/day) Mean (range)	Stage of lactation	Chromium concentration (µg/L)			Reference
				mean ± SD	median ± SD	range	
USA	11 (109)	Not reported	Day 1	0.24 ± 0.08		0.12-0.53	Casey et al. (1985)
			Day 2	0.23 ± 0.08			
			Day 3	0.23 ± 0.06			
			Day 4	0.25 ± 0.08			
			Day 5	0.34 ± 0.11			
			Day 8 ± 2 (6-10)	0.27 ± 0.05			
			Day 14 ± 3	0.22 ± 0.09			
			Day 21 ± 3	0.28 ± 0.11			
			Day 23 ± 3	0.26 ± 0.07			
			Overall	0.27 ± 0.10			
Italy	21 (123)	Not reported	Mature (≥ 15 days)		≤ 0.3	≤ 0.3-876	Clemente et al. (1982)
Spain	(21)	Not reported	1-10 days	1.80 ± 0.75		0.45-3.00	Cocho et al. (1992)
			> 10 days	1.25 ± 0.74		0.27-2.27	
			Overall	1.56 ± 0.78		0.27-3.00	
Belgium	(9)	Not reported	0-3 days	0.18 ± 0.34		0.09-0.34	Deelstra et al. (1988)
	(7)		5-10	0.21 ± 0.06		0.15-0.33	
	(10)		30-60	0.14 ± 0.05		0.10-0.23	
Finland	10 (10)	30	8-18 days	0.43 ± 0.13			Kumpulanien et al. (1980a)
	5 (5)		47-54 days	0.39 ± 0.21			
	5 (5)		128-159 days	0.34 ± 0.12			
Finland	5 (5)	34-40	6-8 weeks	(0.19-0.69) ± (0.02-0.06) [a, d]			Kumpulanien et al. (1980b)
	4 (5)	21-38	17-22 weeks	(0.24-0.54) ± (0.01-0.06) [a, d]			
USA	6	400 µg ^{53}Cr (as Cr chloride) for 4 days; dietary intake not reported	1-2 months	0.09-0.46 [d] No ^{53}Cr detected		0.05-1.06[b]	Mohamedshah et al. (1998)

Appendix D: Occurrence data of total chromium in breast milk (continued)

Country	n (number of samples)	Total maternal intake (µg/day) Mean (range)	Stage of lactation	Chromium concentration (µg/L)			Reference
				mean ± SD	median ± SD	range	
Nigeria	45	Not reported	6.1 months	110			Okolo et al. (2001)
Guatemala Hungary Nigeria Philippines Sweden Zaire	(51)	Not reported	3 months		1.17 ± 0.14 0.78 ± 0.21 4.35 ± 1.78 3.46 ± 0.60 1.48 ± 0.57 1.07 ± 0.55		Parr et al. (1991)
Germany, Poland, Czech Republic	19 (536)	256 ± 187 [e] Median: 206	3-68 weeks	10.8	10.8	3.1-19.4	Wappelhorst et al. (2002)
Japan	(1166)	Not reported	1-5 days 6-10 days 11-20 days 21-89 days 90-180 days 181-365 days Summer Winter Overall	17 ± 10 35 ± 54 45 ± 53 50 ± 33 76 ± 54 25 ± 17 67 ± 39 51 ± 52 59 ± 47			Yamawaki et al. (2005)
Japan	79 (64) [f]	Not reported	5-191 days	1.73 ± 2.57	1.00	< 0.1-18.7	Yoshida et al. (2008)

(a): mean ± SE
(b): calculated using molecular weight of chromium 52.9961
(c): mean ± SEM
(d): individual means
(e): mean ± SD
(f): 15 samples were below the limit of detection (< 0.1 µg/L)

APPENDIX E: AVERAGE CHROMIUM OCCURRENCE VALUES (µG/KG) IN THE DIFFERENT FOODS USED TO CALCULATE DIETARY EXPOSURE TO CR(III)

As described in the text chromium concentrations in food were considered as Cr(III). Occurrence values were rounded up to one decimal place.

FOODEX[a]	Average values (µg/kg)			Groups
	N	LB	UB	
Alcoholic beverages (unspecified)	115	4.4	47.6	Alcoholic beverages
Alcoholic mixed drinks	9	0.6	19.2	
Beer and beer-like beverage (unspecified)	106	9.9	17.5	
Beer, strong	26	3.6	9.5	
Beer, regular	328	6.6	10.8	
Beer, alcohol-free	15	52.4	57.4	
Beer-like beverages (Malt drink)[b]	16	0.0	8.0	
Liqueur	20	24.5	38.9	
Spirits	11	25.4	41.6	
Wine	671	26.4	45.3	
Wine-like drinks (e.g. Cider, Perry)	273	6.1	37.6	
Butter	23	175.6	178.5	Animal and vegetable fats and oils
Pork lard (Schmaltz)	20	262.4	264.2	
Margarine and similar products	43	8.6	31.1	
Peanuts butter	2	282.5	282.5	
Vegetable oil (unspecified)[c]	90	430.7	490.7	
Olive oil	15	7.1	24.4	
Rapeseed oil	11	425.0	426.8	
Sunflower oil	57	591.5	680.2	
Composite food (including frozen products)(unspecified)[c]	295	66.6	69.3	Composite food (including frozen products)
Prepared salads	7	102.0	102.0	
Vegetable-based meals	26	176.5	180.0	
Eggs and egg products	82	21.8	29.1	Eggs and egg products
Fish and other seafood (including amphibians, reptiles, snails and insects) (unspecified)[c]	1616	95.0	108.0	Fish and other seafood (including amphibians, reptiles, snails and insects)
Crustaceans	169	46.1	54.2	
Fish meat (unspecified)[c]	931	60.2	71.6	
Sole (Limanda; Solea)	20	103.5	108.2	
Bass (Marone)[b]	1	637.0	637.0	
Bream (Charax)	17	157.5	157.5	
Sea catfish and wolf-fish (Anarhichas)	6	145.2	146.7	
Roach (Rutilus)	5	378.8	378.8	
Plaice (Pleuronectes)	17	144.8	157.2	
Fish offal	29	55.4	62.5	
Fish products	53	182.4	183.3	
Water molluscs	380	205.2	218.8	
Food for infants and small children (unspecified)	65	41.6	68.1	Food for infants and small children
Cereal-based food for infants and young children	150	57.7	68.6	
Follow-on formulae, powder	116	52.7	61.3	
Follow-on formulae, liquid[d]	116	6.5	7.7	
Infant formulae, powder	177	61.6	78.9	
Infant formulae, liquid[d]	177	7.7	9.9	
Ready-to-eat meal for infants and young children	145	46.8	52.1	
Yoghurt, cheese and milk-based dessert for infants and young children	28	37.3	37.3	
Fruit juice and herbal tea for infants and young children	13	2.4	15.0	

Appendix E: Average chromium occurrence values (µg/kg) in the different foods used to calculate dietary exposure to Cr(III) (continued)

FOODEX[a]	N	Average values (µg/kg) LB	UB	Groups
Fruit and fruit products (unspecified)[c]	1448	21.7	38.7	Fruit and fruit products
Citrus fruits	79	18.4	25.2	
Pome fruits	255	9.5	21.9	
Stone fruits	126	8.8	28.1	
Berries and small fruits	596	11.7	25.45	
Miscellaneous fruits[c]	209	15.1	42.5	
Table olives (Olea europaea)	2	145.5	145.5	
Dried fruits	80	71.4	126.3	
Jam	5	64.0	74.0	
Marmalade[c]	1	0.0	50.0	
Other fruit spreads	40	169.2	169.2	
Other fruit products (excluding beverages)	55	62.9	65.4	
Fruit and vegetable juices[c]	1216	9.7	24.0	Fruit and vegetable juices
Grains and grain-based products	17	12.3	35.5	Grains and grain-based products
Bread and rolls (unspecified)	32	50.0	72.8	Bread and rolls
Wheat bread and rolls	295	88.8	115.0	
Rye bread and rolls	87	39.6	46.0	
Mixed wheat and rye bread and rolls	57	36.5	42.6	
Multigrain bread and rolls	39	30.1	42.1	
Unleavened bread, crisp bread and rusk	8	56.6	97.25	
Other bread	25	69.3	78.1	
Bread products	10	106.6	108.6	
Breakfast cereals (unspecified)	10	75.2	75.2	Breakfast cereals
Cereal flakes	131	31.5	83.8	
Popped cereals	8	37.0	78.3	
Grits	3	75.3	75.3	
Porridge	22	24.2	31.1	
Muesli	33	194.7	208.9	
Mixed breakfast cereals	2	263.0	263.0	
Cereal bars	6	317.7	317.7	
Fine bakery wares (unspecified)	25	114.0	116.1	Fine bakery wares
Pastries and cakes (unspecified)	76	84.0	86.4	Pastries and cakes
Croissant, filled with chocolate	2	358.0	358.0	
Biscuits (cookies) (unspecified)	32	151.2	152.1	Biscuits (cookies)
Biscuits, chocolate filling	4	298.7	298.7	
Biscuits, sweet, plain	8	221.6	231.6	
Sticks, salty	2	230.0	275.0	
Grain milling products (unspecified)[c]	559	37.2	65.6	Grain milling products
Wheat milling products	308	44.2	77.5	
Rye milling products	131	17.8	34.4	
Corn milling products	52	28.9	56.4	
Oat milling products	15	109.7	139.7	
Rice milling products	6	27.3	47.3	
Spelt milling products	29	21.3	60.7	
Other milling products	7	71.4	71.4	

Appendix E: Average chromium occurrence values (µg/kg) in the different foods used to calculate dietary exposure to Cr(III) (continued)

FOODEX[a]	N	Average values (µg/kg) LB	UB	Groups
Grains for human consumption (unspecified)[c]	2165	66.4	107.3	Grains for human consumption
Wheat grain	973	55.4	106.4	
Barley grain	152	50.1	96.2	
Corn grain	73	94.8	97.2	
Rye grain	322	101.9	132.0	
Spelt grain	152	32.0	85.3	
Buckwheat grain	132	61.0	99.0	
Millet grain	31	21.7	78.1	
Oats, grain	36	172.4	213.5	
Other grains[b]	3	0.0	36.7	
Rice	289	59.6	79.9	
Pasta (Raw) (unspecified)[c]	52	53.3	61.3	Pasta (Raw)
Pasta, wheat wholemeal, without eggs	9	90.0	143.3	
Glass noodle	14	256.3	262.8	
Noodle, rice[b]	1	14.0	14.0	
Pasta, wheat flour, with eggs	12	79.1	106.8	
Pasta, wheat flour, without eggs	52	49.9	70.0	
Baking ingredients	107	167.2	173.4	Baking ingredients
Condiment	21	28.7	83.9	Condiment
Dressing	3	53.3	70.0	Dressing
Flavourings or essences (unspecified)	23	31.3	69.1	Flavourings or essences
Liquorice (Glycyrrhiza glabra)	61	130.4	206.6	
Herb and spice mixtures	34	1503.4	1521.1	Herb and spice mixtures
Herbs (unspecified)[c]	114	211.6	217.5	Herbs
Parsley, herb (Petroselinum crispum)	65	97.1	99.8	
Sage, herb (Salvia officinalis)	6	296.0	296.0	
Rosemary, herb (Rosmarinus officinalis)[e]	1	211.6	217.5	
Thyme, herb (Thymus spp.)[e]	1	211.6	217.5	
Basil, herb (Ocimum basilicum)	13	220.3	225.3	
Tarragon, herb (Artemisia dracunculus)	2	150.0	150.00	
Chives, herb (Allium schoenoprasum)	11	163.6	187.1	
Dill, herb (Anethum graveolens)	6	70.7	87.3	
Seasoning or extracts	32	302.5	548.6	Seasoning or extracts
Spices (unspecified)	10	419.1	459.1	Spices
Turmeric (Curcuma) (Curcuma domestica syn. C. longa)	2	2380.0	2380.0	
Paprika powder	71	3200.0	3270.6	
Pepper, black and white (Piper nigrum)	105	2608.6	2610. 6	
Caraway (Carum carvi)	4	35.0	140.0	
Cinnamon (Cinnamonum verum syn. C. zeylanicum)	4	84250.0	84250.0	
Chilli powder	19	1833.6	1865.2	
Legumes, beans, dried (unspecified)[c]	407	151.7	168.0	Legumes, nuts and oilseeds
Peanut (Arachis hypogea)	137	121.0	158.7	
Beans (Phaseolus vulgaris)	59	163.4	165.4	
Lentils (Lens culinaris syn. L. esculenta)	61	184.3	192.1	
Peas (Pisum sativum)	39	258.0	264.9	
Scarlet runner bean (Phaseolus coccineus)	26	7.3	7.3	
Black eye bean (Vigna unguiculata)	4	222.5	222.5	
Soya beans (Glycine max)	57	190.6	199.6	
Soya beans flour	2	500.0	500.0	

Appendix E: Average chromium occurrence values (µg/kg) in the different foods used to calculate dietary exposure to Cr(III) (continued)

FOODEX[a]	N	Average values (µg/kg) LB	UB	Groups
Chick pea (Cicer arietinum)	13	74.7	74.7	
Beans, green, without pods (*Phaseolus vulgaris*)	72	58.0	63.9	
Peas, green, without pods (*Pisum sativum*)	82	28.0	40.4	
Oilseeds	455	214.0	227.3	
Tree nuts (unspecified)[c]	138	175.0	192.5	
Almond, sweet (Prunus amygalus dulcis)	106	209.0	226.1	
Cashew nuts (Anacardium occidentale)[b]	1	210.0	210.0	
Chestnuts (Castanea sativa)[b]	2	0.0	22.5	
Coconuts (Cocos nucifera)[b]	2	0.0	20.0	
Pistachios (Pistachia vera[b]	2	0.0	40.0	
Hazelnuts (Corylus avellana)	15	101.9	122.6	
Walnuts (Juglans regia)[b]	2	57.0	57.0	
Meat and meat products (including edible offal) (unspecified)[c]	2088	52.9	63.7	
Edible offal, farmed animals	771	54.6	64.4	
Edible offal, game animals	49	44.9	63.1	
Game birds	7	46.2	47.9	Meat and meat products (including edible offal)
Meat specialities	8	16.4	26.4	
Mixed meat[e]	1	51.5	66.0	
Pastes, pâtés and terrines	27	103.3	108.4	
Poultry	176	54.5	66.5	
Preserved meat	13	73.4	73.4	
Sausages	210	56.6	74.2	
Cow milk	229	13.0	17.9	
Sheep milk	7	15.0	17.1	
Milk based beverages	8	17.2	32.5	Milk and dairy products
Dried milk	3	62.0	62.0	
Cream and cream products	10	22.3	32.3	
Fermented milk products	175	24.4	100.1	
Cheese	102	53.7	70.8	
Milk and milk product imitates	34	22.2	29.9	
Tofu	15	96.0	126.1	Milk and milk product imitates
Soya cheese	4	121.0	132.5	
Soya drink	5	23.4	29.4	
Non-alcoholic beverages (excepting milk-based beverages) (unspecified)	119	7.9	23.5	
Soft drinks	260	4.0	16.0	
Tea (Infusion)[f]	231	6.6	6.6	
Tea and herbs for infusions (Solid) (unspecified)	32	2526.6	2526.6	
Tea (dried leaves and stalks, fermented or otherwise of Camellia sinensis)	46	338.3	338.3	
Camomile flowers (Matricaria recutita)	12	1150.0	1150.0	Non-alcoholic beverages (excepting milk-based beverages)
Peppermint (Mentha × piperita)	104	309.4	309.4	
Rooibos leaves (Aspalathus spp.)	33	0.1	5.6	
Maté (Ilex paraguariensis)[b]	1	6930.0	6930.0	
Ginseng root (Panax ginseng)	3	1326.7	1326.7	
Cocoa beverage[g]	239	72.4	72.4	
Cocoa powder	239	4345.2	4345.2	
Coffee beans, roasted	30	108.2	119.2	
Coffee beans, roasted and ground	16	231.2	231.6	
Coffee (Beverage) (unspecified)[h]	46	8.4	8.8	
Coffee drink, café américano[h]	46	8.4	8.8	

Appendix E: Average chromium occurrence values (µg/kg) in the different foods used to calculate dietary exposure to Cr(III) (continued)

FOODEX[a]	N	Average values (µg/kg) LB	Average values (µg/kg) UB	Groups
Coffee drink, cappuccino[h]	46	8.4	8.8	
Coffee drink, café macchiato[h]	46	8.4	8.8	
Iced coffee[h]	46	8.4	8.8	
Coffee with milk (café latte, café au lait)[h]	46	8.4	8.8	
Coffee drink, espresso[i]	46	21.6	22.6	
Instant coffee, powder	7	71.7	84.6	
Instant coffee, liquid[j]	7	1.2	1.4	
Products for special nutritional use (unspecified)[k]	107	2931.0	2987.3	
Food for weight reduction (unspecified)	90	112.1	355.9	
Products presented as a replacement for one or more meals of the daily diet	1	740.0	740.0	
Dietary supplements (unspecified)	173	21591.1	21636.3	
Vitamin supplements	56	10078.3	10097.0	
Mineral supplements	176	16516.1	16590.3	
Combination of vitamins and minerals supplements	582	23440.5	23513.5	
Supplements containing special fatty acids (e.g. omega-3, essential fatty acids)	42	959.5	1024.3	
Protein and amino acids supplements	3	379.3	379.3	
Fiber supplements	8	1446.2	1521.2	
Plant extract formula	135	4108.7	4208.2	
Coenzyme Q10 supplement	7	1346.0	1383.1	
Yeast based supplement	27	107.0	330.3	
Algae formula (e.g. Spirulina, Chlorella)	71	4243.3	4298.4	
Pollen-based supplement	6	93.3	106.7	
Food for sports people (labelled as such) (unspecified)	158	6343.1	6541.4	Products for special nutritional use[k]
Carbohydrate-rich energy food products for sports people	17	85.3	107.7	
Carbohydrate-electrolyte solutions for sports people	1	43230.0	43230.0	
Protein and protein components for sports people	39	51.0	628.2	
Carnitine-based supplement for sports people	1	17500.0	17500.0	
Dietetic food for diabetics (labelled as such) (unspecified)	32	66788.5	66788.5	
Chocolate and chocolate products for diabetics	69	1226.0	1226.0	
Ready-to-eat meal for diabetics	11	77.2	179.0	
Medical food (are specially formulated and intended for the dietary management of a disease that has distinctive nutritional needs that cannot be met by normal diet alone; intended to be used under medical supervision) (unspecified)	90	140.4	142.9	
Nutritionally complete formulas	152	77.0	139.8	
Nutritionally incomplete formulas	48	9014.1	9096.6	
Formulas for metabolic disorders	16	8.4	247.8	
Oral rehydration products	3	1671.3	1671.3	
Snack food	4	84.9	84.9	
Ices and desserts (unspecified)	23	26.5	57.8	
Ice cream, milk-based	6	34.2	115.9	Snacks, desserts, and other foods
Ice cream, not milk-based	132	11.5	88.9	
Starchy pudding	7	321.1	325.4	
Custard	4	63.5	71.0	

Appendix E: Average chromium occurrence values (µg/kg) in the different foods used to calculate dietary exposure to Cr(III) (continued)

FOODEX[a]	N	Average values (µg/kg) LB	UB	Groups
Other foods (foods which cannot be included in any other group)	20	235.3	236.1	Other foods
Potatoes and potatoes products (unspecified)	319	9.6	18.7	Potatoes and potatoes products
New potatoes	54	5.4	18.6	
Main-crop potatoes	216	59.1	66.9	
French fries	3	85.3	95.3	
Mashed potato powder	2	220.0	220.0	
Potato boiled	6	28.0	38.0	
Potato baked	6	24.0	46.9	
Potato croquettes	2	74.5	74.5	
Other starchy roots and tubers	23	43.2	49.3	Other starchy roots and tubers
Sugar and confectionary (unspecified)	21	218.4	230.8	Sugar and confectionary (non chocolate products)
Sugars (unspecified)	16	72.5	97.6	
White sugar	3	59.6	61.0	
Cane sugar	19	17.7	75.1	
Fructose	2	23.0	38.0	
Glucose	9	99.5	146.2	
Sugar substitutes	2	165.0	185.0	
Sugar beet syrup	5	417.0	417.7	
Honey (unspecified)	115	30.4	42.2	
Honey, monofloral	86	25.7	54.7	
Honey, polyfloral	59	7.1	24.1	
Honey, blended [e]	1	27.5	55.4	
Honeydew honey	17	105.4	129.1	
Dessert sauces	14	206.0	206.0	
Confectionery (non-chocolate) (unspecified)	123	106.0	161.5	
Candies, with sugar	14	73.7	98.8	
Dragée, sugar coated	16	562.5	562.5	
Foamed sugar products (marshmallows)	13	21.2	39.8	
Liquorice candies	8	33.8	93.8	
Gum drops	56	70.3	82.6	
Jelly candies	4	25.0	26.8	
Chocolate (Cocoa) products (unspecified)	421	1427.8	1428.0	Chocolate (Cocoa) products
Chocolate bar	5	886.2	886.2	
Chocolate, cream	17	669.9	669.9	
Chocolate coated confectionery	14	677.9	677.9	
Milk chocolate	39	488.0	489.0	
White chocolate	19	480.8	482.9	
Pralines	1	455.0	455.0	
Vegetables and vegetable products (including fungi)(unspecified)	13	41.4	62.0	Vegetables and vegetable products (including fungi)
Brassica vegetables	361	28.1	36.7	
Garlic, bulb (Allium sativum)[b]	1	580.0	580.0	
Onions, bulb (Allium cepa)	220	52.5	58.7	
Shallots, bulb (Allium ascalonicum, Allium cepa var. aggregatum)	3	67.3	80.7	
Spring onions, bulb (Allium cepa)	24	4.5	13.2	
Cocoa beans and cocoa products (unspecified)	10	3280.2	3280.2	

Appendix E: Average chromium occurrence values (µg/kg) in the different foods used to calculate dietary exposure to Cr(III) (continued)

FOODEX[a]	N	Average values (µg/kg) LB	UB	Groups
Cocoa mass	4	2272.0	2272.0	
Tomatoes (Lycopersicum esculentum)	135	16.4	28.8	
Peppers, paprika (Capsicum annuum, var. grossum and var. longum)	101	1.4	28.2	
Aubergines (Egg plants) (Solanum melongena)	8	28.2	38.0	
Okra, lady's fingers (Hibiscus esculentus)	4	473.5	473.5	
Cucumbers (Cucumis sativus)	83	5.3	22.9	
Gherkins (Cucumis sativus)	8	10.9	17.4	
Courgettes (Zucchini) (Cucurbita pepo var. melopepo)	62	2.7	17.4	
Pumpkins (Cucurbita maxima)	7	11.1	22.4	
Sweet corn (Zea mays var. saccharata)	6	72.5	75.0	
Chilli pepper (Capsicum frutescens)	3	113.3	1137.8	
Fungi, cultivated (unspecified)	24	28.8	41.8	
Cultivated mushroom (syn. Button mushroom) (Agaricus bisporus)	404	14.4	24.2	
Oyster mushroom (Pleurotus ostreatus)	55	6.3	20.0	
Shiitake mushroom (Lentinus edodes)	25	345.4	364.4	
Fungi, wild, edible (unspecified)	45	24.8	37.0	
Boletus (Boletus (and other) spp.)	20	19.9	27.5	
Cantharelle (Cantharellus cibarius)	83	63.8	70.4	
Leaf vegetables (unspecified)	94	14.1	43.2	
Lamb's lettuce (Valerianella locusta)	162	100.5	111.6	
Lettuce, excluding Iceberg-type lettuce (Lactuca sativa)	276	32.5	51.1	
Iceberg-type lettuce	55	16.9	48.9	
Endive, scarole (broad-leaf endive)	49	46.9	65.2	
Rocket, Rucola (Eruca sativa, Diplotaxis spec.)	502	76.4	92.0	
Spinach (fresh) (Spinacia oleracea)	168	119.6	124.5	
Spinach (Spinacia oleracea), preserved, deep-frozen or frozen	112	129.1	130.1	
Beet leaves (Beta vulgaris)	6	64.2	75.2	
Vine leaves (grape leaves) (Vitis euvitis)	10	272.0	272.0	
Witloof (Cichorium intybus. var. foliosum)	10	5.6	22.3	
Mustard seedling (Sinapis alba)[b]	1	105.4	105.4	
Dandelion leaf (Taraxacum officinalis)	2	49.0	56.5	
Legume vegetables	8	30.7	37.7	
Root vegetables	574	23.2	35.8	
Sea weeds	3	441.0	441.0	
Asparagus (Asparagus officinalis)	137	14.1	24.2	
Celery (Apium graveolens var. dulce)	33	6.3	38.3	
Fennel (Foeniculum vulgare)	7	180.1	189.6	
Globe artichokes (Cynara scolymus)	12	47.3	58.2	
Leek (Allium porrum)	27	13.0	23.3	
Rhubarb (Rheum × hybridum)	60	34.1	41.1	
Sugar plants	20	48.3	51.6	

Appendix E: Average chromium occurrence values (µg/kg) in the different foods used to calculate dietary exposure to Cr(III) (continued)

FOODEX[a]	N	Average values (µg/kg) LB	UB	Groups
Vegetable products (unspecified)	26	130.3	133.7	
Tomato purée	9	203.3	205.0	
Mixed vegetable purée[c]	1	160.5	163.6	
Pickled vegetables[c]	1	160.5	163.6	
Chesnut purée[c]	2	160.5	163.6	
Sauerkraut	11	127.0	127.0	
Sun-dried tomatoes	3	422.7	422.7	
Mashed vegetables	2	24.5	39.5	
Hops (dried), including hop pellets and unconcentrated powder (Humulus lupulus)	3	388.3	388.3	

(a): Within each food category and depending on their reported occurrence values, the samples were grouped at Level 1 (bold), Level 2 (normal), Level 3 (*italics*). Foods were grouped slightly different from FoodEx classification to better explain their contribution to the exposure.
(b): These foods with all reported data as left-censored or with just one sample reported were not considered for exposure.
(c): Occurrence values calculated using the average occurrence value from all foods at the inmediate lower FoodEx level.
(d): Occurrence values were calculated using a dilution factor of 8 on the occurrence values from the corresponding samples of follow-on formulae, powder and infant formulae, powder.
(e): Occurrence value assigned from the food group at the inmediate upper FoodEx level.
(f): Occurrence values were calculated using a dilution factor of 100 on the occurrence value from 231 samples of tea and herbs for infusions (solid).
(g): Occurrence values were calculated using a dilution factor of 60 on the occurrence value from 239 samples of cocoa powder.
(h): Occurrence values were calculated using a dilution factor of 18 on the occurrence value from 49 samples of coffee beans roasted and coffee beans roasted and ground.
(i): Occurrence values were calculated using a dilution factor of 7 on the occurrence value from 49 samples of coffee beans roasted and coffee beans roasted and ground.
(j): Occurrence values were calculated using a dilution factor of 63 on the occurrence value from 7 samples of instant coffee powder.
(k): Contribution of the food group 'Products for special nutritional use' to the dietary exposure to Cr(III) was not considered as the Comprehensive database contains limited information on their consumption. A separate scenario is presented in the main text to evaluate the potential contribution of this type of products (Section 6.1.3).

APPENDIX F: DIETARY SURVEYS CONSIDERED FOR THE CHRONIC EXPOSURE ASSESSMENT WITH THE AVAILABLE NUMBER OF SUBJECTS IN THE DIFFERENT AGE CLASSES

Code[a]	Country	Dietary survey[b]	Method	Days	Age	Number of subjects						
						Infants	Toddlers	Other children	Adolescent	Adults	Elderly	Very elderly
BE/1	Belgium	Diet National 2004	24 h dietary recall	2	15-105				584	1304	518	712
BE/2	Belgium	Regional Flanders	Food record	3	2-5		36[c]	625				
BG/1	Bulgaria	NUTRICHILD	24-hour recall	2	0.1-5	860	428	433				
CY	Cyprus	Childhealth	Dietary record	3	11-18				303			
CZ	Czech Republic	SISP04	24-hour recall	2	4-64			389	298	1666		
DE/1	Germany	DONALD 2006	Dietary record	3	1-10		92	211				
DE/2	Germany	DONALD 2007	Dietary record	3	1-10		85	226				
DE/3	Germany	DONALD 2008	Dietary record	3	1-10		84	223				
DE/4	Germany	National Nutrition Survey	24-hour recall	2	14-80				1011	10419	2006	490
DK	Denmark	Danish Dietary Survey	Food record	7	4-75			490	479	2822	309	20[c]
EL	Greece	Regional Crete	Dietary record	3	4-6			839				
ES/1	Spain	AESAN	24-hour recall	2	18-60					410		
ES/2	Spain	AESAN-FIAB	Food record	3	17-60				86	981		
ES/3	Spain	NUT INK05	24-hour recall	2	4-18			399	651			
ES/4	Spain	enKid	24-hour recall	2	1-14		17[c]	156	209			
FI/1	Finland	DIPP	Food record	3	1-6		497	933				
FI/2	Finland	FINDIET 2007	48-hour recall	2	25-74					1575	463	
FI/3	Finland	STRIP	Food record	4	7-8			250				
FR	France	INCA2	Food record	7	3-79			482	973	2276	264	84
HU	Hungary	National Repr Surv	Food record	3	18-96					1074	206	80
IE	Ireland	NSFC	Food record	7	18-64					958		
IT	Italy	INRAN-SCAI 2005–06	Food record	3	0.1-98	16[c]	36[c]	193	247	2313	290	228
LV	Latvia	EFSA_TEST	24-hour recall	2	7-66			189	470	1306		
NL/1	The Netherlands	DNFCS 2003	24 h dietary recall	2	19-30					750		
NL/2	The Netherlands	VCP kids	Food record	3	2-6		322	957				
SE/1	Sweden	RIKSMATEN 1997-98	Food record	7	18-74					1210		
SE/2	Sweden	NFAn	24-hour recall	4	3-18			1473	1018			
UK	United Kingdom	NDNS	Food record	7	19-64					1724		

(a): Abbreviations to be used consistently in all tables on exposure assessment.
(b): More information on the dietary surveys is given in the Guidance of EFSA 'Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment' (EFSA, 2011b);
(c): 95th percentile calculated over a number of observations lower than 60 require cautious interpretation as the results may not be statistically robust.

APPENDIX G: MEAN AND 95TH PERCENTILE DIETARY EXPOSURE ESTIMATES OF CR(III) IN FOOD AND CR(VI) IN WATER CALCULATED FOR EACH OF THE 26 DIETARY SURVEYS

Table G1: Mean and 95th percentile (P95) chronic dietary exposure to Cr(III) (µg/kg b.w. per day) for total population in lower-bound (LB) and upper-bound (UB) scenario.

Code[a]	Range of dietary exposure (LB – UB) (µg/kg b.w. per day)													
	Infants		Toddlers		Other children		Adolescents		Adults		Elderly		Very elderly	
	Mean	P95	Mean	P95	Mean	P95	Mean	P95	Mean	P95	Mean	P95	Mean	P95
BE/1							1.29-1.64	2.69-3.10	1.02-1.33	1.98-2.40	0.88-1.15	1.54-1.93	0.90-1.15	1.56-1.98
BE/2			4.39-5.89	-[b]	3.12-4.39	5.78-7.50								
BG	2.21-3.63	4.76-9.45	3.77-5.63	5.88-9.02	3.50-4.86	6.06-7.91								
CY							1.04-1.32	1.82-2.27						
CZ					2.94-3.77	5.62-6.94	1.98-2.53	3.85-4.80	1.10-1.41	1.90-2.42				
DE/1			2.44-3.35	3.95-5.02	2.14-2.95	3.89-4.74								
DE/2			2.30-3.20	3.40-4.50	2.17-2.96	3.77-4.71								
DE/3			2.25-3.15	3.39-4.54	2.10-2.87	3.78-4.49								
DE/4							0.90-1.22	1.84-2.28	0.81-1.10	1.48-1.93	0.75-1.01	1.30-1.70	0.76-1.01	1.30-1.72
DK					1.87-2.79	2.92-4.24	1.01-1.53	1.71-2.53	0.78-1.13	1.22-1.75	0.75-1.08	1.16-1.68	0.75-1.09	-[b]
EL					1.86-2.45	3.16-4.03								
ES/1									0.79-1.14	1.39-1.95				
ES/2							1.02-1.38	1.84-2.40	0.87-1.20	1.49-2.01				
ES/3					3.19-4.06	5.54-6.73	1.89-2.34	3.78-4.34						
ES/4			4.65-5.89	-[b]	3.53-4.37	7.32-7.94	2.06-2.49	4.08-4.79						
FI/1			2.37-3.70	5.07-8.44	2.35-3.57	4.20-5.99								
FI/2									0.77-1.15	1.37-2.02	0.62-0.96	1.12-1.70		
FI/3					2.22-3.02	3.72-4.61								
FR							1.33-1.69	2.52-3.05	0.93-1.24	1.59-2.07	0.90-1.21	1.47-1.94	0.91-1.21	1.58-1.95
HU									1.22-1.56	1.99-2.48	1.05-1.35	1.54-1.96	1.16-1.47	1.81-2.25
IE									0.97-1.26	1.61-2.06				
IT	1.47-1.88	-[b]	2.41-3.45	-[b]	1.88-2.55	3.16-4.21	1.11-1.51	1.95 -2.66	0.79-1.09	1.27-1.74	0.75-1.04	1.18-1.60	0.74-1.03	1.17-1.65
LV					1.59-2.05	3.26-3.75	1.15-1.48	2.19-2.79	0.80-1.00	1.49-1.82				
NL/1									1.12-1.53	2.02-2.64				
NL/2			3.30-4.88	5.75-8.54	2.90-4.24	5.26-7.03								
SE/1									1.00-1.33	1.68-2.18				
SE/2					2.47-3.37	5.33-6.32	1.62-2.11	3.33-3.99						
UK									0.82-1.10	1.33-1.72				

b.w.: body weight; P95: 95th percentile; BE: Belgium; BG: Bulgaria; CY: Cyprus; CZ: the Czech Republic; DE: Germany; DK: Denmark; EL: Greece; ES: Spain; FI: Finland; FR: France; HU: Hungary; IE: Ireland; IT: Italy; LV: Latvia; NL: the Netherlands; SE: Sweden; UK: the United Kingdom.

(a): Details on the dietary surveys and the number of subjects are given in Table 4.

(b): 95th percentile calculated over a number of observations lower than 60 require cautious interpretation as the results may not be statistically robust (EFSA, 2011b).

Table G2: Mean and 95th percentile (P95) chronic exposure to Cr(VI) (ng/kg b.w. per day) through the consumption of water intended for human consumption and mineral waters for total population in lower-bound (LB) and upper-bound (UB) scenario.

Code[a]	Range of chronic exposure (LB – UB) (ng/kg b.w. per day)													
	Infants		Toddlers		Other children		Adolescents		Adults		Elderly		Very elderly	
	Mean	P95	Mean	P95	Mean	P95	Mean	P95	Mean	P95	Mean	P95	Mean	P95
BE/1							4.7-26.1	15.9-79.1	5.2-27.9	16.2-79.0	3.9-21.6	13.0-62.7	3.3-18.4	10.5-51.8
BE/2			8.5-34.8	-[b]	9.5-39.9	28.4-111.7								
BG	14.1-106.2	49.8-320.2	7.5-56.6	27.0-145.0	7.2-52.0	21.7-150.9								
CY							0.01-0.02[c]	0.0-0.0[c]						
CZ					8.5-56.3	23.5-130.7	7.4-44.2	20.7-97.2	7.7-43.7	19.7-90.8				
DE/1			30.4-84.4	103.1-186.6	11.8-57.1	34.4-128.9								
DE/2			37.5-96.6	104.1-194.1	12.4-56.1	32.6-125.8								
DE/3			30.4-89.0	99.6-239.3	13.6-60.8	33.9-133.7								
DE/4							10.2-39.3	29.7-110.3	10.9-42.4	29.3-108.3	8.4-33.4	24.1-89.8	7.4-29.9	21.0-76.4
DK					3.3-38.1	6.9-78.5	2.3-25.6	5.4-61.3	2.3-25.5	5.9-66.9	1.9-21.0	4.8-55.1	1.3-15.3	-[b]
EL					0.03-0.04[c]	0.0-0.0[c]								
ES/1									1.4-15.8	4.7-50.8				
ES/2							1.5-16.8	4.0-46.4	1.4-16.1	4.3-49.1				
ES/3					6.1-48.4	23.5-106.1	3.7-31.0	11.1-64.0						
ES/4			8.2-60.2	-[b]	6.7-43.7	22.2-126.1	2.8-22.7	9.3-64.1						
FI/1			8.2-94.2	16.1-184.2	3.2-37.0	7.0-80.3								
FI/2									2.2-24.8	5.4-60.2	2.0-23.2	4.9-56.2		
FI/3					0.7-7.4	2.8-28.1								
FR					7.9-49.5	20.2-99.1	4.3-27.7	12.0-67.3	4.9-30.5	14.3-77.9	4.8-29.8	13.0-65.3	5.6-33.0	17.0-87.4
HU									1.7-6.5[c]	8.3-31.0[c]	1.4-5.3[c]	6.6-24.6[c]	1.0-3.7[c]	5.1-18.8[c]
IE									8.5-9.5	29.0-32.3				
IT	33.2-159.1	-[b]	15.2-82.2	-[b]	10.3-56.5	22.8-113.6	6.4-34.5	14.1-73.1	5.4-28.3	13.7-64.5	4.1-23.4	10.2-50.7	4.8-26.2	10.6-51.5
LV					1.6-7.2[c]	8.6-39.5[c]	1.2-5.2[c]	5.0-20.5[c]	0.8-3.9[c]	3.9-18.4[c]				
NL/1									1.6-13.2	5.9-49.1				
NL/2			39.6-44.7	113.3-126.5	26.6-30.3	76.0-86.6								
SE/1									1.9-15.9	6.3-48.5				
SE/2					1.1-12.1	3.8-37.4	0.9-8.8	2.9-28.5						
UK									8.9-18.0	28.8-49.5				

b.w.: body weight; P95: 95th percentile; BE: Belgium; BG: Bulgaria; CY: Cyprus; CZ: the Czech Republic; DE: Germany; DK: Denmark; EL: Greece; ES: Spain; FI: Finland; FR: France; HU: Hungary; IE: Ireland; IT: Italy; LV: Latvia; NL: the Netherlands; SE: Sweden; UK: the United Kingdom.

(a): Details on the dietary surveys and the number of subjects are given in Table 4.

(b): 95th percentile calculated over a number of observations lower than 60 require cautious interpretation as the results may not be statistically robust (EFSA, 2011b).

(c): Dietary surveys where consumers of drinking water were less than 50 % of the total number of participants.

Table 28: Table G3: Summary statistics of the chronic intake of Cr(III) (μg/day) across European dietary surveys. Estimates were rounded up to one decimal place.

	Mean intake (μg/day)					
	Lower bound (LB)			Upper bound (UB)		
	Min	**Median**	**Max**	**Min**	**Median**	**Max**
Infants	12.3	-[(a)]	17.7	16.0	-[(a)]	29.1
Toddlers	23.6	30.1	67.8	35.4	42.9	85.7
Other children	40.2	54.3	85.4	53.1	71.2	106.5
Adolescents	49.4	63.5	98.7	65.5	83.4	119.6
Adults	54.1	60.9	86.5	74.5	83.8	112.6
Elderly	47.3	57.3	77.0	72.6	77.1	99.3
Very Elderly	49.4	59.3	78.4	68.7	79.2	99.7
	95th percentile intake[(b)] (μg/day)					
	Lower bound (LB)			Upper bound (UB)		
	Min	**Median**	**Max**	**Min**	**Median**	**Max**
Infants	41.0	-[(b)]	-[(b)]	74.4	-[(b)]	-[(b)]
Toddlers	37.6	48.5	82.1	51.8	71.7	122.1
Other children	65.0	93.3	164.4	84.3	114.9	179.0
Adolescents	75.4	116.4	182.6	94.0	141.4	212.7
Adults	86.2	107.3	144.7	117.5	140.3	190.2
Elderly	78.2	95.6	111.2	110.3	126.6	140.8
Very Elderly	75.7	103.3	111.2	106.0	126.4	139.2

(a): Details on the dietary surveys and the number of subjects are given in Appendix F.
(b): 95th percentile calculated over a number of observations lower than 60 require cautious interpretation as the results may not be statistically robust (EFSA, 2011b).

APPENDIX H: OVERVIEW OF CHROMIUM TOXICITY STUDIES

Table H1: Repeated toxicity studies with Cr(III) compounds

Study*	NOAEL	LOAEL	Effect	Reference
13-week oral (diet) B6C3F1 mice 10M + 10 F/group 0, 80, 240, 2000, 10000 or 50000 mg/kg diet chromium picolinate monohydrate (≅ M: 0, 17, 50, 450, 2300 or 11900 and F: 0, 14, 40, 370, 1775 or 9140 mg chromium picolinate monohydrate/kg b.w. per day) **Doses:** **M: 0, 2, 6.2, 54, 273, 1419 mg Cr(III)/kg b.w. per day[(a)]** **F: 0, 1.7, 4.9, 44, 212, 1090 mg Cr(III)/kg b.w. per day[(a)]**	50000 mg/kg diet **M: 1419 and F: 1090 mg Cr(III)/kg b.w. per day**	-	No adverse effect.	Rhodes et al. (2005) NTP (2010)
90-day (5 days/week) oral (diet) rat (Becton Dickinson) 0 %, 2 % or 5 % Cr2O3 baked in bread 6/14/5 M, resp. and 6/5/10 F, resp. **Doses:** **M: 0; 570; 1368 mg Cr(III)/kg b.w. per day[(a)]** **F: 0; 547; 1217 mg Cr(III)/kg b.w. per day[(a)]**	5 % (50000 mg/kg diet) **M: 1368 and F: 1217 mg Cr(III)/kg b.w. per day**	-	Reductions in absolute liver and spleen weights at HD not considered as an adverse effect.	Ivankovic and Preussman (1975)
14-week oral (diet) F344/N rats 10M + 10 F/group 0, 80, 240, 2000, 10000 or 50000 mg/kg diet chromium picolinate monohydrate (≅ M: 0, 7, 20, 160, 800 or 4240 and F: 0, 6, 20, 160, 780 or 4250 mg chromium picolinate monohydrate/kg b.w. per day) **Doses:** **M: 0, 0.8, 2.4, 19.1, 95.4, 506 mg Cr(III)/kg b.w. per day[(a)]** **F: 0, 0.7, 2.4, 19.1, 93, 507 mg Cr(III)/kg b.w. per day[(a)]**	50000 mg/kg diet **M: 506 and F: 507 mg Cr(III)/kg b.w. per day**	-	No adverse effect.	Rhodes et al. (2005) NTP (2010)

Table H1: Repeated toxicity studies with Cr(III) compounds (continued)

Study*	NOAEL	LOAEL	Effect	Reference
90-day oral (diet) Sprague-Dawley rats 0, 5, 50 or 125 mg/kg diet chromium nicotinate (0, 200, 2000 or 5000 µg Cr(III) human equivalency dose per day) **Doses:** **M: 0, 0.04, 0.40, 1.0 mg Cr(III)/kg b.w. per day[(b)]** **F: 0, 0.04, 0.42, 1.1 mg Cr(III)/kg b.w. per day[(b)]**	125 mg/kg diet **1 mg Cr(III)/kg b.w. per day**	-	No adverse effect.	Shara et al. (2005)
24-weeks oral (diet) rat (Harlan Sprague Dawley) 0, 5, 25, 50 or 100 mg Cr(III) /kg diet (as chromium chloride or chromium picolinate) **Doses: 0, 0.45, 2.25, 4.5, 9 mg Cr(III)/kg b.w. per day[(c)]**	100 mg /kg diet **9 mg Cr(III)/kg b.w. per day**	-	No toxicity observed (b.w., organ weights, blood and histological measurements). Animals fed chromium picolinate were found to have liver and kidney chromium concentrations two- to threefold greater than those fed chromium chloride, demonstrating the higher absorption of chromium picolinate.	Anderson et al. (1997)
52-week oral (diet) Sprague-Dawley rats 0 or 25 mg/kg diet chromium nicotinate (0, 1000 µg Cr(III) human equivalency dose per day) **Doses:** **M: 0, 0.17 mg Cr(III)/ kg b.w. per day[(b)]** **F: 0, 0.22 mg Cr(III)/ kg b.w. per day[(b)]**	-	25 mg/kg diet **M: 0.17/F: 0.22 mg Cr(III)/kg b.w. per day**	Signif. decrease b.w. gain at 26, 39 or 52 weeks: 7.7, 8.1 and 14.9 % in M and 5.5, 11.4 and 9.6 % in F, respectively).	Shara et al. (2007)
2-year (5 days/week = 600 feeding days) oral (diet) rat (Becton Dickinson) 60 M + 60 F/group 0 %, 1 %, 2 % or 5 % Cr2O3 baked in bread (0, 360, 720 and 1800 g total Cr2O3/kg b.w.) Animals maintained on control diets following termination of exposure until they became moribund or died. 60M + 60 F/group **Doses: 0; 293; 586; 1466 mg Cr(III)/kg b.w. per day[(a)]**	5 % (50000 mg/kg diet) **1466 mg Cr(III)/kg b.w. per day**	-	No adverse effect.	Ivankovic and Preussman (1975)

Table H1: Repeated toxicity studies with Cr(III) compounds (continued)

Study*	NOAEL	LOAEL	Effect	Reference
2-year oral (diet) F344/N rats 50M + 50 F/group 0, 2000, 10000 or 50000 mg/kg diet chromium picolinate monohydrate (≅ M: 0, 90, 460 or 2400 and F: 0, 100, 510 or 2630 mg chromium picolinate monohydrate/kg b.w. per day) **Doses:** **M: 0, 10.7, 55, 286 mg Cr(III)/kg b.w. per day(a)** **F: 0, 12, 61, 314 mg Cr(III)/kg b.w. per day(a)**	50000 mg/kg diet **M: 286 and F: 314 mg Cr(III)/kg b.w. per day**	-	Increased incidence of preputial gland adenoma in M at 10000 and 50000 ppm (> hist. C range) (1/50, 1/50, 7/50 and 4/50). The CONTAM Panel do not consider this benign lesion to be treatment-related.	NTP (2010)
2-year oral (diet) B6C3F1 mice 50M + 50 F/group 0, 2000, 10000 or 50000 mg/kg diet chromium picolinate monohydrate (≅ M: 0, 250, 1200 or 6565 and F: 0, 240, 1200 or 6100 mg chromium picolinate monohydrate/kg b.w. per day) **Doses:** **M: 0, 30, 143, 783 mg Cr(III)/kg b.w. per day(a)** **F: 0, 29, 143, 728 mg Cr(III)/kg b.w. per day(a)**	50000 mg/kg diet **M: 783 and F: 728 mg Cr(III)/kg b.w. per day**	-	Decrease mean b.w. of 50000 ppm females (10 %) at 1-year, but similar to control group at 2-year.	NTP (2010)

b.w.: body weight; NOAEL: no-observed-adverse-effect level; LOAEL: lowest-observed-adverse-effect level; MW: molecular weight; M: male; F: female.
* In the conversions from concentration to daily doses, the MW of the anhydrous salts were used when no information on hydration number was available in the original publication.
(a): Conversion using the data reported in the original publication.
(b): Conversion using drinking water/feed consumption data and average body weight reported in the publication.
(c): Conversion using the default correction factor for subacute/subchronic/chronic exposure via drinking water/feed from EFSA SC (2012).

Table H2: Developmental and reproductive toxicity studies with Cr(III) compounds

Study*	NOAEL	LOAEL	Effect	Reference
1-generation reproductive toxicity				
90-day (5 days/week) oral (diet) rat (Becton Dickinson) 0 %, 2 % or 5 % Cr2O3 baked in bread 9F paired with M from same dosage group 60 days after start of feeding **Doses:** **M: 0; 570; 1368 mg Cr(III)/kg b.w. per day[(a)]** **F: 0; 547; 1217 mg Cr(III)/kg b.w. per day[(a)]**	M: 1368 mg Cr(III)/kg b.w. per day F: 1217 mg Cr(III)/kg b.w. per day	-	No effect on fertility, gestation length or litter size. Pups: no malformations or other adverse effects observed.	Ivankovic and Preussman, (1975)
Fertility studies				
12 weeks oral exposure of sexually mature M Sprague-Dawley rats 0, 1000 mg chromium chloride/L (0, 328.4 mg Cr(III)/L) **Doses: 0 and 30 mg Cr(III)/kg b.w. per day[(b)]** X untreated F	-	**30 mg Cr(III)/kg b.w. per day**	Inhibitory effect on sexual and aggressive behaviour: reduction number of mounts, increased post-ejaculatory interval, decrease number of M ejaculating, decreased aggressive behaviour towards other M. Decrease b.w., absolute testes, seminal vesicles and preputial glands weights. No effect on fertility of treated M. Increase number of resorptions and dead fetuses in F mated with treated M. No histopathology performed.	Bataineh et al. (1997)
12 weeks oral exposure of sexually mature M Swiss mice 0, 1000 or 5000 mg chromium chloride/L (0, 328.4 or 1641.8 mg Cr(III)/L) **Doses: 0, 49, 246 mg Cr(III)/kg b.w. per day[(b)]** X untreated F	-	**49 mg Cr(III)/kg b.w. per day**	Decrease b.w., testes weight in treated M, decrease seminal weight in M at HD. Reduction preputial glands in treated M. Decrease fertility in M at 5000 mg/L. Increase number of resorptions and dead fetuses in F impregnated with exposed M. No histopathology performed.	Elbetieha and Al-Hamood (1997)
12 weeks oral exposure of sexually mature F Swiss mice 0, 2000 or 5000 mg chromium chloride/L (0, 656.6 or 1641.8 mg Cr(III)/L) **Doses: 0, 98, 246 mg Cr(III)/kg b.w. per day[(b)]** X untreated M	-	**98 mg Cr(III)/kg b.w. per day**	Increase ovarian weight and reduction uterine weights in treated F No effect on F fertility (pregnancy rate). Decrease number of implantations and viable fetuses in treated F. Increase number of resorptions in treated F. No histopathology performed.	Elbetieha and Al-Hamood (1997)

Table H2: Developmental and reproductive toxicity studies with Cr(III) compounds (continued)

Study*	NOAEL	LOAEL	Effect	Reference
Male CD-1 mice Oral (diet) 0, 200 mg chromium picolinate/kg b.w. per day. **Doses:** **0, 25 mg Cr(III)/kg b.w. per day[(a)]** for 4 weeks before mating X untreated F F sacrificed on GD 17	25 mg Cr(III)/kg b.w. per day	-	No significant effect on mating and fertility indices. No effect on the average number of implantations in F. No effect on prenatal mortality, fetal weight or gross or skeletal morphology.	McAdory et al. (2011)
Developmental toxicity studies				
Mated F Swiss mice Oral (drinking water) 0 or 1000 mg/L chromium chloride day 12 of gestation – day 20 of lactation **Doses: 0, 79 mg Cr(III)/kg b.w. per day[(c)]**	-	**79 mg Cr(III)/kg b.w. per day**	Offsprings: M: decrease number of pregnant females, reduction b.w., testes, seminal vesicles and preputial glands weights. F: delayed sexual maturation (delayed vaginal opening), reduction of fertility (decrease number pregnant females, implantations (not stat signif.) viable fetuses (not stat signif.), b.w., ovaries and uteri weights, increase number of resorptions. impairment of reproductive functions and fertility in adulthood. No histopathology performed	Al-Hamood et al. (1998)
Mated F CD-1 mice Oral (diet) GD 6-17 Dams sacrificed GD 17 **Doses:** **0, 200 mg chromium picolinate (25 mg Cr(III)/kg b.w. per day), 200 mg/kg $CrCl_3$ (39 mg Cr(III)/kg b.w. per day)[(a)]**	-	**25 mg Cr(III)/kg b.w. per day**	No effect on maternal toxicity, no effect on b.w. gain or food consumption. No effect on maternal fertility (number of implantations, resorbed or dead fetuses). No effect on fetal weight. Significant increase in incidence of bifurcated cervical arches in chromium picolinate group (effect not reproducible in other studies). No effect in $CrCl_3$ group.	Bailey et al. (2006)
Mated F CD-1 mice Oral (diet) GD 6-17 Dams sacrificed GD 17 **Doses:** **0, 200 mg chromium picolinate (25 mg Cr(III)/kg b.w. per day) or as Cr(III)cation $Cr_3O(O_2CCH_2CH_3)_6(H_2O)_3)^+$ 3.3 or 26 mg Cr(III)/kg b.w. per day[(a)]**	**25 mg Cr (III)/kg b.w./day**	-	No signs of maternal toxicity, no effect on b.w. gain or food consumption. No decrease in fetal weight, no effect on number of resorbed or dead fetuses and no difference in the number of implantations/litter or significantly increased incidence of skeletal defects, no effect on gross malformations.	Bailey et al. (2008a)

Table H2: Developmental and reproductive toxicity studies with Cr(III) compounds (continued)

Study*	NOAEL	LOAEL	Effect	Reference
Mated F CD-1 mice From implantation through weaning Oral (diet) 0, 200 mg chromium picolinate/kg **Doses:** **0, 25 mg Cr(III)/kg b.w. per day[(a)]**	**25 mg Cr(III)/kg b.w./day**	-	No significant effects on a variety of tests assessing motor and sensory functions, as well as memory performed between the ages of 5 and 60 days.	Bailey et al. (2008b)
Mated F Sprague-Dawley rats 25 mg chromium chloride /rat by gavage **Doses: 25 mg CrCl3/rat** **= 33.6 mg Cr(III)/kg b.w. per day[(c)]** GD 1-3 or GD 4-6	-	33.6 mg Cr(III)/kg b.w. per day	Decrease pregnancy rate for exposure on GD 1-3.	Bataineh et al. (2007)
Toxicity on reproductive organs				
13-week oral (diet) B6C3F1 mice 10M + 10 F/group 0, 80, 240, 2000, 10000 or 50000 mg/kg diet chromium picolinate monohydrate (≅ M: 0, 17, 50, 450, 2300 or 11900 and F: 0, 14, 40, 370, 1775 or 9140 mg chromium picolinate monohydrate/kg b.w. per day) **Doses:** **M: 0, 2, 6.2, 54, 273, 1419 mg Cr(III)/kg b.w. per day[(a)]** **F: 0, 1.7, 4.9, 44, 212, 1090 mg Cr(III)/kg b.w. per day[(a)]**	50000 mg/kg diet **M: 1419 and F: 1090 mg Cr(III)/kg b.w. per day**	-	No significant changes in reproductive organ weights in M or F, in sperm parameters in M or in estrous cyclicity in F.	Rhodes et al. (2005) NTP (2010)

Table H2: Developmental and reproductive toxicity studies with Cr(III) compounds (continued)

Study*	NOAEL	LOAEL	Effect	Reference
14-week oral (diet) F344/N rats 10M + 10 F/group 0, 80, 240, 2000, 10000 or 50000 mg/kg diet chromium picolinate monohydrate (≙ M: 0, 7, 20, 160, 800 or 4240 and F: 0, 6, 20, 160, 780 or 4250 mg chromium picolinate monohydrate/kg b.w. per day) **Doses:** **M: 0, 0.8, 2.4, 19.1, 95.4, 506 mg Cr(III)/kg b.w. per day[(a)]** **F: 0, 0.7, 2.4, 19.1, 93, 507 mg Cr(III)/kg b.w. per day[(a)]**	50000 mg/kg diet **M: 506 and F: 507 mg Cr(III)/kg b.w. per day**	-	No significant changes in reproductive organ weights in M or F, in sperm parameters in M or in estrous cyclicity in F.	Rhodes et al. (2005) NTP (2010)
24-weeks oral (diet) rat (Harlan Sprague Dawley) 0, 5, 25, 50 or 100 mg Cr(III) /kg diet (as chromium chloride or chromium picolinate) **Doses: 0, 0.45, 2.25, 4.5, 9 mg Cr(III)/kg b.w. per day[(b)]**	**9 mg Cr(III)/kg b.w. per day**	-	No toxicity observed (b.w., organ weights, blood and histological measurements). No changes in testis or epididymis weight.	Anderson et al. (1997)
Male Balb-c albino Swiss mice 7-week (35 days) oral (diet) 0, 100, 200 and 400 mg/kg food chromium sulphate **Doses:** **0, 9.2, 19, 46 mg Cr(III)/kg b.w. per day[(c)]**	-	**9.2 mg Cr(III)/kg b.w. per day**	No effect on b.w. gain, mean food consumption, testes and epididymis weights. Degeneration of outer cellular layer of seminiferous tubules, significant reduction of number of spermatogonia/tubule, accumulation of germ cells in resting spermatocytes stage, decrease number of cells at leptotene and zygotene stages and significant increases in the number of germ cells at the pachytene stage of meiosis. Significant reduction of sperm count in epididymis, dose-dependent increase in % of morphologically abnormal sperm.	Zahid et al. (1990)

b.w.: body weight; NOAEL: no-observed-adverse-effect level; LOAEL: lowest-observed-adverse-effect level; MW: molecular weight; M: male; F: female; GD: gestation day.
* In the conversions from concentration to daily doses, the MW of the anhydrous salts were used when no information on hydration number was available in the original publication.
(a): Data reported in the original publication.
(b): Conversion using the default correction factor for subacute/subchronic/chronic exposure via drinking water/feed from EFSA SC (2012).
(c): Conversion using drinking water/feed consumption data and average body weight reported in the publication.

Table H3: Summary of *in vivo* genotoxicity of Cr(III) - oral route

Test system/ Endpoint	Compound	Dose/route	Exposure time/evaluation time	Tissue	Response*	Reference
Rat (F344/N) Micronuclei	Cr picolinate	Oral exposure by gavage 156 to 2500 mg/kg b.w. **Doses: 19.4-310.7 mg Cr(III))/kg b.w. per day**	three times at 24 hr intervals	Bone marrow erythrocytes	Negative 2500 mg Cr-pic/kg b.w. 310.7 mg Cr(III))/kg b.w. per day	NTP (2010)
Mouse (B6C3F1) Micronuclei	Cr picolinate monohydrate	Oral exposure in feed 80 to 50.000 mg/kg diet **Doses: M:2-1419 mg Cr(III)/kg b.w. per day F: 1.7-1090 mg Cr(III)/kg b.w. per day**	3 months feeding	Peripheral blood erythrocytes	Negative - 50.000 mg/kg 1419 mg Cr(III)/kg b.w. per day	NTP (2010)
Mouse (BDF1) Micronuclei	Chromic potassium sulphate dodeca-hydrate $CrK(SO_4)_2x\ 12H_2O$	Drinking water 500mg/l **Doses: M:165 mg Cr(III)/kg b.w. per day F: 140 mg Cr(III)/kg b.w. per day**	for 7 months	Bone marrow and peripheral blood cells	Negative 165 mg Cr(III)//kg b.w. per day	De Flora et al. (2006)
Rats (Sprague–Dawley) Micronuclei	Cr picolinate	Single oral dose of 33, 250, 2000 mg/kg b.w. **Doses: 4.1, 30.8, 246 mg Cr(III)/kg b.w. per day**	18 and 42 hrs after administration	Bone marrow cells	Negative 246 mg Cr(III)//kg b.w. per day	Komorowski et al. (2008)
Mouse (C57BL/6J) DNA deletions (pun reversion assay)	Cr(III) chloride salt	Drinking water to dams 1875 or 3750 mg/l **Doses: 375 or 750 mg Cr(III)/kg b.w. day**	Transplacental effect in the embryos harvested at 17.5 days postcoitum	Developing embryos (embryo Cr(III) concentrations were 8.72 and 18.77 ng/g, respectively)	Positive 375 mg Cr(III)//kg b.w. per day	Kirpnick-Sobol et al. (2006)

b.w.: body weight.
* The lowest effective dose is indicated for positive results and the highest dose tested for negative results.

Table H4: Summary of *in vivo* genotoxicity of Cr(III) – non-oral route

Test system/ Endpoint	Compound	Dose/route	Exposure time/evaluation time	Tissue	Response*	Reference
Mouse (Slc:ddY) Micronuclei	Cr chloride	i.p. injection **Doses: 0, 20.5, 41 mg Cr(III)/kg b.w. per day**	once a day for 2d 24 hrs after the 2nd administration	Bone marrow cells	Negative 62.5 mg/kg b.w. 20.5 mg Cr(III)/kg b.w. per day	Itoh and Shimada (1996)
Mouse (CBA/Ca) Micronuclei Comet assay	Cr picolinate	i.p. injection (up to 3 mg Cr-pic/kg b.w.) **Doses: 0, 0.4 mg Cr(III)/kg b.w. per day**	42 hrs after injection 16 hrs after injection	Peripheral blood cells Lymphocytes Hepatocytes	Negative 3 mg Cr-pic/kg b.w. 0.4 mg Cr(III)/kg b.w. per day Negative in both cell types 3 mg Cr-pic/kg b.w.	Andersson et al. (2007)

b.w.: body weight; i.p.: intraperitoneal;
* The lowest effective dose is indicated for positive results and the highest dose tested for negative results.

Table H5: Repeated dose toxicity studies with Cr(VI) compounds

Study*	NOAEL	LOAEL	Effect	Reference
20-day study Albino rats (Rattus rattus albino) Oral (gavage) 0 and 50 mg potassium chromate/kg b.w. per day **Doses:** **0, 13.4 mg Cr(VI)/kg b.w. per day[(a)]**	-	50 mg potassium chromate/kg b.w. per day **13.4 mg Cr(VI)/kg b.w. per day**	Liver: lipid accumulation particularly at perilobular zone, increase triglycerides and phospholipids, inhibition of alkaline phosphatase, acid phosphatase glucose-6-phosphatase and cholinesterase, stimulation of lipase. Kidney: lipid accumulation, increases triglycerides and phospholipids mainly in epithelium of distal tubules, inhibition of alkaline phosphatase, acid phosphatase glucose-6-phosphatase and lipase.	Kumar and Rana (1982, 1984) Kumar et al. (1985)
28-day study Male Wistar rats Oral (drinking water) 0, 0.07 g Cr(VI)/L (sodium chromate) **Doses: 4.8 mg Cr(VI)/kg b.w. per day[(b)]** 0, 0.7 g Cr(VI)/L (sodium chromate) **Doses: 48 mg Cr(VI)/kg b.w. per day[(b)]**	-	0, 0.07 g Cr(VI)/L (sodium chromate) **4.8 mg Cr(VI)/kg b.w. per day** 0, 0.7 g Cr(VI)/L (sodium chromate) **48 mg Cr(VI)/kg b.w. per day**	Slight effects on b.w. No effects on motor activity. Decrease urinary excretion, proteinuria. Decrease motor activity.	Diaz-Mayans et al. (1986)

Table H5: Repeated dose toxicity studies with Cr(VI) compounds (continued)

Study*	NOAEL	LOAEL	Effect	Reference
28-day range finding study B6C3F1 mice Oral (drinking water) 0, 15.6, 31.25, 62.5, 125 and 250 mg sodium dichromate dihydrate/L, corresponding to 0, 5.4, 10.9, 21.8, 43.6, 87.2 mg Cr(VI)/L **Doses:** **0, 1, 2, 3.9, 7.8, 15.7 mg Cr(VI)/kg b.w. per day[(c)]** Evaluation of the potential to modulate immune function	125 mg sodium dichromate dihydrate/L **7.8 mg Cr(VI)/kg b.w. per day**	250 mg sodium dichromate dihydrate/ L **15.7 mg Cr(VI)/kg b.w. per day**	Reductions in final mean b.w. and b.w. gain at HD. In HD mice decrease erythroid parameters. Minimal effects in the various immunological parameters evaluated.	NTP (2008)
28-day range finding study F344/N and Sprague-Dawley rats Oral (drinking water) 0, 14.3, 57.3, 172 and 516 mg sodium dichromate dihydrate/L, corresponding to 0, 5, 20, 60, 180 mg Cr(VI)/L **Doses:** **0, 0.6, 2.4, 7.2, 21.6 mg Cr(VI)/kg b.w. per day[(c)]** Evaluation of the potential to modulate immune function	57.3 mg sodium dichromate dihydrate/L **2.4 mg Cr(VI)/kg b.w. per day**	172 mg sodium dichromate dihydrate/ L **7.2 mg Cr(VI)/kg b.w. per day**	Reductions in final mean b.w. and b.w. gain at HD. Decrease water consumption. Minimal effects in the various immunological parameters evaluated.	NTP (2008)
9-week exposure Male and female BALB/c mice + 8-week recovery Oral (diet) Potassium dichromate 0, 15, 50, 100 and 400 mg/kg food, corresponding to 0, 4, 13, 28, 115 mg potassium dichromate/kg b.w. per day **Doses:** **0, 1.4, 4.6, 9.9, 40.7 mg Cr(VI)/kg b.w. per day[(a)]**	15 mg/kg food **1.4 mg Cr(VI)/kg b.w. per day**	50 mg/kg food **4.6 mg Cr(VI)/kg b.w. per day**	Hepatocytes: cytoplasmic vacuolation. Slight reduction of b.w., MCV and MCH values at 400 mg/kg food. No effect on spermatogenesis has been reported.	NTP (1996a, 1997)

Table H5: Repeated dose toxicity studies with Cr(VI) compounds (continued)

Study*	NOAEL	LOAEL	Effect	Reference
9-week exposure Male and female Sprague-Dawley rats+ 8-week recovery Oral (diet) Potassium dichromate 0, 15, 50, 100 and 400 mg/kg food corresponding to 0, 5.3, 17.7, 35.3, 141 mg Cr(VI)/kg food **Doses:** **0, 0.4, 1.1, 2.3, 9.2 mg Cr(VI)/kg b.w. per day**[(a)]	100 mg/kg food **2.3 mg Cr(VI)/kg** b.w. per day	400 mg/kg food **9.2 mg Cr(VI)/kg b.w. per day**	Changes in MCV and MCH values in M and F at 400 mg/kg food. No effect on testis, epididymus or spermatogenesis has been reported.	NTP (1996a, 1997)
3-month exposure M and F B6C3F1 mice Oral (drinking water) 0, 62.5, 125, 250, 500 or 1000 mg sodium dichromate dihydrate/L, corresponding to 0, 9, 15, 26, 45, and 80 mg sodium dichromate dihydrate /kg b.w. per day **Doses:** **0, 3.1, 5.2, 9.1, 15.7, 27.9 mg Cr(VI)/kg b.w. per day**[(a)]	-	62.5 mg sodium dichromate dihydrate/ L **3.1 mg Cr(VI)/kg b.w. per day**	Dose-dependent reduction of b.w. and water consumption from 125 mg/L. Reduction of absolute liver weight in 2 upper doses, increased relative kidney weight in HD M, increase thymus weight and increase testis weight. Haematological changes: mycrocytic hypochromic anemia. Duodenum: increased incidence of epithelial hyperplasia in all exposed groups and of histiocytic cellular infiltration from 125 mg/L. Mesenteric lymph node: histiocytic hyperplasia from 125 mg/L. Stomach lesions were observed in HD M and in F of the two HD dose group. No clinical chemistry or urinalysis were performed.	NTP (2007)

Table H5: Repeated dose toxicity studies with Cr(VI) compounds (continued)

Study*	NOAEL	LOAEL	Effect	Reference
3-month exposure M and F F344 rats Oral (drinking water) 0, 62.5, 125, 250, 500 or 1000 mg sodium dichromate dihydrate/L, corresponding to: M: 0, 5, 9, 17, 32, and 60 mg sodium dichromate dihydrate /kg b.w. per day F: 0, 5, 10, 18, 33, and 61 mg sodium dichromate dihydrate/kg b.w. per day **Doses:** **M: 0, 1.7, 3.1, 5.9, 11.1, 20.9 mg Cr(VI)/kg b.w. per day[(a)]** **F: 0, 1.7, 3.5, 6.3, 11.5, 21.3 mg Cr(VI)/kg b.w. per day[(a)]**	-	62.5 mg sodium dichromate dihydrate/ L **1.7 mg Cr(VI)/kg b.w. per day**	Reduction of mean b.w. in M at 2 HD and in F at HD. Reduction of water consumption in M and F at 3 upper doses. Reduction of liver weight, increase spleen weight and kidney weight Haematological changes: mycrocytic hypochromic anemia. Clinical chemistry: reduced serum cholesterol and triglycerides and increased levels of alanine aminotransferase and sorbitol dehydrogenase in M & F rats. Reduced urine volume and increased specific gravity and creatinine conc. in M & F. Histiocytic cellular infiltration was observed in the duodenum in M and F, in the liver of F from 125 mg/L, in the pancreatic lymph node in M. Increased incidence of lymphoid hyperplasia and ectasia in pancreatic lymph node at HD. Stomach lesions: focal ulceration, regenerative epithelial hyperplasia and squamous epithelial metaplasia at HD. Chronic liver inflammation in F at HD. Bone marrow hyperplasia in F at HD.	NTP (2007)

Table H5: Repeated dose toxicity studies with Cr(VI) compounds (continued)

Study*	NOAEL	LOAEL	Effect	Reference
3-month comparative study in 3 strains of male mice, B6C3F1, BALB/c, am3-C57BL/6 Oral (drinking water) 0, 62.5, 125, 250, mg sodium dichromate dihydrate/L B6C3F1: 8, 15, and 26 mg sodium dichromate dihydrate /kg b.w. per day **Doses:** **2.8, 5.2, 9.1 mg Cr(VI)/kg b.w. per day[(a)]** BALB/c: 9, 14, and 24 mg sodium dichromate dihydrate /kg b.w. per day **Doses:** **3.1, 4.9, 8.4 mg Cr(VI)/kg b.w. per day[(a)]** am3-C57BL/6: 8, 15, and 25 mg sodium dichromate dihydrate /kg b.w. per day **Doses:** **2.8, 5.2, 8.7 mg Cr(VI)/kg b.w. per day[(a)]**	-	62.5 mg sodium dichromate dihydrate/ L **2.8/3.1/2.8 mg Cr(VI)/kg b.w. per day**	Decreases in final mean b.w. and b.w. gain. Decrease water consumption Decrease kidney weight at 125 and kidney, lung, spleen and thymus at 250 mg/L in B6C3F1 mice attributed to changes in b.w. with the exception of thymus weight changes. Haematological changes: mycrocytic hypochromic anemia. Dose-related increased incidences of histiocytic cellular infiltrates and mucosal epithelial hyperplasia were observed in the duodenum. Increases of incidences of glycogen depletion in the liver and minimal secretory depletion in the pancreas. Increases in alanine aminotransferase activity occurred in HD B6C3F1 and am3-C57BL/6 mice and total protein and albumin conc. decreases in the two HD groups B6C3F1 mice. Decreases in heart, kidney and liver were consistent with the reductions in b.w. Reproductive tissue evaluations: decrease in left testis weight related to decreased b.w. in HD am3-C57BL/6 mice.	NTP (2007)

Table H5: Repeated dose toxicity studies with Cr(VI) compounds (continued)

Study*	NOAEL	LOAEL	Effect	Reference
90-day study B6C3F1 mice Oral (drinking water) 0, 0.3, 4, 14, 60, 170 and 520 mg sodium dichromate dihydrate/L **Doses:** **0, 0.03, 0.3, 1.1, 4.7, 12.2 and 31 mg Cr(VI)/kg b.w. per day[(a)]**	1.1	4.7	Water consumption: significantly lower in the two HC groups. No treatment-related gross lesions. No microscopic lesions in the oral cavity. Significant increases Cr at ≥ 60 mg/L in the oral cavity, glandular stomach, jejunum and ileum. Duodenum: Significant increase Cr at ≥ 14 mg/L Cr. Significant decreases in reduced-to-oxidized glutathione ratio (GSH/GSSG). Intestinal lesions: villous cytoplasmic vacuolisation at ≥ 60 mg/L and atrophy, apoptosis and crypt hyperplasia at ≥170 mg/L. Multinucleated syncitia (fused cells) in the villous lamina propria at 520 mg/L. Increase protein carbonyls at ≥ 4 mg/L. Jejunum: Significant decreases in GSH/GSSG ratio and similar histopathological lesions as in duodenum.	Thompson et al. (2011a)
90-day study F344 rats Oral (drinking water) 0, 0.3, 4, 60, 170 and 520 mg sodium dichromate dihydrate/L **Doses:** **0, 0.02, 0.2, 3.6, 8.7 and 24 mg Cr(VI)/kg b.w. per day[(a)]**	0.2	3.6	Water consumption: significantly lower in the two HC groups No treatment-related gross lesions No microscopic lesions in the oral cavity. Significant increases Cr at ≥ 60 mg/l in the oral cavity, duodenum and jejunum. Significant increases Cr in the glandular stomach and ileum at ≥ 170 mg/L and 520 mg/L, respectively. Duodenum: Apoptosis at ≥ 60 mg/L and crypt cell hyperplasia at ≥ 170 mg/L. Histiocytic infiltration at ≥ 60 mg/L. Jejunum: Apoptosis, crypt cell hyperplasia and villous atrophy at concentrations as low as 4 mg/L (incidences not statistically different from control animals and in many instances the lesions were not observed at higher concentrations). Histiocytic infiltration at ≥ 60 mg/L. Significant decreases in GSH/GSSG ratio at 60 mg/L	Thompson et al. (2012b)

Table H5: Repeated dose toxicity studies with Cr(VI) compounds (continued)

Study*	NOAEL	LOAEL	Effect	Reference
22-week study Female Wistar rats Oral (drinking water) 0 and 25 mg potassium dichromate/L corresponding to 0, 8.8 mg Cr(VI)/L **Doses:** **0, 0.8 mg Cr(VI)/kg b.w. per day[(c)]**	-	25 mg potassium dichromate /L **0.8 mg Cr(VI)/kg b.w. per day**	Liver: degeneration with reticular arrangement of hepatocytes, increased sinusoidal space, vacuoation and necrosis, increase serum AST and ALT, decreased level glycogen. Kidney: diffused glomerulus, degeneration of basement membrane in Bowman's capsule, renal tubular epithelial degeneration. Decreased serum cholesterol, increased serum triglycerides and glucose levels.	Chopra et al. (1996)
22-week study Male Wistar rats Oral (drinking water) 0, 25 mg potassium dichromate/L corresponding to 0, 8.8 mg Cr(VI)/L **Doses:** **0, 0.8 mg Cr(VI)/kg b.w. per day[(c)]**	-	25 mg potassium dichromate /L **0.8 mg Cr(VI)/kg b.w. per day**	Decrease serum succinate dehydrogenase. Liver: degeneration, vacuolation, increased sinusoidal space and necrosis, increase serum AST and ALT, decresed levels triglycerides and glycogen, increased levels cholesterol. Kidney: vacuolation in glomeruli, degeneration of basement membrane in Bowman's capsule, renal tubular epithelial degeneration.	Acharya et al. (2001)
6-month study Wistar rats Oral (drinking water) 0, 25 mg potassium dichromate/L **Doses:** **M: 1.79 mg Cr(VI)/kg b.w. per day[(a)]** **F: 2.11 mg Cr(VI)/kg b.w. per day[(a)]**	-	25 mg potassium dichromate /L **1.79 mg Cr(VI)/kg b.w. per day**	No effect on b.w. gain. Increased urinary excretion of albumin (marker of glomerular dysfunction) and β2-microglobulin (marker of tubular dysfunction) in F rats. No similar nephrotoxic effects observed in male rats.	Vyskocil et al. (1993)

Table H5: Repeated dose toxicity studies with Cr(VI) compounds (continued)

Study*	NOAEL	LOAEL	Effect	Reference
2-year study B6C3F1 mice Oral (drinking water) M: 0, 14.3, 28.6, 85.7 and 257.4 mg sodium dichromate dihydrate/L, F: 0, 14.3, 57.3, 172 and 516 mg sodium dichromate dihydrate/L, corresponding to: M: 0, 1.1, 2.6, 7, 17 mg sodium dichromate dihydrate /kg b.w. per day F: 0, 1.1, 3.9, 9, 25 mg sodium dichromate dihydrate /kg b.w. per day **Doses:** **M: 0, 0.38, 0.91, 2.4 and 5.9 mg Cr (VI)/kg b.w. per day[(a)]** **F: 0, 0.38, 1.4, 3.1 and 8.7 mg Cr(VI)/kg b.w. per day[(a)]**		14.3 mg sodium dichromate dihydrate/ L **0.38 mg Cr (VI)/kg b.w. per day**	Decrease mean b.w. gain and water consumption at HD. Erythrocyte microcytosis in F mice. Anemia in F mice. Haematology not performed in M. Duodenum: dose-related increase in diffuse hyperplasia of epithelium, and increased incidence of hystiocytic cellular infiltration at 2 HD. Liver: dose-related increase of incidence of histiocytic cellular infiltration in M and F and of chronic inflammation in F at 2 HD. Mesenteric lymph node: increased incidence of histiocytic cellular infiltration. Pancreatic lymph node: increased incidence of histiocytic cellular infiltration at 2 HD. Pancreas: increased incidence of cytoplasmic alteration in acini in M at 2 HD and in all exposed F.	NTP (2008)

Table H5: Repeated dose toxicity studies with Cr(VI) compounds (continued)

Study*	NOAEL	LOAEL	Effect	Reference
2-year study F344/N rats Oral (drinking water) M & F: 0, 14.3, 57.3, 172 and 516 mg sodium dichromate dihydrate/L, corresponding to: M: 0, 0.6, 2.2, 6, 17 mg sodium dichromate dihydrate /kg b.w. per day F: 0, 0.7, 2.7, 7, 20 mg sodium dichromate dihydrate /kg b.w. per day **Doses:** **M: 0, 0.21, 0.77, 2.1 and 5.9 mg Cr (VI)/kg b.w. per day(a)** **F: 0, 0.24, 0.94, 2.4 and 7.0 mg Cr (VI)/kg b.w. per day(a)**	F: - M: 14.3 mg sodium dichromate dihydrate/L **0.21 mg Cr (VI)/kg b.w. per day**	F: 14.3 mg sodium dichromate dihydrate/ L **0.24 mg Cr (VI)/kg b.w. per day** M: 57.3 mg sodium dichromate dihydrate/ L **0.77 mg Cr (VI)/kg b.w. per day**	Signif decrease mean b.w. gains and reduced water consumption. Erythrocyte microcytosis in M rats at 3 upper doses. Anemia in M rats. Haematology not performed in F. Increased serum ALT at 3 upper doses (< enzyme induction). Liver: increased incidence of histiocytic cellular infiltration in M at HD and in F at the 3 upper doses, increased incidence of chronic inflammation in M at 172 mg /L and in all exposed groups of F, with an increase in severity in HD F, dose-related increases in incidences of fatty change in F at the 3 upper doses. Duodenum: increased incidence of histiocytic cellular infiltration in M at 3 upper doses and at 2 HD in F. Mesenteric lymph nodes: increased incidence of histiocytic cellular infiltration in M at 3 upper doses and in F at 2 HD, increased incidence of minimal lymph node hemorrhage in M at 3 upper doses and in F at HD. Pancreatic lymph node: increased incidence of histiocytic cellular infiltration in M at HD and in F at 3 upper doses. Salivary gland: atrophy in F at 2 HD.	NTP (2008)

b.w.: body weight; M: male; F: female; HD: highest dose; NOAEL: no-observed-adverse-effect level; LOAEL: lowest-observed-adverse-effect level; MW: molecular weight; ALT: alanine aminotransferase; AST: aspartate aminotransferase; GSH/GSSG: reduced-to-oxidized glutathione ratio; MCV: mean corpuscular volume; MCH: mean corpuscular haemoglobin.

* In the conversions from concentration to daily doses, the MW of the anhydrous salts were used when no information on hydration number was available in the original publication.

(a): Data reported in the original publication.

(b): Conversion using drinking water/feed consumption data and average body weight reported in the publication.

(c): Conversion using the default correction factor for subacute/subchronic/chronic exposure via feed/drinking water from EFSA SC (2012).

Table H6: Developmental and reproductive toxicity studies with Cr(VI) compounds

Study*	NOAEL	LOAEL	Effect	Reference
Multigeneration reproductive toxicity				
Continuous breeding study 2-generation BALB/c mice Oral (diet) potassium dichromate 0, 100, 200 and 400 mg/kg diet F0: expo: 1 week before mating, continuous mating for 12 weeks (20 pairs) F0: 0, 19.4, 38.6 and 85.7 mg potassium dichromate/kg b.w. per day. **Doses: 0, 6.9, 13.6, 30.3 mg Cr(VI)/ kg b.w. per day[(a)]** Litters examined PND 1. F1 litters reared by dams until weaning on PND 21, then separated, allowed to mature for about 74 days, 20 pairs allowed to mate and produce F2 F1:0, 22.4, 45.5, 104.9 mg potassium dichromate/kg b.w. per day. **Doses: 0, 7.9, 16.1, 37 mg Cr(VI)/ kg b.w. per day[(a)]** F2 litters reared by dams until weaning on PND 21 and then sacrificed	F0: Parental: 13.6 mg/ kg b.w. per day Cr(VI) Reproduction: 30.3 mg/kg b.w. per day Cr(VI) F1: Parental: 200 ppm (16.1 mg Cr(VI)/kg b.w. per day Reproduction: 400 ppm (37 mg Cr(VI)/kg b.w. per day	F0: Parental: 30.3 mg/ kg b.w. per day Cr(VI) Reproduction: - F1: Parental: 400 ppm (37 mg Cr(VI)/kg b.w. per day Reproduction: -	No treatment-related effects on fertility or reproductive performance. No effect on oestrous cyclicity of F1 animals. Parents: Slight decrease mean b.w. of HD F0 & F1 M & F. Decrease mean absolute liver weights in HD F0 M &F Treatment-related changes in haematology (decrease MCV, MCH and Hb) for F1 animals.	NTP (1997)

Table H6: Developmental and reproductive toxicity studies with Cr(VI) compounds (continued)

Study*	NOAEL	LOAEL	Effect	Reference
		Male reproductive toxicity studies		
Adult male Sprague Dawley rats Oral (drinking water) 0 or 1000 mg potassium dichromate/L for 12 weeks. **Doses: 0 and 32 mg Cr(VI)/kg b.w. per day[(b)]** Mated with untreated females	-	1000 mg/L **32 mg Cr(VI)/kg b.w. per day**	Inhibitory effect on sexual and aggressive behaviour: reduction number of mounts, increased post-ejaculatory interval, decrease number of M ejaculating, decreased aggressive behaviour towards other M. Decrease b.w., absolute and relative testes, absolute seminal vesicles and preputial gland weights. No effect on fertility of treated M (number pregnant females, implantations or viable fetuses). Increase number of resorptions. No histopathology parformed.	Bataineh et al. (1997)
Male Wistar rats Oral (diet) 0, 10 or 20 mg chromium trioxide/ kg b.w. per day. **Doses: to 0, 5, 10 mg Cr (VI)/kg b.w. per day[(a)]** 6 days treatment Animals sacrificed 6 weeks after treatment	-	10 mg/kg per day CrO_3 **5 mg Cr (VI)/kg b.w. per day**	Dose-related reduction epididymal sperm counts and increase frequency abnormal sperm. Decrease diameter seminiferous tubules, disruption germ cell arrangement (equivocal given uncertainty in sampling and sectioning methods).	Li et al. (2001)

Table H6: Developmental and reproductive toxicity studies with Cr(VI) compounds (continued)

Study*	NOAEL	LOAEL	Effect	Reference
Mature male Charles Foster rats Oral (gavage) 0, 20, 40 or 60 mg sodium dichromate/kg b.w. per day for 90 days **Doses: 0, 7.9, 15.9, 23.8 mg Cr(VI)/kg b.w. per day[(a)]**	-	20 mg sodium dichromate/kg b.w./day **7.9 mg Cr(VI)/kg b.w. per day**	Lower final b.w. and b.w. gain at 2 HD. Lower mean testis weights, Lower testicular DNA and RNA content reduction seminiferous tubule diameter, reduction Leydig cell populations, degenerative changes in Leydig cells, and reduction. Leydig cell nuclear diameter at 2 HD. Dose-related reduction testicular protein content at all doses. Reduction resting spermatocyte counts at HD. Reduction pachytene spermatocyte counts and stage 7 spermatid counts at 2 HD. Increase testicular cholesterol and decrease succinic dehydrogenase at 2 HD. Decrease 3βΔ5-HSH and serum testosterone at all doses.	Chowdhury and Mitra (1995)
Adult male swiss mice Oral (drinking water) 0, 1000, 2000, 4000 or 5000 mg potassium dichromate/L, **Doses: 0, 53, 106, 212 and 265 mg Cr(VI)/kg b.w. per day[(b)]** for 12 weeks X untreated F (10 days) F sacrificed 1-week after end mating	-	1000 mg/L potassium dichromate **53 mg Cr(VI)/kg b.w. per day**	Reduction b.w. and testis weight at 2000 and 5000 mg/L. Reduction seminal vesicles and preputial glangs weight at 5000 mg/L. Decrease frequency pregnant F at HD. Decrease implantation frequency or number of live fetuses at 2000 and 4000 mg/L Resorptions at 1000 and 5000 mg/L.	Elbetieha and Al-Hamood (1997)

Table H6: Developmental and reproductive toxicity studies with Cr(VI) compounds (continued)

Study*	NOAEL	LOAEL	Effect	Reference
Pregnant BALB/c mice Oral (drinking water) 0 or 1000 mg potassium dichromate/L **Doses: 0 and 76 mg Cr(VI)/kg b.w. per day[(c)]** GD 12- lactation D 20 litters culled to 8 pups/litter on first day from PND 20: examination for vaginal opening PND 60: M caged with untreated F, mating for 10 days Sacrifice F 1 wk after mating period for examination of uterine contents Additional animals sacrificed on PND 50	1000 mg/L Potassium dichromate **76 mg Cr(VI)/kg b.w. per day**	-	No signif. change in fertility for M. No signif. diffrences in number of implantations, viable foetuses or resorptions. Additional M sacrificed on PND 50: no effect on b.w., testis weight or seminal vesicle or preputial gland weights.	Al-Hamood et al. (1998)
Male BALB/c albino Swiss mice Oral (diet) 0, 100, 200 and 400 mg potassium dichromate /kg feed for 35 days Animals sacrificed at end of treatment **Doses:** **M: 16, 28, 63 mg Cr(VI)/kg b.w. per day[(c)]**	-	100 mg potassium dichromate/kg feed **16 mg Cr5VI)/kg b.w. per day**	No effect on food consumption, b.w. gain, mean testes and epididymis weights. Dose-related increases in % degenerated tubules and undegenerated tubules without spermatogonia. Dose-related reduction in mean numbers spermatogonia. Dose-related increases in number of resting spermatocytes. Increases in frequency of cells in pachytene phase at all doses and in zygotene phase at 2 low dose. Reduction epididymal sperm counts and increases % abnormal sperm in the mid and high doses. (findings appeared inconsistent)	Zahid et al. (1990)

Table H6: Developmental and reproductive toxicity studies with Cr(VI) compounds (continued)

Study*	NOAEL	LOAEL	Effect	Reference
9-week exposure Male and female BALB/c mice + 8-week recovery Oral (diet) Potassium dichromate 0, 15, 50, 100 and 400 mg/kg food, corresponding to 0, 4, 13, 28, 115 mg potassium dichromate/kg b.w. per day **Doses:** **0, 1.4, 4.6, 9.9, 40.7 mg Cr(VI)/kg b.w. per day[(a)]**	Systematic tox: 15 mg/kg food **1.4 mg Cr(VI)/kg b.w. per day** Reproduction tox: **40.7 mg Cr(VI)/kg b.w. per day**	Systematic tox: 50 mg/kg food **4.6 mg Cr(VI)/kg b.w. per day** Reproduction tox: -	No effect on spermatogenesis has been reported.	NTP (1996a, 1997)
-week exposure Male and female Sprague-Dawley rats+ 8-week recovery Oral (diet) Potassium dichromate 0, 15, 50, 100 and 400 mg/kg food corresponding to 0, 5.3, 17.7, 35.3, 141 mg Cr(VI)/kg food **Doses:** **0, 0.4, 1.1, 2.3, 9.2 mg Cr(VI)/kg b.w. per day[(a)]**	Systematic tox: 100 mg/kg food **2.3 mg Cr(VI)/kg b.w. per day** Reproduction tox: **9.2 mg Cr(VI)/kg b.w. per day**	Systematic tox: 400 mg/kg food **9.2 mg Cr(VI)/kg b.w. per day** Reproduction tox: -	No effect on testis, epididymus or spermatogenesis has been reported.	NTP (1996b, 1997)
Adult male New Zealand white rabbits Oral (gavage) 0, 5 mg potassium dichromate/kg b.w. per day for 10 weeks **Doses: 0, 1.8 mg Cr(VI)/kg b.w. per day[(a)]**	-	5 mg/kg b.w. potassium dichromate **1.8 mg Cr(VI)/kg b.w. per day**	No adverse clinical signs. Decrease b.w., mean testes and epididymis weights. Reduction mean plasma testosterone. Increases in reaction time, pH and % of dead sperm. Decreases in packed sperm volume, sperm concentratin, total sperm output, sperm motility, total motile sperm, % normal sperm, total functional sperm fraction. Seminal plasma parameters were also affected.	Yousef et al. (2006)

Table H6: Developmental and reproductive toxicity studies with Cr(VI) compounds (continued)

Study*	NOAEL	LOAEL	Effect	Reference
Adult male bonnet monkeys (*Macaca radiata* Geoffrey) Oral (drinking water) 0, 50, 100, 200 or 400 mg potassium dichromate/L For 180 days Add. Group: 400 mg/L potassium dichromate for 180 days + recovery period of 180 days **Doses: 0, 0.8, 1.7, 3.4, and 6.8 mg Cr(VI)/kg b.w. per day**	50 mg/L potassium dichromate **0.8 mg Cr(VI)/kg b.w. per day**	100 mg/L potassium dichromate **1.7 mg Cr(VI)/kg b.w. per day**	Decrease sperm counts at doses ≥ 100 mg/L (dose-related). Sperm counts returned to control after 3-month recovery. Decrease activity superoxide dismutase in seminal plasma and sperm at doses ≥ 100 mg/L (effect reversible). Decrease catalase activity in seminal plasma and sperm at doses ≥ 100 mg/L (effect reversible). Decrease glutathione level in seminal plasma and sperm at doses ≥ 200 mg/L (effect reversible). Increase hydrogen peroxide concentration in seminal plasma and sperm at doses ≥ 100 mg/L (effect reversible). Dose-related increase in plasma chromium concentration by the end of 1-month treatment (partially reversible, remained above control levels). Data to support hypothesis that chronic Cr(VI) exposure caused reversible oxidative stress in the seminal plasma and sperm, leading to sperm death and reduced motility of live sperm.	Subramanian et al. (2006)
Adult male macaque monkeys (Macaca radiata) Oral (drinking water) 0, 100, 200 or 400 mg/L potassium dichromate for 180 days **Doses: 0, 1.7, 3.4, and 6.8 mg Cr(VI)/kg b.w. per day**	-	100 mg/L potassium dichromate **1.7 mg Cr(VI)/kg b.w. per day**	Accumulation of sperm-derived lipofuscian material in principal cells, basal cells and intraepithelial macrophages of the epithelium of epididymal tissues. Principal cells apparently phagocytosed dead sperm from the lumen.	Aruldhas et al. (2006)

Table H6: Developmental and reproductive toxicity studies with Cr(VI) compounds (continued)

Study*	NOAEL	LOAEL	Effect	Reference
Adult male bonnet monkeys (*Macaca radiata*) Oral (drinking water) 0, 100, 200 or 400 mg/L potassium dichromate for 180 days + recovery period of 180 days (half of animals) **Doses: 0, 1.7, 3.4, and 6.8 mg Cr(VI)/kg b.w. per day**	-	100 mg/L potassium dichromate **1.7 mg Cr(VI)/kg b.w. per day**	Increase plasma chromium levels at 24h following last day of treatment (up to 10 fold), values declined to control values after 180 days recovery. Decrease relative testes weights at end of treatment, returned to normal following 180 days recovery. Disorganized seminiferous tubules, dose-related decrease in diameter. Depletion of germ cells and hyperplasia of Leydig cells, absence of spermatids in some tubules, Sertoli cell fibrosis, vacuoles surrounding spermatids still adherent to the epithelium, multinucleate giant cells in adluminal compartment, lumen filled with prematurely released germ cells and cell debris and abnormal appearance of chromatin in postzygotene spermatocyte. These effects disappeared after recovery period. Treatment-related changes in testicular structure. The specific activities of testicular superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, and glucose-6-phosphate dehydrogenase, considered to indicate the status of oxidative stress in the testis, were all significantly decreased. The authors concluded that Cr(VI) disrupts spermatogenesis by inducing free-radical toxicity.	Aruldhas et al. (2005)
Adult male bonnet monkeys (*Macaca radiata*) Oral (drinking water) 0, 100, 200 or 400 mg/L potassium dichromate for 180 days + recovery period of 180 days (half of animals) **Doses: 0, 1.7, 3.4, and 6.8 mg Cr(VI)/kg b.w. per day**	-	100 mg/L potassium dichromate **1.7 mg Cr(VI)/kg b.w. per day**	Two types of 'microcanals' in epididymal epithelium. Effect dose-related. The authors hypothesize that the first type of microcanal provides passage for spermatozoa to bypass the blocked main duct. The second type of microcanal was proposed as a means by which spermatozoa reaching the core of the epithelium are sequestered, as a mechanism to avoid an autoimmune response. (effects were not quantified, but the authors' believed that the incidence and severity of microcanalisation increased with increasing Cr(VI) concentration). They interpreted their findings as indicative of Cr(VI)-induced obstruction of the distal portion of the cauda epididymis.	Aruldhas et al. (2004)

Table H6: Developmental and reproductive toxicity studies with Cr(VI) compounds (continued)

Study*	NOAEL	LOAEL	Effect	Reference
Female reproductive toxicity studies				
Pregnant Wistar rats allowed to deliver normally (18/group) litters culled to 4 F pups/dam on first day treatment during lactation PND 1-20 oral (drinking water) 0, 200 mg potassium dichromate/L. **Doses: 0 and 24 mg Cr(VI)/kg b.w. per day**[(b)] Sacrifice F offsprings on PND 21 (weaning), PND 45 or PND 65 Blood and ovaries were collected	-	200 mg/L **24 mg Cr(VI)/kg b.w. per day**	Offspring: Increase chromium levels in plasma and ovaries Increase time vaginal opening (marker for onset of puberty). Signif. lengthening of estrous cycle, specif. diestrous. Reduction numbers of ovarian follicles. Signif. changes in circulationg levels of steroid and pituitary hormones.	Banu et al. (2008)
Wistar rats Oral (drinking water) 0, 50 or 200 mg potassium dichromate/L **Doses: 0, 6 and 24 mg Cr(VI)/kg b.w. per day**[(b)] litters culled to 4 F pups/dam on first day treatment during lactation PND 1-21 (weaning) Sacrifice F offsprings on PND 21, 45 or 65 Blood and uterus were collected	-	50 mg/L **6 mg Cr(VI)/kg b.w. per day**	Dose-related reductions in antioxidant enzymes activities in uterine tissue (oxidative stress) associated with delayed puberty and alterated steroids and gonadotrophin levels.	Samuel et al. (2011)

Table H6: Developmental and reproductive toxicity studies with Cr(VI) compounds (continued)

Study*	NOAEL	LOAEL	Effect	Reference
Pregnant BALB/c mice Oral (drinking water) 0 or 1000 mg potassium dichromate/L **Doses: 0 and 76 mg Cr(VI)/kg b.w. per day[(c)]** GD 12- lactation D 20 litters culled to 8 pups/litter on first day from PND 20: examination for vaginal opening PND 60: F caged with untreated M, mating for 10 days Sacrifice F 1 wk after mating period for examination of uterine contents Additional animals sacrificed on PND 50	-	1000 mg/L **76 mg Cr(VI)/kg b.w. per day**	No effect on b.w. of F offsprings. Delayed time vaginal opening (delay in onset of puberty) by about 3 days. Reduction pregnancy rate, number of implantations, viable fetuses 3 resorptions among treated F (none in C). On PND 50: no effect on b.w., ovarian weight or uterine weight.	Al-Hamood et al. (1998)
Developmental toxicity				
Gestational exposure				
Pregnant Wistar rats Oral (drinking water) 0, 50 mg/L potassium chromate GD 6-15 **Doses: 0 and 1.6 mg Cr (VI)/kg b.w. per day[(b)]**	Maternal & Developmental toxicity: -	Maternal & Developmental toxicity: 50 mg/L **1.6 mg Cr (VI)/kg b.w. per day**	Dams: Decrease b.w. gain mainly attributed to retarded fetal growth and resorptions Histopathological lesions in placenta. Litters: Increase number of pre- and post-implantation loss, resorption frequency and frequency dead fetuses/litter. Fetuses: Decrease number live fetuses/litter, fetal weight. Increase frequencies of visceral and skeletal anomalies, in particular renal pelvis dilatation and incomplete ossification of skull bones. Chromium passed placental barrier and accumulated in fetal tissues. Signif. increase chromium levels in blood, placenta and fetal tissues.	Elsaieed and Nada (2002)

Table H6: Developmental and reproductive toxicity studies with Cr(VI) compounds (continued)

Study*	NOAEL	LOAEL	Effect	Reference
Pregnant Swiss albino mice Oral (drinking water) 0, 250, 500 or 750 mg potassium dichromate/L **Doses: 0, 53, 101, and 152 mg Cr(VI)/kg b.w. per day**[(c)] GD 6-14 Sacrifice on GD 19	Maternal toxicity: 250 mg/L **53 mg Cr(VI)/kg b.w. per day** Developmental toxicity: -	Maternal toxicity: 500 mg/L **101 mg Cr(VI)/kg b.w. per day** Developmental toxicity: 250 mg/L **53 mg Cr(VI)/kg b.w. per day**	Dams: Dose-related decrease b.w. gain in 2HD animals. Litter data: Dose-related increase number of resorptions at all doses. Decrease number of fetuses (live and dead)/litter in 2HD. Dose-related increase post-implantation loss in 2 HD. Fetuses: No effect on fetal Crown-rump length (CRL). Decrease fetal weight in 2HD. Signif. increase of gross external abnormalities at HD (drooping wrist, subdermal hemorrhagic patches). No gross visceral abnormalities. Signif. increase frequency of reduced caudal ossification in 2 HD. Signif. increase frequency of reduced ossification nasal, frontal, parietal interparietal and tarsals in HD group. Dose-related increase chromium levels in maternal blood, placentas and fetuses.	Junaid et al. (1996a)

Table H6: Developmental and reproductive toxicity studies with Cr(VI) compounds (continued)

Study*	NOAEL	LOAEL	Effect	Reference
Pregnant Swiss albino mice Oral (drinking water) 0, 250, 500 or 750 mg potassium dichromate/L GD 14-19 Sacrifice on GD 19 **Doses: 0, 45, 90, 135 mg Cr(VI)/kg b.w. per day**[(b)]	Maternal toxicity: 250 mg/L **45 mg Cr(VI)/kg b.w. per day** Developmental toxicity: -	Maternal toxicity: 500 mg/L **90 mg Cr(VI)/kg b.w. per day** Developmental toxicity: 250 mg/L **45 mg Cr(VI)/kg b.w. per day**	Dams: Dose-related decrease b.w. gain in 2HD animals. Litter data: Dose-related signif. increase post-implantation loss, placental weights in 2 HD. Fetuses: Dose-related decrease fetal weights and CRL (all doses). No gross visceral abnormalities. Signif. increase of gross external abnormalities at HD (drooping wrist, subdermal hemorrhagic patches, kinky tail, short tail) and drooping wrist at 500 mg/L. Signif. increase frequency reduced caudal ossification at all doses, reduced tarsal ossification in 2 HD and reduced ossification nasal, parietal, interparietal, carpal, metacarpals in HD. Dose-related signif; increase chromium levels in maternal blood, placentas and foetuses. Chromium appeared to accumulate in placenta (slower rate of transfer from placenta to fetus than from maternal blood to placenta).	Junaid et al. (1995)

Table H6: Developmental and reproductive toxicity studies with Cr(VI) compounds (continued)

Study*	NOAEL	LOAEL	Effect	Reference
Pregnant ITRC-bred albino mice Oral (drinking water) 0, 250, 500 or 1000 mg potassium dichromate/L GD 1-19 Sacrifice GD 20 **Doses: 48; 99 and 239 mg Cr (VI)/kg b.w. per day[(c)]**	Maternal toxicity: 250 mg/L **48 mg Cr (VI)/kg b.w. per day** Developmental toxicity: -	Maternal toxicity: 500 mg/L **99 mg Cr (VI)/kg b.w. per day** Developmental toxicity: 250 mg/L **48 mg Cr (VI)/kg b.w. per day**	Dams: Signif. lower b.w. gain in 2 HD. Litter data: Signif. decrease litter size and signif. increase pre-implantation loss in 500 mg/L group. Signif. increase resorption frequency and post-implantation loss at 250 and 500 mg/L. Fetuses: Signif. decrease fetal weights and CRL. Increase frequency kinked tail and subdermal hemorrhages at 500 mg/L. Increase frequency reduced ossification (cranial, forelimb, hind limb, sternebrae, thoracic vertebrae, caudal vertebrae), reduced ribs at 500 mg/L. Reduced cranial ossification also at 250 mg/L. No internal soft-tissue anomalies. Stat. signif. chromium levels in maternal blood at HD, placentas at 2 HD and foetuses at 500 mg/L.	Trivedi et al. (1989)
Mated female Sprague-Dawley rats Oral (gavage) 0, 25 mg potassium dichromate/rat GD 1-3 or GD 4-6 Sacrifice on GD 20 **Doses: 0, 36 mg Cr(VI)/kg b.w. per day**	Maternal toxicity: NR Developmental toxicity: -	Maternal toxicity: NR Developmental toxicity: 25 mg/rat **36 mg Cr(VI)/kg b.w. per day**	GD 1-3: 0 females/10 were pregnant, no implantations were observed. GD 4-6: decreased number of pregnant females (70 % compared to 90 %), of implantations (81 % of controls) and statistically significant decrease in number of viable fetuses (31 % of controls) and increased number of resorptions/total number of implantations (77.3 % compared to 2.4 % in controls).	Bataineh et al. (2007)

Table H6: Developmental and reproductive toxicity studies with Cr(VI) compounds (continued)

Study*	NOAEL	LOAEL	Effect	Reference
Dams exposed prior to mating				
Female rats (Druckery) Oral (drinking water) 0, 250, 500 and 750 mg potassium dichromate/L Day 50 of age for 3 months X untreated M Sacrifice F on GD 19 **Doses: 0, 45, 89, 124 mg Cr(VI)/kg b.w. per day**[(c)]	-	250 mg/L **45 mg Cr(VI)/kg b.w. per day**	Dams: Mortality: 15 % at 500 and 10 % at 750 mg/L. End of 90 days treatment: all F acyclic and in persistent diestrous phase, during subsequent 15-20 day mating period, estrous cycles returned and animals began to mate. Signif. and dose-related lengthening of estrous cycles. Dose-related decrease mating and fertility indices. Dose-related decrease maternal b.w. at end of gestation and b.w. gain during gestation (stat signif at 2 HD). Litter data: Dose-related decrease number corpora lutea, implantations, live fetuses/litter (stat signif at 2 HD). Dose-related increase resorption frequency (stat signif at 2 HD), frequency of pre- and post-implantation loss (all doses). Dose-related decrease placental weights (stat. signif. at 2 HD) Fetuses: Dose-related decrease fetal weight (all doses), CRL (stat. signif. at 2 HD). No gross visceral anomalies. Signif. increase frequency of drooping wrist and subdermal hemorrhagic patches (all doses). Signif. increase frequency kinky tail and short tail at 2 HD. Dose-related increase frequency reduced caudal ossification (all doses). Dose-related increase Cr concentrations in maternal blood, placenta and fetal tissues.	Kanojia et al. (1998)

Table H6: Developmental and reproductive toxicity studies with Cr(VI) compounds (continued)

Study*	NOAEL	LOAEL	Effect	Reference
Female Swiss albino rats Oral (drinking water) 0, 250, 500 and 750 mg potassium dichromate/L treatment for 20 days X untreated M Sacrifice F on GD 19 **Doses: 0, 31, 60, 75 mg Cr(VI)/kg b.w. per day**[(c)]	Maternal toxicity: 250 mg/L **31 mg Cr(VI)/kg b.w. per day** Developmental toxicity: -	Maternal Toxicity: 500 mg/L **60 mg Cr(VI)/kg b.w. per day** Developmental toxicity: 250 mg/L **31 mg Cr(VI)/kg b.w. per day**	Dams: Dose-related lengthening of estrous cycles. (stat signif at HD). Dose-related decrease mating and fertility indices. Dose-related decrease maternal b.w. gain during gestation (stat signif at 2 HD). Litter data: Dose-related decrease number corpora lutea, and implantations (stat signif at 2 HD) and live fetuses/litter (all doses). Dose-related increase resorption frequency (all doses), frequency of pre- implantation loss (2 HD) and post-implantation loss (all doses). Dose-related increase placental weights. Fetuses: No effect on fetal weight and CRL. No gross visceral abnormalities. Increase frequency gross abnormalities and skeletal anomalies at HD (dermal hemorrhagic patches, kinky tail, short tail, reduced parietal and inter-parietal ossification, reduced caudal ossification). Reduced caudal ossification also seen at 500 mg/L. Dose-related increase Cr concentrations in maternal blood, placenta and fetal tissues.	Kanojia et al. (1996)

Table H6: Developmental and reproductive toxicity studies with Cr(VI) compounds (continued)

Study*	NOAEL	LOAEL	Effect	Reference
Adult female Swiss mice Oral (drinking water) 0, 2000 or 5000 mg potassium dichromate/L for 12 weeks X untreated M (10 days) Sacrifice F 1 wk after mating period for examination of uterine contents Additional animals were not mated and sacrificed at end of treatment period for determinayion of b.w. and organ weights **Doses: 0, 106 and 265 mg Cr(VI)/kg b.w. per day**[(b)]	Maternal toxicity: 2000 mg/L **106 mg Cr(VI)/kg b.w. per day** Developmental toxicity: -	Maternal toxicity: 5000 mg/L **265 mg Cr(VI)/kg b.w. per day** Developmental toxicity: 2000 mg/L **106 mg Cr(VI)/kg b.w. per day**	Dams: Increase ovarian weights at HD. Number of pregnant animals/total mated F: 17/18, 14/15 and 9/11. Litter data: Reduction of number of implantations/litter, number of viable foetuses. Increase number of litters with resorptions.	Elbetieha and Al-Hamood (1997)

Table H6: Developmental and reproductive toxicity studies with Cr(VI) compounds (continued)

Study*	NOAEL	LOAEL	Effect	Reference
Female Swiss albino mice Oral (drinking water) 0, 250, 500 or 750 mg potassium dichromate/L, 20 days X untreated M Sacrifice on GD 19 **Doses:0, 52, 98, and 169 mg Cr(VI)/kg b.w. per day**[(c)]	Maternal toxicity: 500 mg/L **98 mg Cr(VI)/kg b.w. per day** Developmental toxicity: -	Maternal Toxicity: 750 mg/L **169 mg Cr(VI)/kg b.w. per day** Developmental toxicity: 250 mg/L **52 mg Cr(VI)/kg b.w. per day**	Litter data: Decrease number corpora lutea, no implantation sites, no resorptions, no live foetuses at HD. Dose-related decrease number implantations /litter and live foetuses/litter at 2 LD. Dose-related increase pre-implantation loss and resorptions/litter (stat signif at 500 mg/l) and post- implantation loss (at 2 LD). Decrease placental weight at 250 mg/l and increase at 500 mg/L. Fetuses: Dose-related decrease fetal weight and CRL. Signif. increase frequency of sub-dermal hemorrhagic patches, kinky tail, short tail and reduced parietal, inter-parietal and caudal ossification at 500 mg/L. Signif. increase frequency of reduced caudal ossification at 250 mg/L. Significant and dose-related increase of Cr(VI) levels in maternal blood. Dose-related increase Cr(VI) levels in placentas in 2 LD and in foetuses at 500 mg/L.	Junaid et al. (1996b)

b.w.: body weight; M: male; F: female; HD: highest dose; NOAEL: no-observed-adverse-effect level; LOAEL: lowest-observed-adverse-effect level; MW: molecular weight; ALT: alanine aminotransferase; AST: aspartate aminotransferase; GSH/GSSG: reduced-to-oxidized glutathione ratio; MCV: mean corpuscular volume; MCH: mean corpuscular haemoglobin; CRL: Crown-rump length; PND: postnatal day; Hb: Haemoglobin; LD: low dose.

* In the conversions from concentration to daily doses, the MW of the anhydrous salts were used when no information on hydration number was available in the original publication.

(a): Data reported in the original publication.

(b): Conversion using the default correction factor for subacute/subchronic/chronic exposure via drinking water/feed from EFSA SC (2012).

(c): Conversion using drinking water/feed consumption data and average body weight reported in the publication.

Table H7: Summary of *in vivo* genotoxicity of Chromium (VI) – oral route

Test system/ Endpoint	Compound	Dose/route	Exposure time/evaluation time	Tissue	Response*	Reference
Female C57BL/ 6J*pun*/*pun* mouse DNA deletions	Potassium dichromate	drinking water at 62.5, or 125 mg Cr(VI)/L **Doses: 12.5 or 25 mg Cr(VI)//kg b.w. per day**	10.5 to 20.5 days postcoitum 20d old offspring analysed	20-day-old offspring were harvested	Positive 62.5 mg Cr(VI)/L 12.5 mg Cr(VI)//kg b.w. per day Dose-response;	Kirpnick-Sobol et al. (2006)
Pregnant Swiss albino mouse Micronuclei	Potassium dichromate	drinking water at 0, 5, or 10 mg Cr(VI)/L **Doses: 0, 0.9, 1.8 mg Cr(VI)/kg b.w. per day**[(a)]	throughout the duration of pregnancy sacrifice on d18 of pregnancy	bone marrow cells from dams; liver and peripheral blood cells from fetuses	Negative 10 mg Cr(VI)/L 1.8 mg Cr(VI)/kg b.w. per day	De Flora et al. (2006)
	Sodium dichromate dihydrate	drinking water at 0, 5, or 10 mg Cr(VI)/L **Doses: 0, 0.9, 1.8 mg Cr(VI)/kg b.w. per day**[(a)]				
BDF1 male mouse	Potassium dichromate	drinking water at 0, 10, or 20 mg Cr(VI)/L **Doses: 0, 3, or 6 mg Cr(VI)/kg b.w. per day**	for 20 d	bone marrow, peripheral blood cells	Negative 20 mg Cr(VI)/L 6 mg Cr(VI)/kg b.w. per day	
BDF1 mouse (male and female)	Sodium dichromate dihydrate	drinking water at 0, 5, 50, and 500 mg Cr(VI)/L **Doses:** **F: 1.4, 14, 140 mg Cr(VI)/kg b.w. per day** **M: 1.65, 16.5, 165 mg Cr(VI)/kg b.w. per day**	for 210 d		Negative 500 mg Cr(VI)/L F: 140 mg Cr(VI)/kg b.w. per day M: 165 mg Cr(VI)/kg b.w. per day	
Swiss-Webster mouse Micronuclei	Potassium dichromate	drinking water at 0, 1, 5, or 20 mg Cr(VI)/L **Doses:** **0.2, 0.9, or 3.6 mg Cr(VI)/ kg b.w. per day**[(a)]	group 1: drinking water ad libitum, for 48 hours; group 2: two bolus doses (20 mL/kg) at 24 and 48 hrs before sacrifice	mouse bone marrow cells	Negative 20 mg Cr(VI)/L 3.6 mg Cr(VI)/ kg b.w. per day	Mirsalis et al. (1996)
B6C3F1 BALB/c *am3*-C57BL/6 male mouse Micronuclei	Sodium dichromate dihydrate	drinking water at 0, 62.5, 125, or 250 mg/L (0, 21.8, 43.6, or 87.2 mg Cr(VI)/L); **Doses: 0, 2.8, 5.2, or 8.7 mg Cr(VI)/kg b.w. per day**	for 3 mo	peripheral red blood cells	Equivocal 87.2 mg Cr(VI)/L (B6C3F1) Negative 87.2 mg Cr(VI)/L (BALB/c) Positive 43.6 mg Cr(VI)/L (*am3*-C57BL/6)	NTP (2007)

Table H7: Summary of *in vivo* genotoxicity of Chromium (VI) - oral route (continued)

Test system/ Endpoint	Compound	Dose/route	Exposure time/evaluation time	Tissue	Response*	Reference
B6C3F1 mouse Micronuclei		drinking water at 0, 62.5, 125, 250, 500, or 1000 mg/L (0, 21.8, 43.6, 87.2, 174.5, or 349 mg Cr(VI)/L); **Doses: 0, 3.1, 5.2, 9.1, 15.7, or 27.9 mg Cr(VI)/kg b.w. per day**	for 3 mo	peripheral red blood cells	Negative 349 mg Cr(VI)/L 27.9 mg Cr(VI)/kg b.w. per day	NTP (2007)
BDF1 male mouse Micronuclei	Potassium dichromate	single gavage dose of 0 or 50 mg /kg **Doses: 17.7 mg Cr(VI)/kg b.w. per day**		bone marrow cells	Negative 50 mg Cr(VI)/kg 17.7 mg Cr(VI)/ kg b.w. per day	De Flora et al. (2006)
Male MS/Ae and CD-1 mouse Micronuclei	Potassium chromate	single gavage doses of 0, 10, 20, 40, 80, 160, or 320 mg/kg **Doses: 0, 5.3, 10.7, 21.4, 42.8, 85.7 mg Cr(VI)/ kg b.w. per day**		Bone marrow cells	Negative Negative up to acutely toxic doses 85.7 mg Cr(VI)/ kg b.w. per day	Shindo et al. (1989)
Swiss albino mouse DNA damage Comet assay	Potassium dichromate	single gavage doses of 0, 0.59,1,19, 2.38, 4.75, 9.5, 19, 38 or 76 mg/kg **Doses: 0, 0.21, 0.42, 0.84, 1.68, 3.37, 6.7, 13,5 or 26.9 mg Cr(VI)/kg b.w. per day**	samples analysed at 24, 48, 72 and 96 hrs, and 1 and 2 wks post-treatement	leukocytes	Positive 0.21 mg Cr(VI)/kg b.w. per day Dose-response	Devi et al. (2001)
Swiss albino mouse DNA damage Comet assay	Potassium dichromate	single gavage doses of 0, 25, 50 and 100 mg/kg **Doses: 0, 8.8, 17.7 and 35.4 Cr(VI)/kg b.w. per day**	for 1 d or 5 consecutive d	peripheral lymphocytes	Positive 8.8 mg Cr(VI)/kg b.w. per day Dose-response	Wang et al. (2006)
ddY mouse DNA damage Comet assay	Potassium dichromate	single gavage doses of 0 or 320 mg/kg **Doses: 0 or 85.7 mg Cr(VI)/kg b.w. per day**	samples analysed at 3, 8 and 24 hrs after treatment	stomach, colon. Liver, kidney, bladder, lung, brain and bone marrow	Positive 85.7 mg Cr(VI)/kg b.w. per day	Sekihashi et al. (2001)

b.w.: body weight.

* The lowest effective dose is indicated for positive results and the highest dose tested for negative results.

(a): Doses calculated using the default correction factor for subacute/subchronic/chronic exposure via drinking water/feed from EFSA SC (2012).

Table H8: Summary of *in vivo* genotoxicity of Chromium (VI) - non-oral route

Test system/ Endpoint	Compound	Dose/route	Exposure time/evaluation time	Tissue	Response*	Reference
Male lacZ transgenic MutaTM mouse Mutation	Potassium chromate	Single i.p. dose, with 24 hrs interval,of 0 or 40 mg/kg **Doses: 0, 10.7 mg Cr(VI)/kg b.w. per day**	sampling at 1 and 7 d after 2nd treatment	liver and bone marrow cells	Positive 10.7 mg Cr(VI)/kg b.w. per day	Itoh and Shimada (1998)
C57BL/6 Big Blue mouse Mutation	Potassium dichromate	single doses (intratracheal instillation) **Doses: 0 or 6.75 mg Cr(VI)/kg b.w. per day**	4 wks for gene expression	lung, kidney, liver	Positive in lung and kidney 6.75 mg Cr(VI)/kg b.w. per day Negative in liver	Cheng et al. (2000)
CBA . C57Bl/6J hybrid male mouse Dominant lethality (frequency of postimplantatio n loss)	Potassium dichromate	single i.p. doses of 0, 0.5, 1.0, 1.5, 2.0, 10, or 20 mg/kg **Doses: 0, 0.18, 0.35, 0.53, 0.71, 3.5, or 7.1 mg Cr(VI)/kg b.w. per day** repeated i.p. doses of 0, 1.0, or 2.0 mg/kg daily for 21 days **Doses: 0, 0.35, 0.71 mg Cr(VI)/kg b.w. per day**	Pregnant dams were sacrificed 12–14 d after conception.		Positive 7.1 mg Cr(VI)/kg (acute exposure) Positive 0.71 mg Cr(VI)/kg b.w. per day (repeated exposure)	Paschin et al. (1982)
CBA . C57Bl/6J hybrid mouse Micronuclei	Potassium dichromate	single i.p. doses of 0, 1, 5, or 10 mg/kg **Doses: 0.35, 1.77, or 3.54 mg Cr(VI)/kg b.w. per day**	samples analysed 24, 48, and 72 hrs after treatment	bone marrow cells	Positive 0.35 mg Cr(VI)/kg b.w. per day	Paschin and Toropzev (1982)
Slc:ddY mouse	Potassium chromate	two i.p. doses with 24 hrs interval of 0, 30, 40, and 50 mg/kg **Doses: 0, 8.0, 10.7, 13.4 mg Cr(VI)/kg b.w. per day**		bone marrow cells	Positive 8.0 mg Cr(VI)/kg b.w. per day	Itoh and Shimada, (1996)
NMRI mouse Micronuclei	Potassium chromate	two i.p. doses with 24 hrs interval of 0, 12.12, 24.25, or 48.5 mg/kg **Doses: 0, 3.2, 6.49, or 13.0 mg Cr(VI)/kg b.w. per day**		bone marrow	Positive 13 mg Cr(VI)/kg b.w. per day	Wild (1978)
MS/Ae and CD-1 male mouse Micronuclei	Potassium chromate	single i.p. doses of 0, 10, 20, 40, or 80 mg/kg **Doses: 0, 2.7, 5.3, 10.7, 21.4 mg Cr(VI)/ kg b.w. per day**		bone marrow cells	Positive 10.7 mg Cr(VI)/kg b.w. per day Dose-response	Shindo et al. (1989)
LacZ transgenic MutaTM male mouse Micronuclei	Potassium chromate	two i.p. doses with 24 hrs interval of 0 or 40 mg/kg **Doses: 0, 10.7 mg Cr(VI)/kg b.w. per day**		peripheral red blood cells	Positive 10.7 mg Cr(VI)/kg b.w. per day	Itoh and Shimada (1997)

Table H8: Summary of *in vivo* genotoxicity of Chromium (VI) - non-oral route (continued)

Test system/ Endpoint	Compound	Dose/route	Exposure time/evaluation time	Tissue	Response*	Reference
MS and ddY mouse Micronuclei	Potassium chromate	single i.p. doses of 0, 12.5, 25, or 50 mg/kg **Doses: 0, 3.3, 6.7, 13.4 mg Cr(VI)/kg b.w. per day**		bone marrow cells	Positive 13.4 mg Cr(VI)/kg b.w. per day Dose-response	Hayashi et al. (1982)
BALB/c mouse Micronuclei	Potassium dichromate	single i.p. doses of 0 or 400 µmol **Doses: 20.8 mg Cr(VI)/kg b.w. per day**		bone marrow	Positive(T) 20.8 mg Cr(VI)/kg b.w. per day	Wronska-Nofer et al. (1999)
Pregnant Swiss albino mouse: Micronuclei	Potassium dichromate	single i.p. doses of 0 or 50 mg $K_2Cr_2O_7$/kg on day 17 of pregnancy **Doses: 0, 17.7 mg Cr(VI)/kg b.w. per day**	Mice were sacrificed on day 18 of pregnancy	bone marrow from dams; liver and peripheral blood from fetuses	Positive 50 mg Cr(VI)/kg	De Flora et al. (2006)
	Sodium dichromate dihydrate	single i.p. doses of 0 or 50 mg SSD/kg on day 17 of pregnancy. **Doses: 0, 17.4 mg Cr(VI)/kg b.w. per day**				
BDF1 male mouse Micronuclei	Potassium dichromate	single i.p. doses of 0 or 50 mg $K_2Cr_2O_7$/kg. **Doses: 0, 17.7 mg Cr(VI)/kg b.w. per day**		bone marrow cells		
Sprague-Dawley rat Chromosomal aberrations	Potassium dichromate	Single i.p. doses of 2.5, 5, 7.5, and 10 mg/kg per day for 5 days **Doses: 0, 0.88, 1.77, 2.65, or 3.54 mg Cr(VI)/kg b.w. per day**		bone marrow cells	Positive 0.88 mg Cr(VI)/kg b.w. per day Dose-response;	Patlolla et al. (2008)
ddY mouse DNA damage Comet assay	Potassium chromate	single i.p. doses of 0 or 120 mg/kg **Doses: 0 or 32.1 mg Cr(VI)/kg b.w. per day**	Samples analysed 3, 8 and 24 hrs after treatemnt	Stomach, colon, liver. kidney, bladder, lung, brain and bone marrow	Positive in stomach, colon, bladder, lung and brain 32.1 mg Cr(VI)/kg b.w. per day Negative in liver, kidney and bone marrow	Sekihashi et al. (2001)
Male albino mouse DNA damage Comet assay	Potassium dichromate	single i.p. **Doses: 0 or 20 mg Cr(VI)/kg b.w. per day**	Samples analysed 15 min and 3 hrs after treatment	liver. kidney, spleen, lung and brain	Positive in liver and kidney 15 min after treatment 20 mg Cr(VI)/kg b.w. per day Negative in spleen, lung and brain marrow	Ueno et al. (2001)

b.w.: body weight; i.p.: intraperitoneal.

* The lowest effective dose is indicated for positive results and the highest dose tested for negative results.

APPENDIX I: OBSERVATION IN HUMANS

I1. Six fatal outcomes accidental or intentional ingestion of hexavalent chromium

Cases of accidental or intentional ingestion of Cr(VI) that have resulted in death have been reported in the past and continue to be reported even in more recent literature. A selection is listed below, even when the amount of ingested Cr(VI) was unknown.

A 22-month-old boy died 18.5 hours after ingesting an unknown amount of a sodium dichromate solution despite gastric lavage. Autopsy revealed generalized edema, pulmonary edema, severe bronchitis, acute bronchopneumonia, early hypoxic changes in the myocardium, liver congestion, and necrosis of the liver, renal tubules, and gastrointestinal tract (Ellis et al., 1982).

A 1-year-old girl died after ingesting an unknown amount of ammonium dichromate with severe dehydration, caustic burns in the mouth and pharynx, blood in the vomitus, diarrhea, irregular respiration, and labored breathing. The ultimate cause of death was shock and hemorrhage into the small intestine (Reichelderfer, 1968).

A 17-year-old male died after ingesting 29 mg Cr(VI)/kg b.w. as potassium dichromate in a suicide. He died 14 hours after ingestion from respiratory distress with severe hemorrhages. Caustic burns in the stomach and duodenum and gastrointestinal hemorrhage were also found (Iserson et al., 1983; Clochesy,1984).

A 35-year-old female died after ingesting approximately 357 mg Cr(VI)/kg b.w. as chromic acid in a suicide (Loubières et al., 1999) and died of multiple organ failure. (metabolic acidosis, gastrointestinal hemorrhage and necrosis, fatty degeneration of the liver, and acute renal failure and necrosis).

A 14-year-old boy died 8 days after admission to the hospital following ingestion of 7.5 mg Cr(VI)/kg b.w. as potassium dichromate from his chemistry set. Death was preceded by gastro-intestinal ulceration and severe liver and kidney damage (Kaufman et al., 1970).

A 44-year-old man died of severe gastrointestinal hemorrhage one month after ingesting 4.1 mg Cr(VI)/kg b.w. as chromic acid (Saryan and Reedy, 1988).

I2. Haematological effects after accidental or intentional ingestion of Cr (VI)

A 18-year-old woman who ingested a few grams of potassium dichromate exhibited decreased hemoglobin content and hematocrit, and increased total white blood cell counts, reticulocyte counts, and plasma hemoglobin 4 days after ingestion. Intravascular hemolysis was suggested (Sharma et al., 1978).

A 25-year-old woman who drank a solution containing potassium dichromate had a clinically significant increase in leukocytes due to a rise in polymorphonuclear cells (Goldman and Karotkin, 1935).

A 44-year-old man had decreased hemoglobin levels 9 days after ingestion of 4.1 mg Cr(VI)/kg b.w. as chromic acid solution that probably resulted from gastrointestinal hemorrhage (Saryan and Reedy, 1988).

Blood coagulation was inhibited in a 17-year-old male who died after ingesting ~ 29 mg Cr(VI)/kg b.w. as potassium dichromate (Iserson et al., 1983; Clochesy, 1984).

I3. Gastrouintestinal effects after accidental or intentional ingestion of Cr (VI)

A 25-year-old woman who drank a solution containing potassium dichromate experienced abdominal pain and vomiting (Goldman and Karotkin, 1935).

Two people who ate oatmeal contaminated with potassium dichromate became suddenly ill with severe abdominal pain and vomiting, followed by diarrhea (Partington, 1950).

Nausea, hemetemesis, and bloody diarrhea were reported in a 24-year-old woman who ingested ammonium dichromate in a suicide attempt (Hasan, 2007).

I4. Hepatic effects after accidental or intentional ingestion of Cr(VI)

Increased alanine and aspartate aminotransferase, γ-glutamyl transferase, and bilirubin levels were observed 4 days after accidental ingestion of 20 % chromic acid (Barešić et al., 2009).

I5. Renal effects after accidental or intentional ingestion of Cr (VI)

Acute renal failure, characterized by proteinuria, and hematuria, and followed by anuria, developed in a chrome plating worker who had accidentally swallowed an unreported volume of a plating fluid containing 300 g Cr trioxide/L (Fristedt et al., 1965).

An adult consuming a nonlethal dose of 20 % chromic acid showed a rapid decrease in urine output progressing to anuria within 4 days of ingestion; an abdominal ultrasound revealed enlarged kidneys with edematous cortex and pronounced pyramids without other pathology (Barešić et al. 2009).

A 18-year-old woman who ingested a few grams of potassium dichromate reported proteinuria, oliguria, and destruction of the tubular epithelium of the kidneys. She regained renal function following dialysis (Sharma et al., 1978).

Proteinuria and oliguria were observed after ingestion of potassium dichromate by a 25-year-old woman (Goldman and Karotkin, 1935).

Acute renal failure that required hemodialysis was reported in a 24-year-old man who ingested an unknown quantity of a dietary supplement (Arsenal X®) containing Cr picolinate daily for 2 weeks (Wani et al., 2006). Serum creatinine was elevated approximately 3 times above the normal range, blood urea nitrogen was elevated slightly above normal range, urinalysis was positive for protein, and renal biopsy showed acute tubular necrosis. The patient developed severe impairment of renal function that required hemodialysis. Renal function improved within 4 weeks of discontinuation of treatment with the supplement.

I6. Renal effects after accidental or intentional ingestion of Cr(VI)

Administration of 0.04 mg Cr(VI)/kg as potassium dichromate in an oral tolerance test exacerbated dermatitis of a building worker who had a 20-year history of Cr contact dermatitis. A double dose led to dyshidrotic lesions (vesicular eruptions) on the hands (Goitre et al., 1982).

APPENDIX J: BMD ANALYSIS ON CRITICAL ENDPOINTS EVALUATED IN SECTION 7.5. DOSE-RESPONSE ASSESSMENT

This appendix reports details on the dose-response analysis using the BMD approach for the data and critical endpoints chosen for dose-response (DR) assessment of Cr(VI) in Section 7.5 in this opinion. This includes the toxicity data, including data on the carcinogenicity of male and female mice and rats exposed to SDD from the studies of the NTP (NTP 2007, 2008), see also Stout et al. (2009), Witt et al. (2013) and Section 7.2.2.5. Parts of these data were also analyzed by ATSDR (ADTSR, 2012) using the BMD approach. However, whereas the CONTAM Panel based the DR analysis on the guidance given by EFSA (2009c) the ATSDR used a different approach such that their reported numerical values of the BMD/Ls do not coincide necessarily with those reported in this opinion but were mostly of the same order of magnitude.

The BMD/L values were calculated by means of the software BMDSv2.4[19] and PROAST[20].

For dichotomous (quantal) data, all models available in BMDS and PROAST, respectively, were selected for the BMD analysis using the default benchmark response (BMR) of 10 % extra risk as advised by the EFSA guidance on the use of benchmark dose (EFSA, 2009c).

The nested exponential and the nested Hill family of models of PROAST was used for continuous data. All model fits were examined for acceptability at the good-of-fit p-value of 0.05 based on the (profile) maximum likelihood criterion. For a DR data set of quantal data the minimum BMDL obtained for all acceptable models was used identified as the BMDL as long as the 90 % confidence interval of the BMD (represented by the BMDL/BMDU interval) and the range of the BMDL values of acceptable models for that data set were both not substantially larger than one order of magnitude (EFSA, 2009c, 2011).

Models allowing for restrictions were run only when the fit of the respective unrestricted models would not allow identifying an acceptable model and/or when their application would be indicated after inspection of the dose-response data.

For continuous data the best fitting model of the two nested families (Exponential and Hill) were identified using PROAST and the minimum BMDL of the two families was chosen to characterized a DR data set. For the benchmark response (BMR) the default value for continuous data recommended by EFSA (2009c) of 5 % was used in the absence of statistical or toxicological considerations supporting a deviation from that default value, defined as a percent change of the magnitude of the response when compared to that predicted at background, i.e. a relative deviation from background. The BMD analysis was based on means and standard deviations or standard errors, respectively, available from studies.

The nested character of the family of models (Exponential or Hill models) makes it possible to formally choose one model for describing a particular data set. In general, when a model is extended by one or more parameters the resulting fit criterion may achieve a higher value compared to the model with fewer parameters. However, it is unfavorable to use a model with too many parameters, as this results in reduced precision of model predictions. Therefore, a formal criterion is needed to decide whether extension in the number of parameters should be accepted or not. A formal decision criterion is to testat the 5 % significance level. In the PROAST software used by the CONTAM Panel for the BMD analysis the appropriate model is automatically selected by consecutively fitting the members of the model family and chosing the model that cannot be significantly improved by a model having more parameters, as determined by the likelihood ratio test (Slob, 2002).

For interpreting the graphs and tables obtained by PROAST it should be noted that the data of each dose group are assumed to be log-normally distributed and the software reconstructs from the reported summary data of (arithmetic) means and standard deviations a lognormal distribution by calculating the corresponding geometric means and geometric standard deviations, fitting each nested model family to

[19] US EPA: http://www.epa.gov/ncea/bmds/
[20] RIVM: http://www.rivm.nl/en/Documents_and_publications/Scientific/Models/PROAST

these data and back-calculating them to the original scale. It should be also noted that the graphs of PROAST software present the 95 % confidence interval of the means using the lognormal distribution such that the whiskers in the graphic do not indicate the range of the data or the range between plus/minus the standard deviation or standard errors of the mean but a 95 % confidence interval.

For quantal data PROAST implemented the multistage models as nested model family and allows such the selection of a best fitting model in that family. Therefore, the best fitting model in this nested family and its BMD/L pair were assessed together with the BMD/L pairs of the other models.

When the observed dose response curves for males and females exhibited a high degree of similarity, when the dose ranges were by design identical or at least comparable and when the means was comparable in both sexes, a combined BMD modeling of the male and female dose-response data using sex as covariate was performed using the PROAST software. It should be also noted that PROAST software automatically tests for a statistically significant difference between the two sexes (based on the fitted models and model parameter values). When there is no statistical significant difference ($p > 0.05$) the data are pooled into one data set and the resulting BMD/L values of that analysis is reported. Therefore no separate curves for males and female are calculated in the graphic of the combined analysis. In that case, the outcome of PROAST could be cross-checked by applying BMDS software to the pooled data. The combined analysis should provide smaller BMDL/BMDU intervals and such the BMDL should be larger than when calculating separate BMDLs for each sex. This reflects the higher precision of the $BMDL_{05}$ when combining data and such increasing the power of statistical modeling. The sensitivity of the combined BMD analysis of the data of the two sexes of rats was investigated by fitting the male and the female data also separately. For the most relevant BMD analysis (epithelial adenoma or carcinoma in the small intestine in males and females combined) the sensitivity of the $BMDL_{10}$ value on the the number of animals planned and realized in the experiment was investigated, too (details not reported).

J1. Chromium (VI) neoplastic lesions

J1.1. BMD analysis of squamous cell neoplastic lesions in male and female rats

The CONTAM Panel identified one data set reported by NTP (NTP 2007, 2008), Stout et al. (2009), and Witt et al. (2013) on the carcinogenicity of SDD in male and female rats as suitable for DR assessment of Cr(VI). This Section informs on the details of the analysis at first for male and females separately using BMDS software and then on result of the analysis when combining males and females. We start with the evaluation of the carcinoma and papilloma data combined and report then also the evaluations of the carcinoma data only following the order of the results reported in Table 20 in Section 7.5.1.

When using the BMDS software, each table informs on the modelling outcome of the non-restricted models applied to the respective data set following the guidance of EFSA (2009c). The corresponding figure shows the fit of the model selected (corresponding to the minimum $BMDL_{10}$). In some cases also figures of other similarly good fitting models are shown for illustrative reasons. In green are the dose-response data with two sided 95 % confidence intervals of the incidences, in red is the fitted curve and in blue the one-sided 95 % confidence curve from which the $BMDL_{10}$ value of the respective model was derived.

Table J1: Squamous cell carcinoma or papilloma combined in oral mucosa or tongue in male rats. The benchmark dose (BMD_{10}), and the 95 % benchmark dose lower confidence limit ($BMDL_{10}$) values are given for a BMR of 10 % extra risk with characteristics of the model fit. The model with lowest $BMDL_{10}$ is given in bold.

Models	Restriction	N of parameters	Minus Log-likelihood	P-value	Accepted	BMD_{10} (mg/kg b.w. per day)	$BMDL_{10}$ (mg/kg b.w. per day)
Full model	na	5	25.00	–	–	–	–
Null (reduced) model	-	1	35.34	-	-	-	-
Probit	na	2	27.11	0.24	yes	5.31	4.33
LogProbit	none	3	26.39	0.25	yes	5.65	4.20
Logistic	na	2	26.85	0.27	yes	5.40	4.56
LogLogistic	none	3	26.39	0.25	yes	5.77	4.39
Quantal-Linear	**na**	**2**	**28.79**	**0.06**	**yes**	**5.87**	**3.30**
Multistage Cancer	na	2	27.35	0.19	yes	5.34	3.99
Multistage	yes	2	27.35	0.19	yes	5.34	3.99
Weibull	none	3	26.39	0.25	yes	5.78	4.44
Gamma	none	2	26.39	0.43	yes	5.61	4.34

b.w.: body weight; na: not applicable.

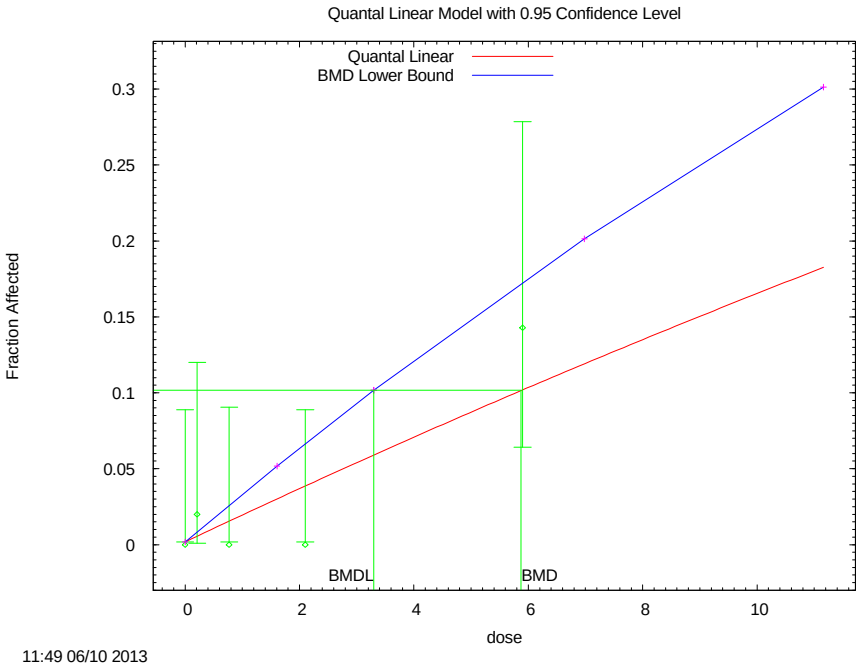


Figure J1: Fit of the quantal-linear model to the dose-response data on the incidence of squamous cell carcinoma or papilloma in oral mucosa or tongue in male rats.

Table J2: Squamous cell carcinoma or papilloma in oral mucosa or tongue in female rats. The benchmark dose (BMD_{10}) and the 95 % benchmark dose lower confidence limit ($BMDL_{10}$) values are given for a BMR of 10 % extra risk with characteristics of the model fit. The model with lowest $BMDL_{10}$ is highlighted in bold.

Models	Restriction	N of parameters	Minus Log-likelihood	P-value	Accepted	BMD_{10} (mg/kg b.w. per day)	$BMDL_{10}$ (mg/kg b.w. per day)
Full model	na	5	44.55	–	–	–	–
Null (reduced) model	-	1	56.74	-	-	-	-
Probit	na	2	45.72	0.50	yes	4.92	4.01
LogProbit	none	3	45.43	0.41	yes	4.58	3.20
Logistic	na	2	45.67	0.52	yes	5.17	4.31
LogLogistic	none	2	45.50	0.39	yes	4.87	3.34
Quantal-Linear	**na**	**2**	**47.22**	**0.15**	**yes**	**4.11**	**2.61**
Multistage Cancer	na	2	45.57	0.50	yes	4.73	3.52
Multistage	none	2	45.36	0.44	yes	4.96	3.65
Weibull	none	3	45.51	0.38	yes	4.95	3.40
Gamma	none	3	45.48	0.39	yes	4.82	3.38

b.w.: body weight; na: not applicable.

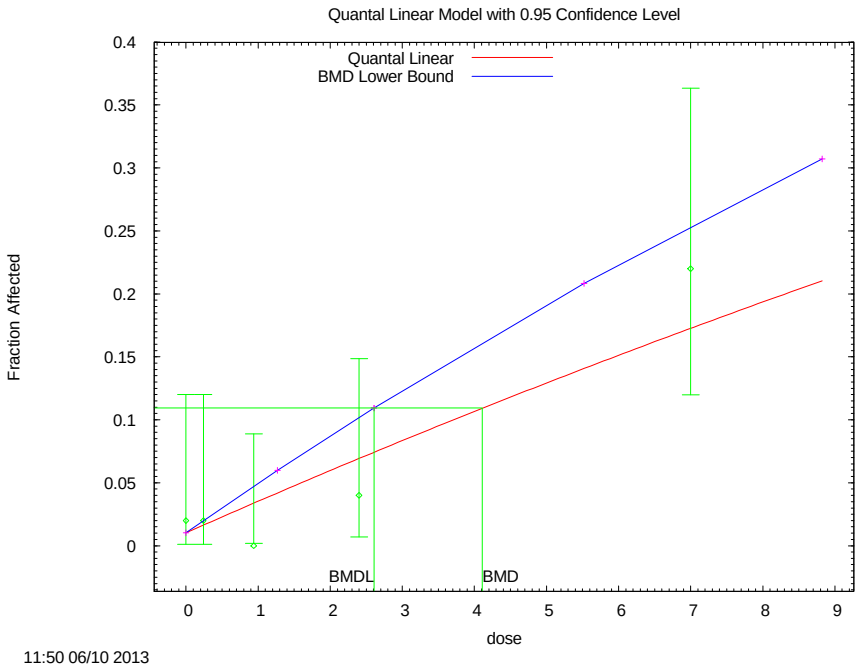
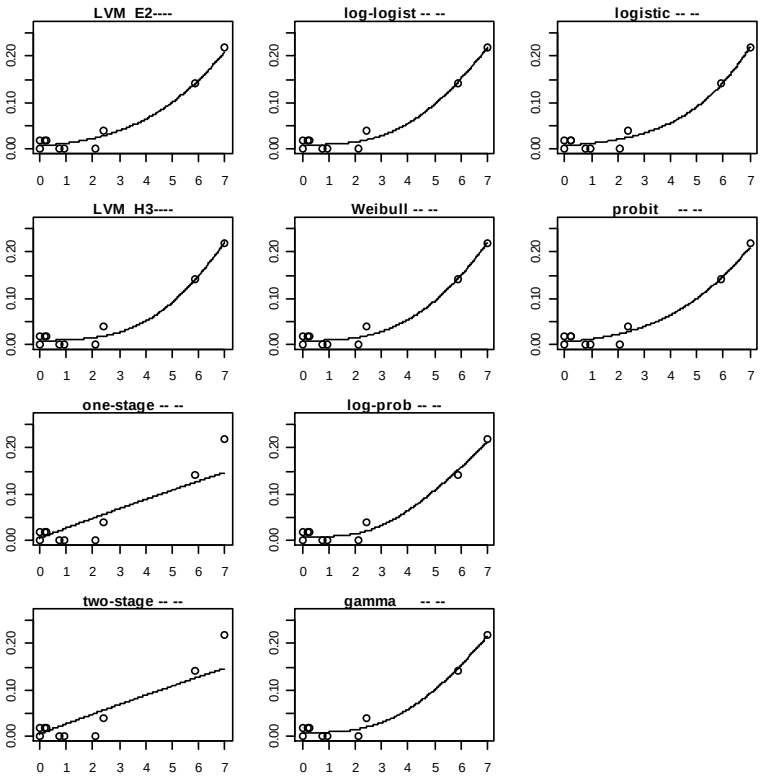


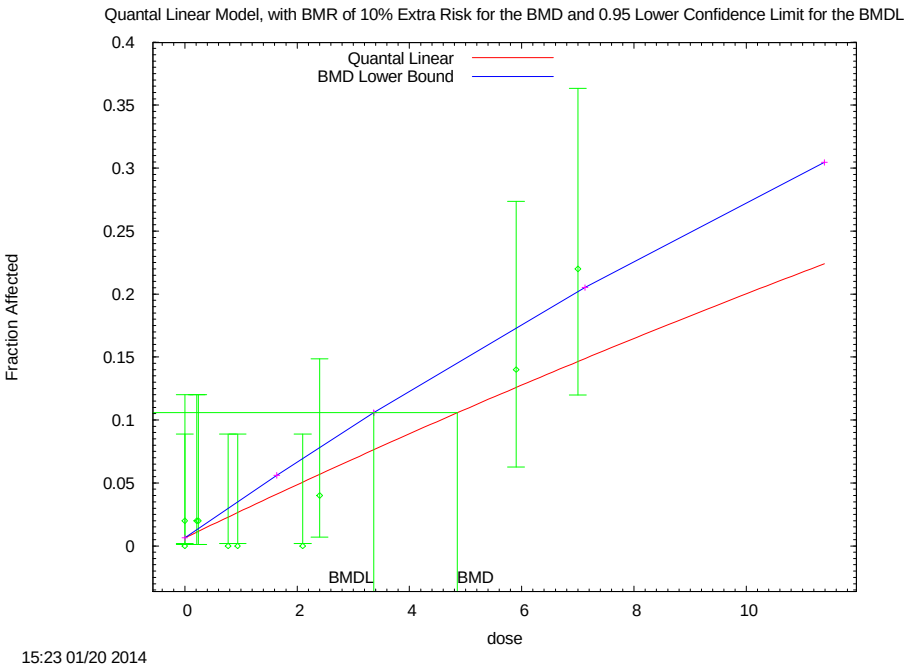
Figure J2: Fit of the quantal-linear model to the dose-response data on the incidence of squamous cell carcinoma or papilloma in oral mucosa or tongue in female rats.

Table J3: Squamous cell carcinoma or papilloma of the oral mucosa or tongue in male and female rats combined using PROAST.The benchmark dose (BMD_{10}) and the 95 % benchmark dose lower confidence limits ($BMDL_{10}$, $BMDU_{10}$ values are given for a BMR of 10 % extra risk with characteristics of the model fit. The selected model based on the model selection in PROAST is highlighted in bold.

Models	N of parameters	Log-likelihood	Accepted	BMD_{10} (mg/kg b.w. per day)	$BMDL_{10}$ (mg/kg b.w. per day)	$BMDU_{10}$ (mg/kg b.w. per day)
null	1	-93.28	–	–	–	–
full	10	-69.70	-	-	-	-
one-stage	**2**	**-77.01**	**yes**	**4.85**	**3.36**	**7.55**
two-stage	3	-77.01	yes	4.85	-	-
log-logist	3	-73.08	yes	5.25	4.22	6.14
Weibull	3	-73.08	yes	5.30	4.29	6.27
log-prob	3	-73.04	yes	5.02	4.01	6.22
gamma	3	-73.06	yes	5.18	4.20	6.24
logistic	2	-73.47	yes	5.35	4.71	6.18
probit	2	-73.66	yes	5.14	4.44	-



a) Result of PROAST for all models



b) Result of BMDS when pooling the data of males and female for the quantal linear model

Figure J3: Fit of all models used in PROAST to the dose-response data on the incidence of squamous cell carcinoma or papilloma in oral mucosa or tongue in rats. Note that the models denoted LVM E2 and H3 are not recommended by EFSA (2009c).

Table J4: Squamous cell carcinoma of the oral mucosa in male rats. The benchmark dose (BMD_{10}) and the 95 % benchmark dose lower confidence limit ($BMDL_{10}$) values are given for a BMR of 10 % extra risk with characteristics of the model fit. The model with lowest $BMDL_{10}$ is highlighted in bold. The p-value of 1 indicates that the model is saturated and its fit equals to a fit of the full model fulfilling therefore the acceptance criterion trivially.

Models	Restriction	N of parameters	Minus Log-likelihood	P-value	Accepted	BMD_{10} (mg/kg b.w. per day)	$BMDL_{10}$ (mg/kg b.w. per day)
Full model	na	5	18.22	–	–	–	–
Null (reduced) model	na	1	28.26	-	-	-	-
Probit	na	2	18.22	1	yes	5.80	4.91
LogProbit	none	2	18.22	1	yes	5.74	4.38
Logistic	na	2	18.22	1	yes	5.85	4.11
LogLogistic	none	2	18.22	1	yes	5.82	4.57
Quantal-Linear	**na**	**1**	**20.91**	**0.25**	**yes**	**7.45**	**4.07**
Multistage Cancer	na	1	19.09	0.79	yes	5.70	4.21
Multistage	none	1	19.09	0.78	yes	5.70	4.21
Weibull	none	2	18.22	1	yes	5.83	4.61
Gamma	none	1	18.22	1	yes	5.72	4.51

na: not applicable, b.w: body weight.

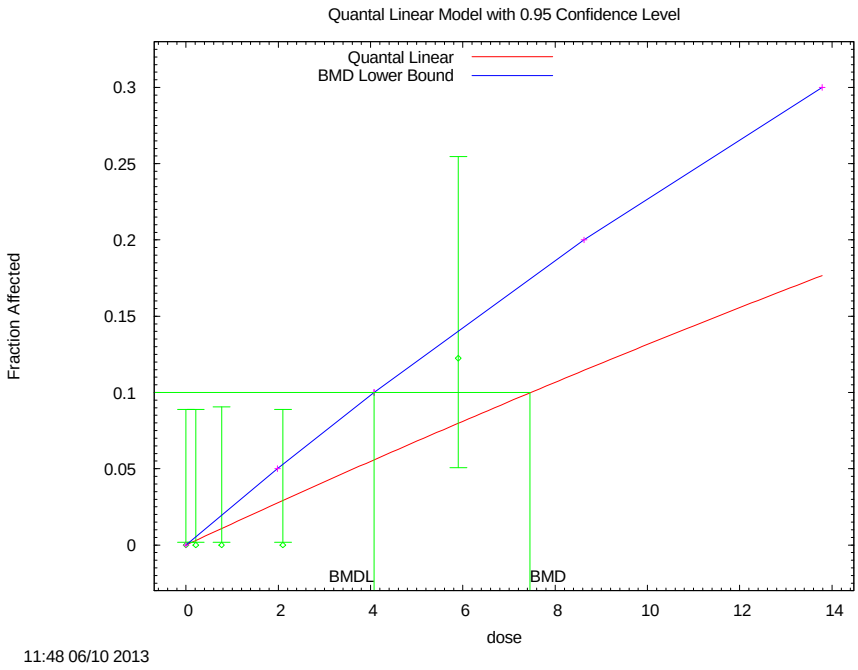


Figure J4: Fit of the quantal-linear model to the dose-response data of squamous cell carcinoma of the oral mucosa in male rats

Table J5: Squamous cell carcinoma of the oral mucosa in female rats. The benchmark dose (BMD_{10}) and the 95 % benchmark dose lower confidence limit ($BMDL_{10}$) values are given for a BMR of 10 % extra risk with characteristics of the model fit. The model with lowest $BMDL_{10}$ is highlighted in bold.

Models	Restriction	N of parameters	Minus Log-likelihood	P-value	Accepted	BMD_{10} (mg/kg b.w. per day)	$BMDL_{10}$ (mg/kg b.w. per day)
Full model	na	5	34.74	–	–	–	–
Null (reduced) model	na	1	51.09	-	-	-	-
Probit	na	2	35.83	0.54	yes	5.19	4.34
LogProbit	none	2	34.92	0.95	yes	4.16	3.00
Logistic	na	2	36.10	0.44	yes	5.48	4.66
LogLogistic	none	2	35.05	0.89	yes	4.41	3.17
Quantal-Linear	**na**	**1**	**36.96**	**0.35**	**yes**	**3.95**	**2.58**
Multistage Cancer	na	1	35.08	0.96	yes	4.50	3.46
Multistage	none	2	35.02	0.91	yes	4.55	3.49
Weibull	none	2	35.08	0.88	yes	4.49	3.24
Gamma	none	2	35.03	0.90	yes	4.36	3.21

na: not applicable, b.w.: body weight

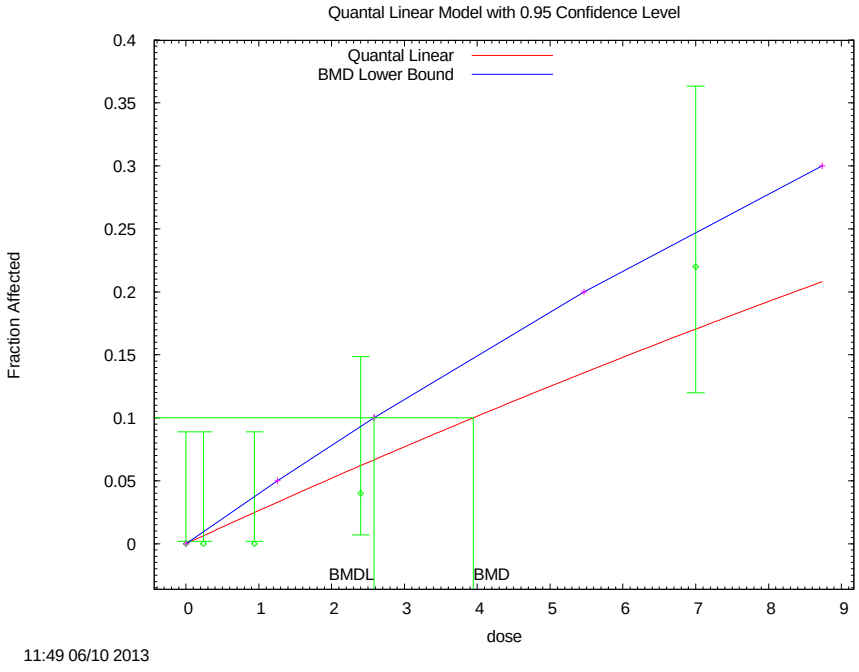
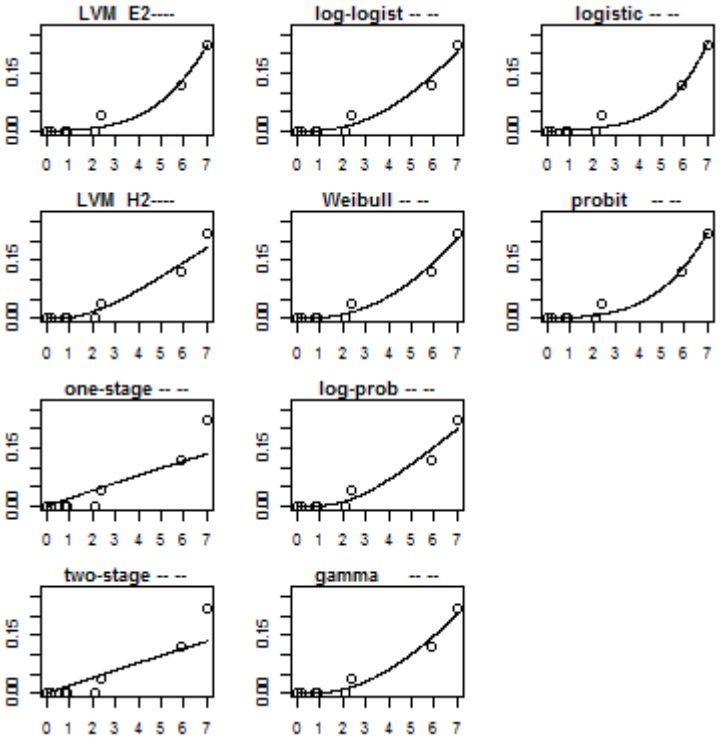


Figure J5: Fit of the quantal-linear model to the dose-response data on squamous cell carcinoma of the oral mucosa in female rats

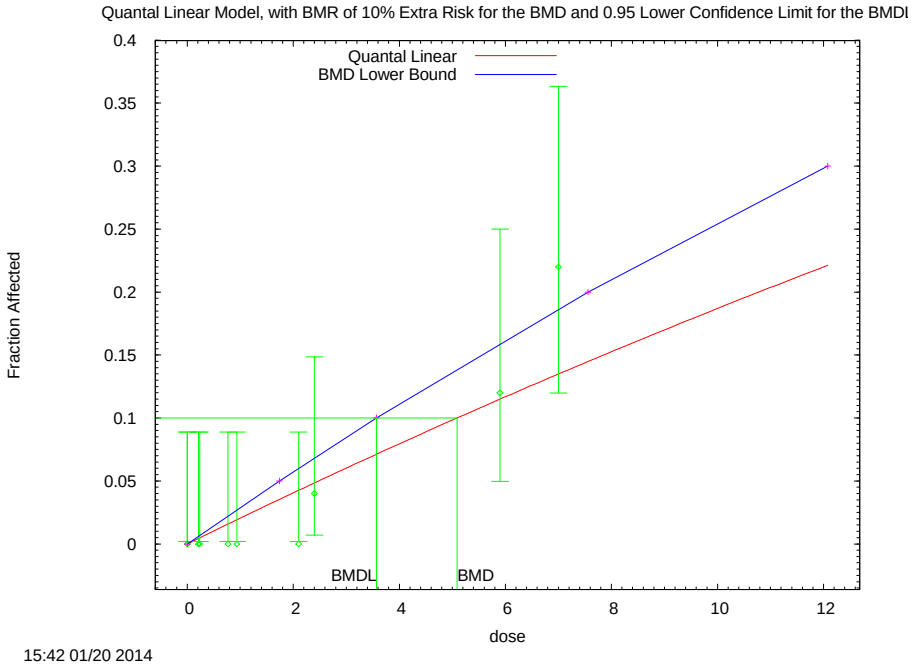
Table J6: Squamous cell carcinoma of the oral mucosa in male and female rats combined using PROAST The benchmark dose (BMD_{10}) and the 95 % benchmark dose lower confidence limit ($BMDL_{10}$) values are given for a BMR of 10 % extra risk with characteristics of the model fit. The selected model based on the model selection used in PROAST is highlighted in bold.

Models	N of parameters	Log-likelihood	Accepted	BMD_{10} (mg/kg b.w. per day)	$BMDL_{10}$ (mg/kg b.w. per day)	$BMDU_{10}$ (mg/kg b.w. per day)
null	1	-80.77	–	–	–	–
full	10	-53.09	-	-	-	-
one-stage	**2**	**-58.89**	**yes**	**5.09**	**3.57**	**7.62**
two-stage	3	-58.89	yes	5.09	3.57	7.62
log-logist	3	-54.56	yes	5.01	4.11	5.96
Weibull	3	-54.56	yes	5.07	4.18	5.98
log-prob	3	-54.49	yes	4.80	3.90	5.85
gamma	3	-54.54	yes	4.96	4.09	5.93
logistic	2	-55.50	yes	5.67	5.01	6.27
probit	2	-55.17	yes	5.45	4.83	-

b.w.: body weight.



a) Result of PROAST for all models



b) Result of BMDS when pooling the data of males and female for the quantal linear model

Figure J6: Fit of all models used in Proast tto the dose-response data on the incidence of squamous cell carcinoma in oral mucosa in rats. Note that the models denoted LVM E2 and H3 are not recommended by EFSA (2009c).

J.1.2. BMD analysis of eptithelial cell neoplastic lesion in the small intestine in male and female mice

The CONTAM Panel identified one data set reported by NTP (NTP 2007, 2008), Stout et al. (2009), and Witt et al. (2013) on the neoplastic effects of SDD in duodenum, jenunum and ileum combined in male and female mice as suitable for DR assessment of Cr(VI). This Section informs on the details of the analysis at first for male and females separately using BMDS software and then on the result of the analysis when combining males and females using PROAST software. We start with the evaluation of the carcinoma or adenoma data and report then also the evaluations of the carcinoma data only following the order of the results reported in Table 20 in Section 7.5.1, see also the comment in Section J1.1 above.

Table J7: Epithelial carcinoma or adenoma in the duodenum, jejunum or ileum in male mice. The benchmark dose (BMD_{10}) and the 95 % benchmark dose lower confidence limit ($BMDL_{10}$) values are given for a BMR of 10 % extra risk with characteristics of the model fit. The model with lowest $BMDL_{10}$ is highlighted in bold.

Models	Restriction	N of parameters	Minus Log-likelihood	P-value	Accepted	BMD_{10} (mg/kg b.w. per day)	$BMDL_{10}$ (mg/kg b.w. per day)
Full model	na	5	78.55	–	–	–	–
Null (reduced) model	na	1	97.54	-	-	-	-
Probit	na	2	79.14	0.76	yes	2.60	2.17
LogProbit	none	3	79.12	0.56	yes	2.36	1.19
Logistic	na	2	79.28	0.69	yes	2.82	2.36
LogLogistic	none	3	79.10	0.57	yes	2.30	1.14
Quantal-Linear	**na**	**2**	**79.74**	**0.50**	**yes**	**1.48**	**1.08**
Multistage Cancer	na	3	79.04	0.61	yes	2.22	1.18
Multistage	none	3	79.04	0.61	yes	2.22	1.15
Weibull	none	3	79.09	0.58	yes	2.26	1.11
Gamma	none	2	79.11	0.57	yes	2.29	1.10

b.w.: body weight; na: not applicable

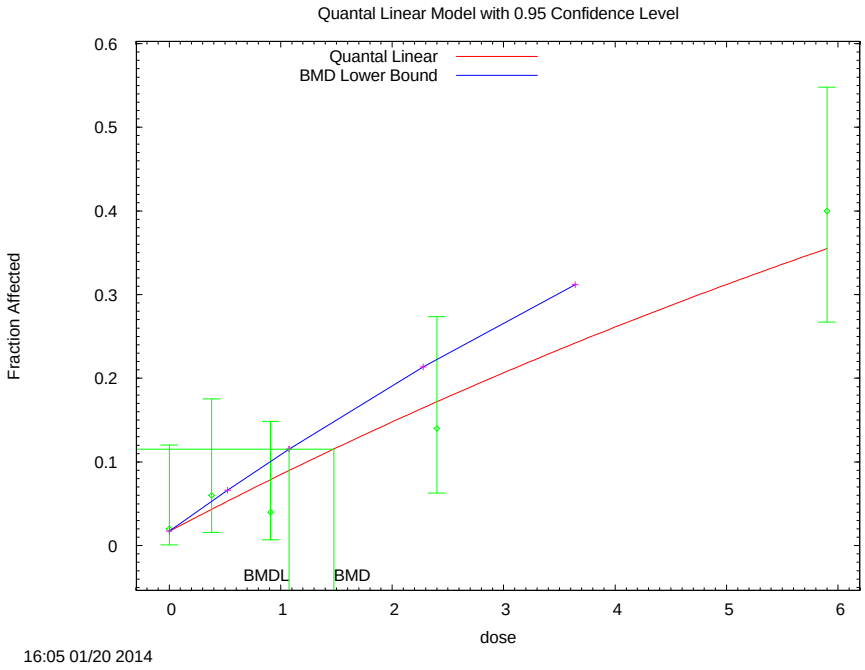


Figure J7: Fit of the Quantal Linear model to the dose-response data on epithelial carcinoma or adenoma combined in the duodenum, jejunum or ileum in male mice

Table J8: Epithelial carcinoma or adenoma combined in the duodenum, jejunum or ileum in female mice. The benchmark dose (BMD_{10}) and the 95 % benchmark dose lower confidence limit ($BMDL_{10}$) values are given for a BMR of 10 % extra risk with characteristics of the model fit. The model with lowest $BMDL_{10}$ is highlighted in bold.

Models	Restriction	N of parameters	Minus Log-likelihood	P-value	Accepted	BMD_{10} (mg/kg b.w. per day)	$BMDL_{10}$ (mg/kg b.w. per day)
Full model	na	5	85.19	–	–	–	–
Null (reduced) model	na	1	116.32	-	-	-	-
Probit	na	2	94.88	< 10-3	no	2.30	2.51
LogProbit	none	3	86.88	0.34	yes	1.19	0.70
Logistic	na	2	95.78	< 10-3	no	3.26	2.72
LogLogistic	none	3	87.22	0.25	yes	1.19	0.66
Quantal-Linear	na	1	87.75	0.27	yes	1.30	1.02
Multistage Cancer	na	1	87.75	0.27	yes	1.30	1.02
Multistage	none	3	87.14	0.27	yes	1.00	0.67
Weibull	**none**	**3**	**87.64**	**0.18**	**yes**	**1.15**	**0.61**
Gamma	none	3	87.69	0.17	yes	1.18	0.61

na: not applicable; b.w.: body weight.

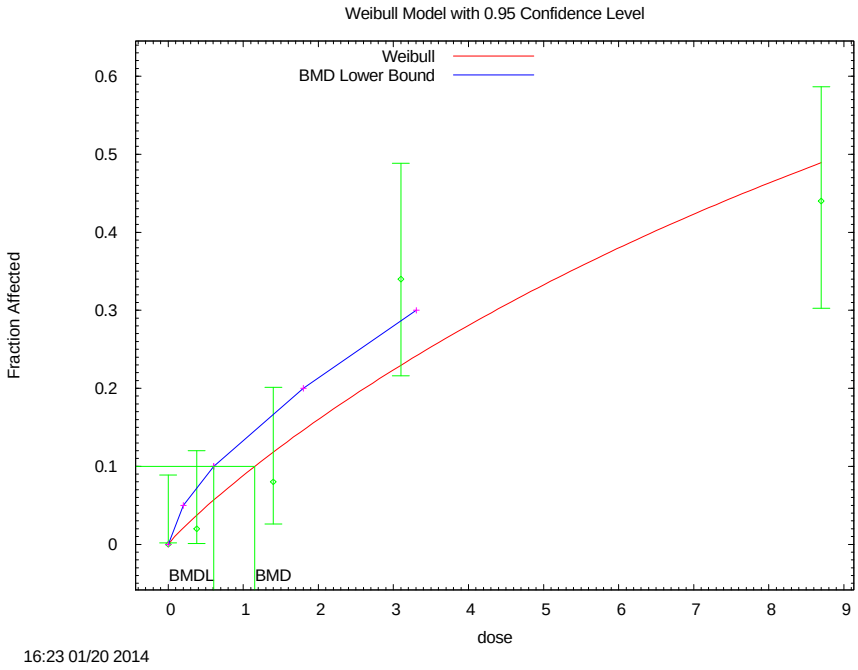
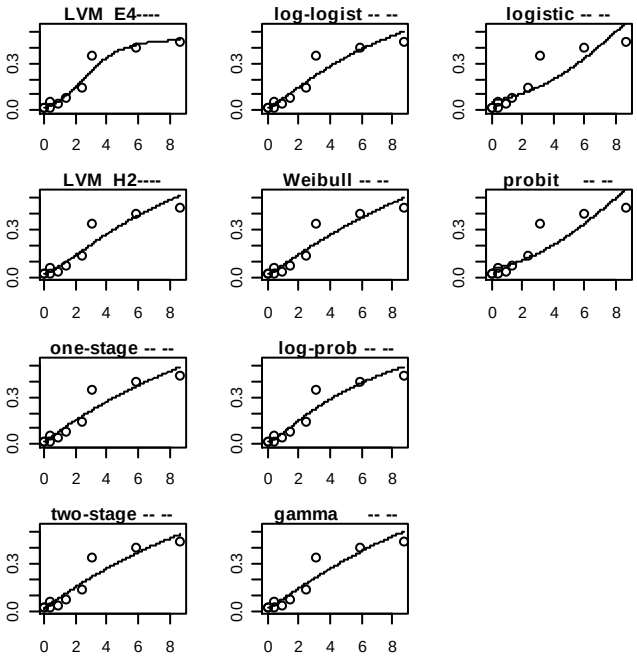


Figure J8: Fit of the Weibull model to the dose-response data on carcinoma or adenoma in the duodenum, jejunum or ileum female mice

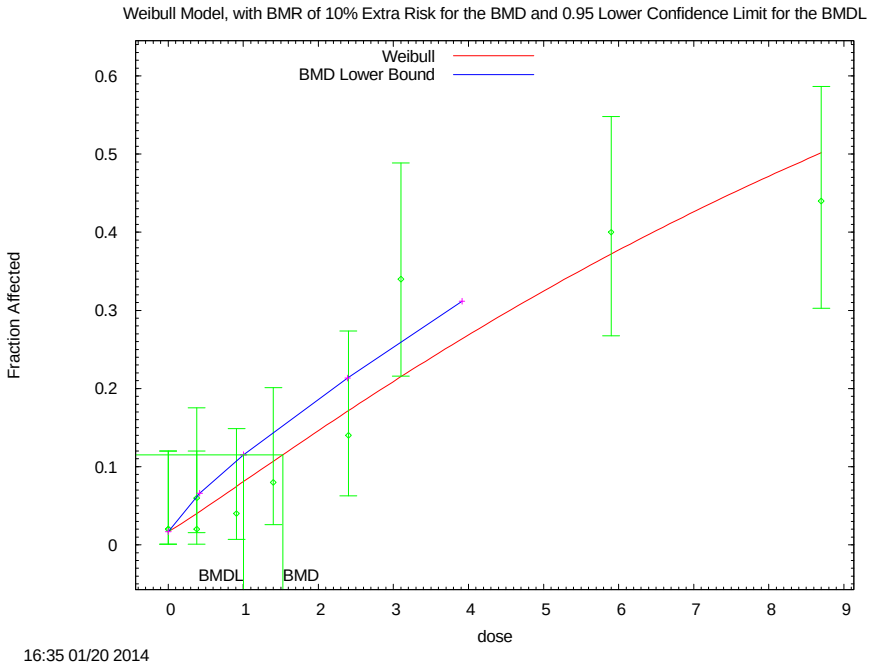
Table J9: Epithelial carcinoma or adenoma in the duodenum, jejunum or ileum in male and female mice combined. The benchmark dose (BMD_{10}) and the 95 % benchmark dose lower confidence limit ($BMDL_{10}$) values are given for a BMR of 10 % extra risk with characteristics of the model fit. The selected model based on the model selection used in PROAST is highlighted in bold.

Models	N of parameters	Log-likelihood	Accepted	BMD_{10} (mg/kg b.w. per day)	$BMDL_{10}$ (mg/kg b.w. per day)	$BMDU_{10}$ (mg/kg b.w. per day)
null	1	-216.49	–	–	–	–
full	10	-168.64	-	-	-	-
one-stage	2	-172.72	yes	1.41	1.15	1.77
two-stage	3	-172.72	yes	1.41	1.15	1.75
log-logist	3	-172.72	yes	1.56	1.04	2.22
Weibull	**3**	**-172.65**	**yes**	**1.53**	**1.00**	**2.21**
log-prob	3	-171.96	yes	1.60	1.06	2.27
Gamma	3	-172.6	yes	1.56	1.02	2.25
Logistic	2	-179.62	No	3.10	2.71	-
probit	2	-178.22	No	2.86	2.50	-

b.w.: body weight.



a) Result of PROAST for all models



b) Result of BMDS when pooling the data of males and female for Weibull model

Figure J9: Fit of all models to the dose-response data on the incidence of epithelial carcinoma or adenoma combined in the duodenum, jejunum or ileum in male and female mice combined. Note that the models denoted LVM E2 and H3 are not recommended by EFSA (2009c).

The BMD calculations for epithelial carcinoma or adenoma in the duodenum, jejunum or ileum in male and female mice were based on the number of animals initially in study (n = 50; see Witt at al., 2013). A sensitivity analysis showed no difference when accounting for early death and drop put as reported by NTP ($BMDL_{10}$ = 0.99) and a slight decrease when accounting for intercurrent mortality based on the method used by NTP for the poly –k test ($BMDL_{10}$ = 0.89). When considering the terminal incidences only as reported by NTP the $BMDL_{10}$ was obtained as 0.79 mg/kg b.w. per day.

Table J10: Epithelial carcinoma in the duodenum, jejunum or ileum carcinoma in male mice. The benchmark dose (BMD_{10}) and the 95 % benchmark dose lower confidence limit ($BMDL_{10}$) values are given for a BMR of 10 % extra risk with characteristics of the model fit. The model with lowest $BMDL_{10}$ is highlighted in bold.

Models	Restriction	N of parameters	Minus Log-likelihood	P-value	Accepted	BMD_{10} (mg/kg b.w. per day)	$BMDL_{10}$ (mg/kg b.w. per day)
Full model	na	5	40.90	–	–	–	–
Null (reduced) model	na	1	45.11	-	-	-	-
Probit	na	2	42.42	0.39	yes	6.26	4.35
LogProbit	**none**	**2**	**41.41**	**0.79**	**yes**	**7.54**	**2.53**
Logistic	na	2	42.47	0.37	yes	6.28	4.53
LogLogistic	none	2	41.38	0.81	yes	7.03	2.61
Quantal-Linear	na	2	42.09	0.50	yes	5.89	3.05
Multistage Cancer	na	2	42.09	0.50	yes	5.89	3.05
Multistage	yes	2	41.86	0.38	yes	5.89	3.05
Weibull	none	2	41.38	0.81	yes	6.94	2.63
Gamma	none	2	41.36	0.81	yes	6.87	2.65

na: not applicable; b.w.: body weight.

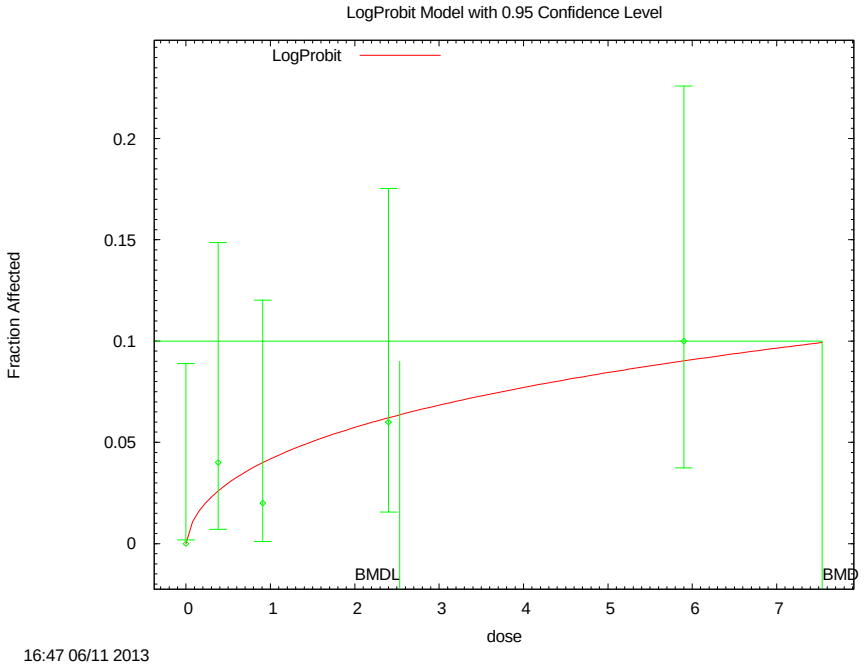


Figure J10: Fit of the logprobit model to the dose-response data on epithelial carcinoma in the duodenum, jejunum or ileum in male mice

Table J11: Epithelial carcinoma in the duodenum, jejunum or ileum carcinoma in female mice. The benchmark dose (BMD_{10}) and the 95 % benchmark dose lower confidence limit ($BMDL_{10}$) values are given for a BMR of 10 % extra risk with characteristics of the model fit. The model with lowest $BMDL_{10}$ is highlighted in bold.

Models	Restriction	N of parameters	Minus Log-likelihood	P-value	Accepted	BMD_{10} (mg/kg b.w. per day)	$BMDL_{10}$ (mg/kg b.w. per day)
Full model	na	5	44.90	–	–	–	–
Null (reduced) model	na	1	51.05	-	-	-	-
Probit	na	2	46.24	0.44	yes	7.46	5.63
LogProbit	none	3	45.82	0.40	yes	6.29	3.59
Logistic	na	3	46.31	0.42	yes	7.66	5.96
LogLogistic	none	2	45.90	0.36	yes	6.51	3.80
Quantal-Linear	na	2	45.94	0.55	yes	6.60	3.93
Multistage Cancer	na	3	45.94	0.35	yes	6.63	3.94
Multistage	**yes**	**3**	**45.94**	**0.36**	**yes**	**6.63**	**3.12**
Weibull	none	3	45.91	0.36	yes	6.57	3.87
Gamma	none	3	45.91	0.36	yes	6.56	3.89

na: not applicable

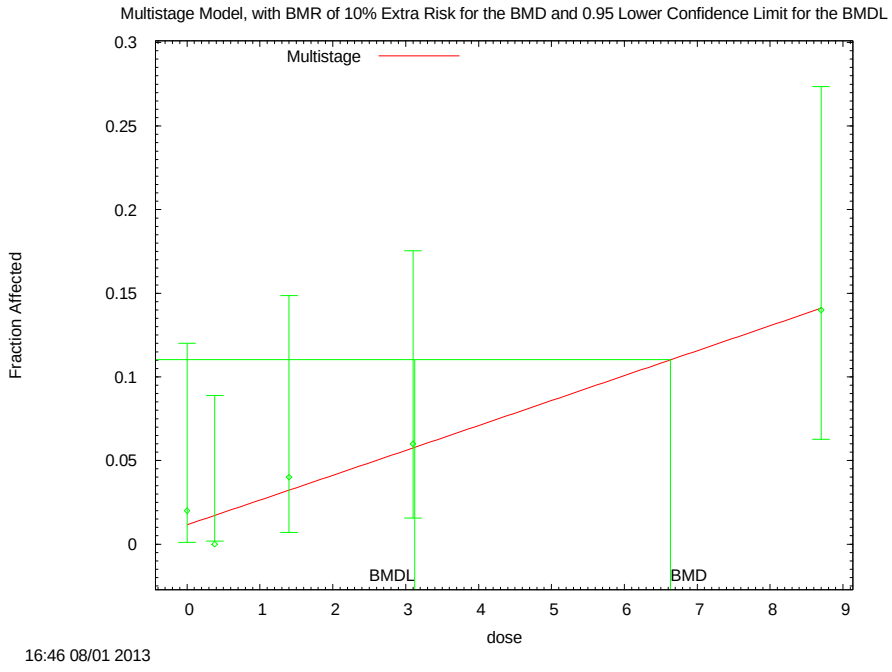
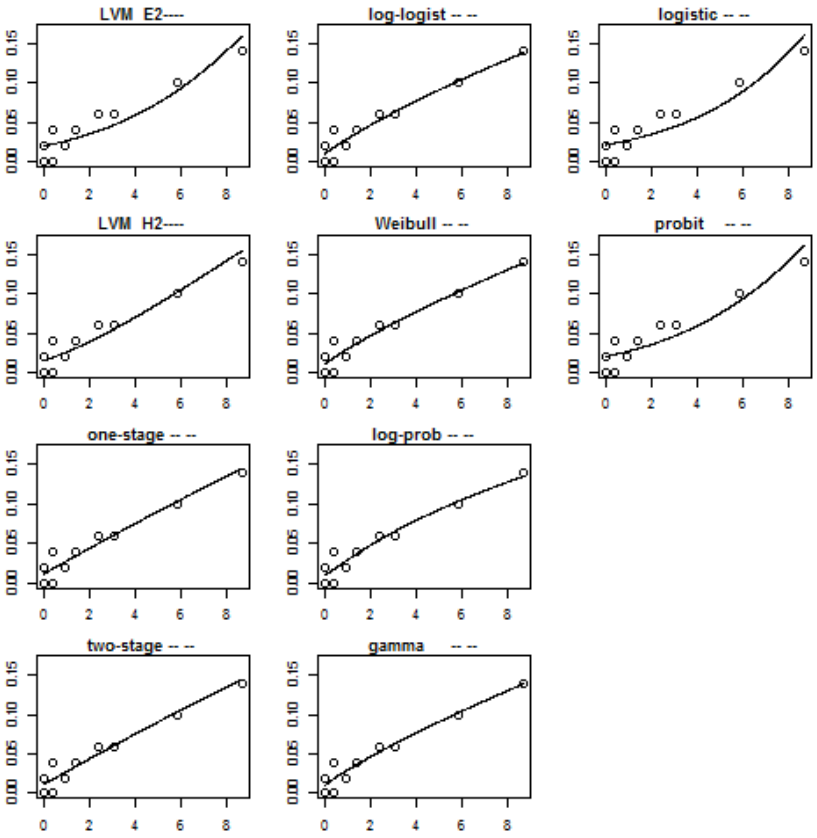


Figure J11: Fit of the Multistage model to the dose-response data on epithelial carcinoma in the duodenum, jejunum or ileum in female mice

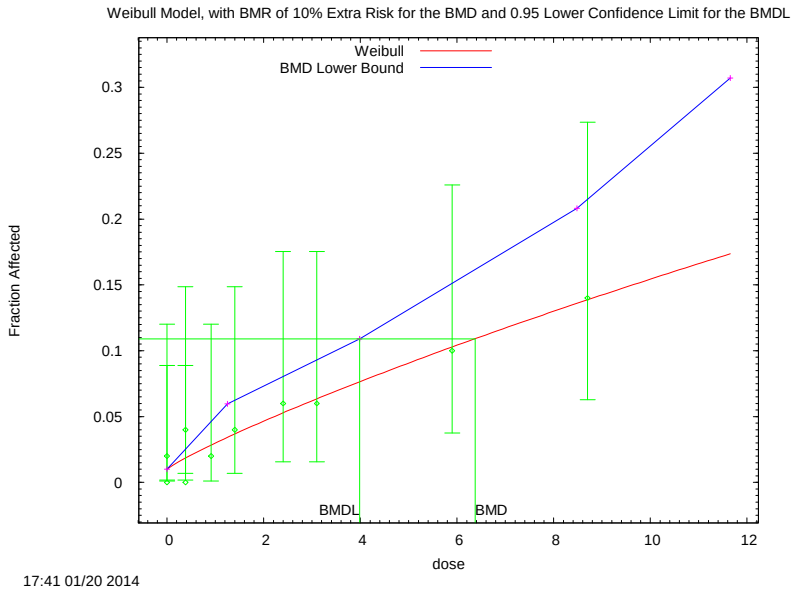
Table J12: Epithelial carcinoma in the duodenum, jejunum or ileum carcinoma in male and female mice combined. The benchmark dose (BMD_{10}) and the 95 % benchmark dose lower confidence limit ($BMDL_{10}$) values are given for a BMR of 10 % extra risk with characteristics of the model fit. The selected model based on the model selection used in PROAST is highlighted in bold. Numerical results of the fit of the probit model were inconsistent.

Models	N of parameters	Log-likelihood	Accepted	BMD_{10} (mg/kg b.w. per day)	$BMDL_{10}$ (mg/kg b.w. per day)	$BMDU_{10}$ (mg/kg b.w. per day)
null	1	-96.29	–	–	–	–
full	10	-85.80	-	-	-	-
one-stage	2	-88.05	yes	6.34	4.16	11.5
two-stage	3	-88.05	yes	6.34	4.16	11.5
log-logist	3	-88.02	yes	6.36	3.94	16.5
Weibull	3	-88.02	yes	6.37	3.98	16.2
log-prob	**3**	**-88.03**	**yes**	**6.38**	**3.81**	**18.5**
gamma	3	-88.02	yes	6.38	4.00	15.9
logistic	2	-88.02	yes	7.32	5.90	10.7
probit	-	-	-	-	-	-

b.w.: body weight.



a) Result of PROAST for all models



b) Result of BMDS when pooling the data of males and female for the Weibull model used to illustrate the data since the log-probit model failed to get fitted with BMDS software

Figure J12: Fit of all models to the dose-response data on the incidence of squamous cell carcinoma or papilloma in oral mucosa or tongue in rats. Note that the models denoted LVM E2 and H3 are not recommended by EFSA (2009c).

J2. BMD analysis of Chromium (VI): non-neoplastic lesions

This part of the appendix informs on the details when applying the BMD approach to the incidences of the following five types of non-neoplastic lesions:

- chronic inflammation of the liver in female rats;
- diffuse epithelial hyperplasia in the duodenum in male and female mice;
- hystiocytic cellular infiltration in mesenteric lymph nodes in male and female mice;
- hystiocytic cellular infiltration in liver in female mice;
- acinus,cytoplasmic alteration in pancreas.

reported in Table 22. The dose-response data of the respevive endpoint are shown on top of the five tables displayed below.

J.2.1. Chronic inflammation of the liver in female rats

For the dose-resposne analysis of the **incidence of chronic inflammation of the liver in female rats** five of the non-restricted models (log-probit, log-logistic, multistage, Weibull, and Gamma) showed an acceptable fit ($p > 0.05$). However the BMD_{10} values ranged from 0.2 to 0.001 mg/kg b.w. per day and the $BMDL_{10}$ values from 0.14 (multistage) to 0.00005 (Gamma) mg/kg b.w. per day, see Table J13. The two graphs in Figure J13 show the fit of the Multistage and the Weibull ($BMDL_{10}$ = 0.0005) models, respectively.

No $BMDL_{10}$was determined from the dose-response data for this endpoint and this data set since the BMD/BMDL ratios and the range of the BMDL values of the acceptable models are larger than one order of magnitude.

Using a modelling policy different from EFSA (2009) and allowing restrictions to the models the ADTSR reported a $BMDL_{10}$ = 0.14 mg/kg b.w. per day, which would correspond to the highest $BMDL_{10}$ value observed among non-restricted models.

Restricted models resulted, as expected, in higher $BMDL_{10}$ values, e.g. 0.37 mg/kg b.w. per day for the multistage and the Weibull model but were not used for dose-response assessment in this opinion.

Table J13: Chronic inflammation of the liver in female rats. The benchmark dose (BMD_{10}) and the 95 % benchmark dose lower confidence limit ($BMDL_{10}$) values are given for a BMR of 10 % extra risk with characteristics of the model fit. The model with lowest $BMDL_{10}$ is highlighted in bold.

Data	Dose	0	0.24	0.94	2.4	7.0
	Response	12/50	21/50	28/50	35/50	39/50

Models	Restriction	N of parameters	Minus Log-likelihood	P-value	Accepted	BMD_{10} (mg/kg b.w. per day)	$BMDL_{10}$ (mg/kg b.w. per day)
Full model	na	5	152.7	–	–	–	–
Null (reduced) model	na	1	172.5	-	-	-	-
Probit	na	2	158.9	0.006	no	0.88	0.70
LogProbit	none	3	1.52.9	0.91	yes	0.052	0.0031
Logistic	na	2	1.58.7	0.008	no	0.84	0.65
LogLogistic	none	3	1.52.8	0.92	yes	0.043	0.0021
Quantal-Linear (QL)	na	2	157.0	0.04	no	0.51	0.37
Multistage Cancer	na	1	157.0	0.04	no	0.52	0.37
Multistage	none	3	153.4	0.51	yes	0.20	0.14
Weibull	none	2	153.0	0.82	yes	0.021	0.0005
Gamma	none	2	153.1	0.72	yes	0.0096	0.00005

b.w.: body weight; na: not applicable; if: invalid fit.

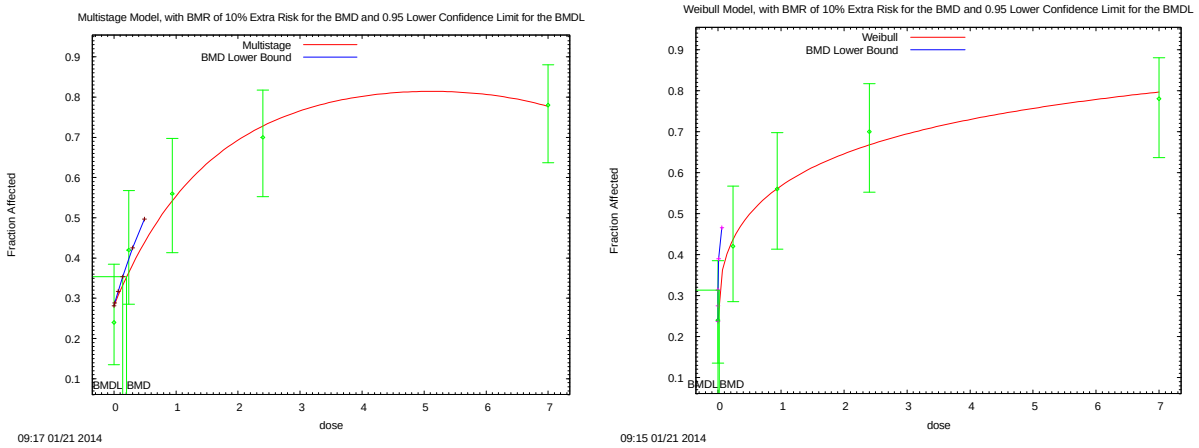


Figure J13: Fits of the Multistage model (left) and Weibull model (right) to the dose-response data on chronic liver inflammation in female rats.

J 2.2. Diffuse epithelial hyperplasia in the duodenum in male mice

For the dose-response analysis of the incidence of **diffuse epithelial hyperplasia in the duodenum in male mice** only one non-restricted model (multistage) showed an acceptable fit ($p > 0.05$) which resulted in a $BMDL_{10}$ = 0.11 mg/kg b.w. per day . Using a different modelling approach, not following EFSA (2009), ADTSR reported a $BMDL_{10}$ of 0.13 mg/kg b.w. per day. Two graphs in Figure J14 show the fit of the unrestricted Multistage model and Weibull model.

The restricted Weibull models resulted, as expected, in a higher $BMDL_{10}$ value of 0.25 mg/kg b.w. per day and was not used for dose-response assessment.

Table J14: Diffuse epithelial hyperplasia in the duodenum in male mice. The benchmark dose (BMD_{10}) and the 95 % benchmark dose lower confidence limit ($BMDL_{10}$) values are given for a BMR of 10 % extra risk with characteristics of the model fit. The model with lowest $BMDL_{10}$ is highlighted in bold.

Data	Dose	0	038	0.91	2.4	5.9
	Response	0/50	11/50	18/50	42/50	32/50

Models	Restriction	N of parameters	Minus Log-likelihood	P-value	Accepted	BMD_{10} (mg/kg b.w. per day)	$BMDL_{10}$ (mg/kg b.w. per day)
Full model	na	5	113.7	–	–	–	–
Null (reduced) model	na	1	169.4	-	-	-	-
Probit	na	2	146.1	< 10-13	no	0.90	0.76
LogProbit	none	2	122.6	0.0004	no	0.11	0.04
Logistic	na	2	1.58.7	<10-13	no	0.90	0.74
LogLogistic	none	3	122.5	0.0005	no	0.10	0.03
Quantal-Linear (QL)	na	2	133.5	< 10-7	no	0.31	0.25
Multistage Cancer	na	same as QL					
Multistage	**none**	**2**	**116.8**	**0.10**	**yes**	**0.14**	**0.11**
Weibull	none	2	123.7	0.0002	no	0.05	0.008
Gamma	none	no fit					

b.w.: body weight; na: not applicable.

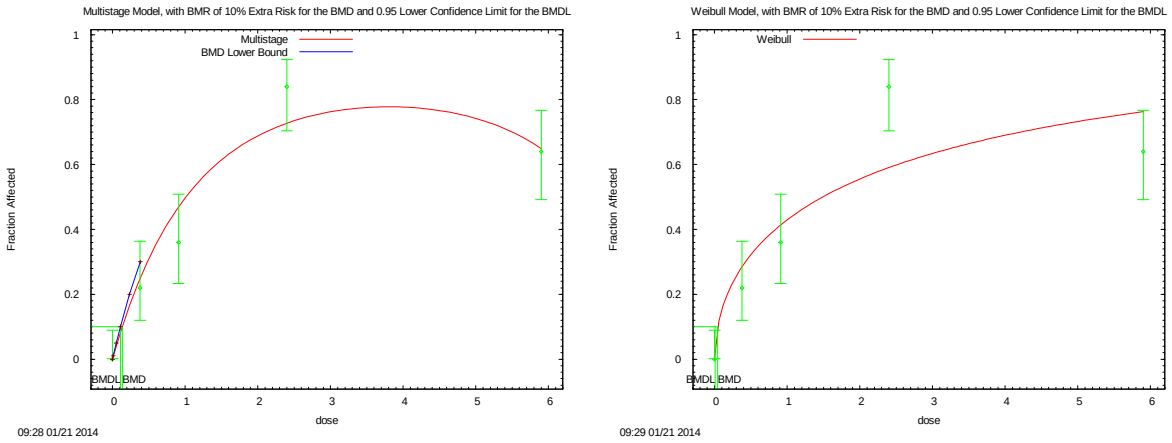


Figure J14: Fits of the Multistage model (left) and Weibull model (right) to the dose-response data on diffuse epithelial hyperplasia in the duodenum in male mice.

J 2.3. Diffuse epithelial hyperplasia in the duodenum in female mice

For the dose-response analysis of the incidence of **diffuse epithelial hyperplasia in the duodenum in female mice** three non-restricted models (log-probit, log-logistic and Weibull) showed an acceptable fit ($p > 0.05$) which resulted in $BMDL_{10}$ values of 0.0065, 0.0052 and 0.0008 mg/kg b.w. per day, respectively.

The graphs in Figure J15 show the fit of the logprobit, the multistage (with not acceptable fit) and the Weibull model (unrestricted with acceptable and restricted with unacceptable fit, $p < 10^{-7}$), respectively.

The restricted Weibull model resulted, as expected, in a higher $BMDL_{10}$ value of 0.27 mg/kg b.w. per day and was not used for dose-response assessment. The restricted log-logistic model showed an acceptable fit (p = 0.05) with a $BMDL_{10}$ = 0.09 mg/kg b.w. per day and was also not used.

No $BMDL_{-10}$ was determined from the dose-response data of this endpoint in this study since the BMD/BMDL ratios ranges between a factor of 6 and 13 and the range of the BMDL values of the acceptable models was larger than one order of magnitude.

Using a different modelling approach not following EFSA (2009) ADTSR reported a $BMDL_{10}$ = 0.09, which corresponds to the $BMDL_{10}$ value of the restricted log-logistic model, which was not used by the CONTAM Panel.

Table J15: Diffuse epithelial hyperplasia in the duodenum in female mice. The benchmark dose (BMD_{10}) and the 95 % benchmark dose lower confidence limit ($BMDL_{10}$) values are given for a BMR of 10 % extra risk with characteristics of the model fit.

Data	Dose	0	0.38	1.4	3.1	8.7
	Response	0/50	16/50	35/50	31/50	42/50

Models	**Restriction**	**N of parameters**	**Minus Log-likelihood**	**P-value**	**Accepted**	**BMD_{10} (mg/kg b.w. per day)**	**$BMDL_{10}$ (mg/kg b.w. per day)**
Full model	na	5	117.1	–	–	–	–
Null (reduced) model	na	1	173.3	-	-	-	-
Probit	na	2	145.0	<10-11	no	0.93	0.78
LogProbit	none	3	119.6	0.16	yes	0.042	0.0065
Logistic	na	2	1.44.6	<10-11	no	0.88	0.72
LogLogistic	none	3	119.6	0.16	yes	0.036	0.0052
Quantal-Linear (QL)	na	2	135.7	<10-7	no	0.34	0.27
Multistage Cancer	na	2	same as QL				
Multistage	none	3	124.1	0.003	no	0.16	0.13
Weibull	none	2	119.9	0.13	yes	0.011	0.0008
Gamma	none	no fit					

b.w.: body weight; na: not applicable.

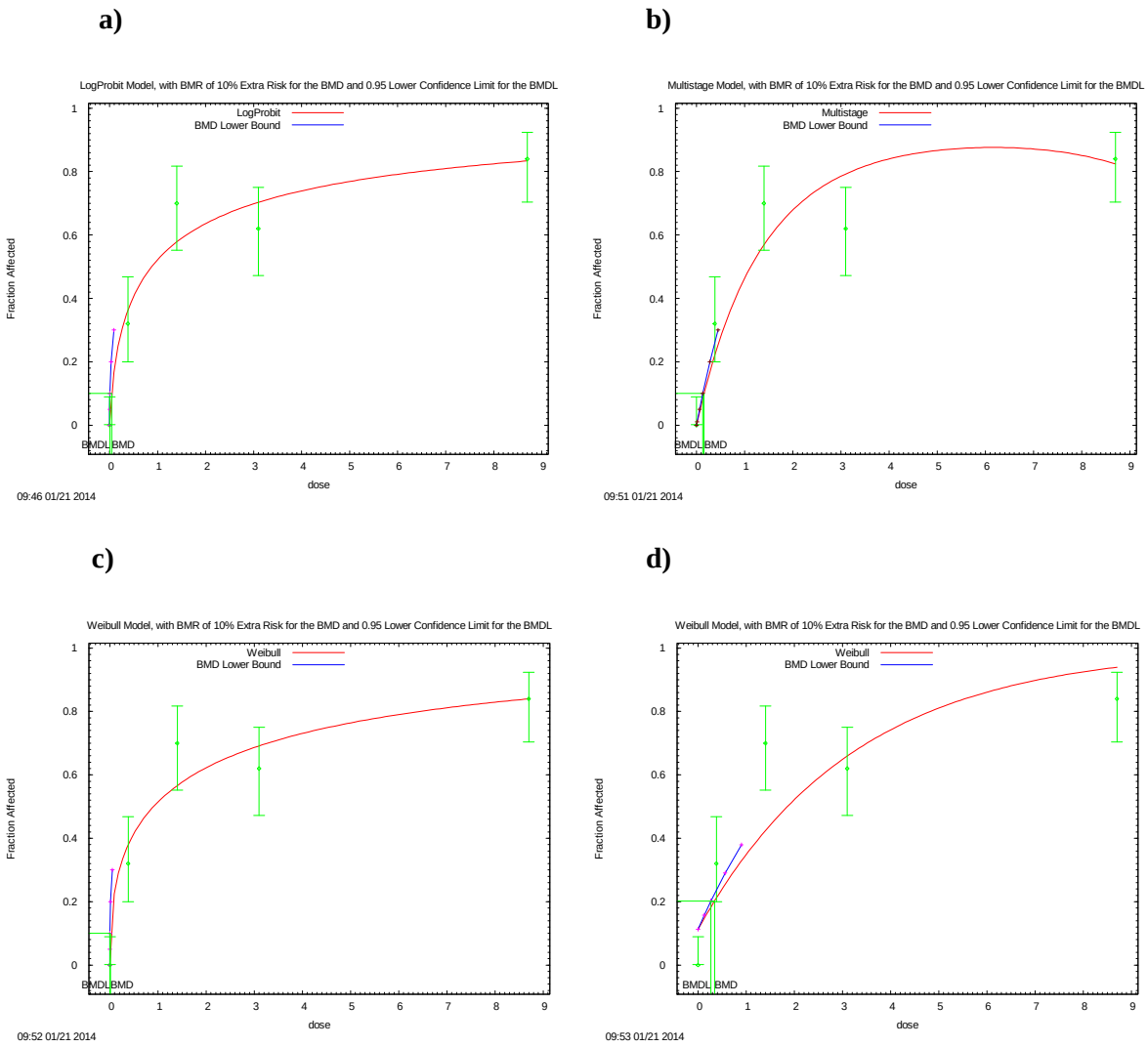


Figure J15: Fits of the Logprobit model (a), Multistage model (b), unrestricted Weibull model (c) and the restricted Weibull model (d) to the dose-response data on diffuse epithelial hyperplasia in the duodennum of female mice.

J 2.4. Hystiocytic cellular infiltration in mesenteric lymph nodes in male mice.

No acceptable model fitted for the dose-response analysis of the incidence of **hystiocytic cellular infiltration in mesenteric lymph nodes in male mice,** see Table J16 and the graph of the multistage model (Figure J16). Also the restricted loglogist, logprobit, multistage, Weibull showed no acceptable fit.

No $BMDL_{10}$ was determined from the dose-response data of this endpoint for male, since no model was acceptable including restricted models. Using a different modelling approach not following EFSA (2009) ADTSR reported also no BMDL.

Table J16: Histiocytic cellular infiltration in mesenteric lymph nodes in male mice. The benchmark dose (BMD_{10}) and the 95 % benchmark dose lower confidence limit ($BMDL_{10}$) values are given for a BMR of 10 % extra risk with characteristics of the model fit.

Data	Dose	0	0.38	0.91	2.4	5.9
	Response	14/47	38/49	31/49	32/49	42/46

Models	**Restriction**	**N of parameters**	**Minus Log-likelihood**	**P-value**	**Accepted**	**BMD_{10} (mg/kg b.w. per day)**	**$BMDL_{10}$ (mg/kg b.w. per day)**
Full model	na	5	132.2	–	–	–	–
Null (reduced) model	na	1	154.8	-	-	-	-
Probit	na	2	142.8.2	< 10-4	no	0.56	0.43
LogProbit	none	3	-	-	if		
Logistic	na	2	142.8	< 10-4	no	0.53	0.39
LogLogistic	none	3	-	-	if		
Quantal-Linear (QL)	na	2	142.5.0	< 10-3	no	0.38	0.26
Multistage Cancer	na	same as QL					
Multistage	none	3	142.5.0	< 10-4	no	0.32	0.16
Weibull	none	2	-	-	if		
Gamma	none	2	-	-	if		

b.w.: body weight; na: not applicable, if: invalid fit;

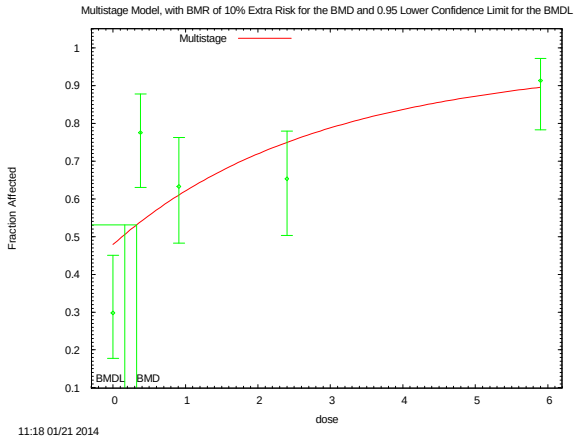


Figure J16: Fits of the Multistage model to the dose-response data on hystiocytic cellular infiltration in the mesenteric lymph node in male mice.

J 2.5. Hystiocytic cellular infiltration in mesenteric lymph nodes in female mice.

For the dose-response analysis of the incidence of **hystiocytic cellular infiltration in mesenteric lymph nodes in female mice** three non-restricted models (log-probit, log-logistic and Weibull) showed an acceptable fit ($p > 0.05$) which resulted in $BMDL_{10}$ values less than 10^{-5} mg/kg b.w. per day. The fit of the restricted models showed, as expected, higher $BMDL_{10}$ values (0.064 and 0.30 mg/kg b.w. per day) than the corresponding unrestricted models but their fit was not acceptable.

The graphs in Figure J17 show the fit of the log-logistic and the Weibull model, both for unrestricted and restricted modeling.

No $BMDL_{10}$ was determined from the dose-response data of this endpoint in female, since the BMD/BMDL ratios and the range of the BMDL values of the acceptable models are larger than one order of magnitude.

Using a different modelling approach not following EFSA (2009) ADTSR (2012) reported also no BMDL.

Table J17: Histiocytic cellular infiltration in mesenteric lymph nodes in female mice. The benchmark dose (BMD_{10}) and the 95 % benchmark dose lower confidence limit ($BMDL_{10}$) values are given for a BMR of 10 % extra risk with characteristics of the model fit.

Data	Dose	0	0.38	1.4	3.1	8.7
	Response	3/46	29/48	26/46	40/50	42/50

Models	**Restriction**	**N of parameters**	**Minus Log-likelihood**	**P-value**	**Accepted**	**BMD_{10} (mg/kg b.w. per day)**	**$BMDL_{10}$ (mg/kg b.w. per day)**
Full model	na	5	121.8	–	–	–	–
Null (reduced) model	na	1	163.0	-	-	-	-
Probit	na	2	143.7	$< 10^{10}$	no	0.85	0.69
LogProbit	none	3	123.8	014	yes	0.003	0.2 10-5
Logistic	na	2	143.1	$< 10^{-10}$	no	0.77	0.61
LogLogistic	none	3	123.8	0.13	yes	0.002	910^{-7}
Quantal-Linear (QL)	na	2	139.2	< 10-6	no	0.41	0.30
Multistage Cancer	na	1	Same	as	QL		
Multistage	none	3	130.9	0.001	no	0.14	0.11
Weibull	none	2	123.6	0.16	yes	0.0003	1.410^{-8}
Gamma	none	3	-	-	if		

b.w.: body weight; na: not applicable; if: invalid fit.

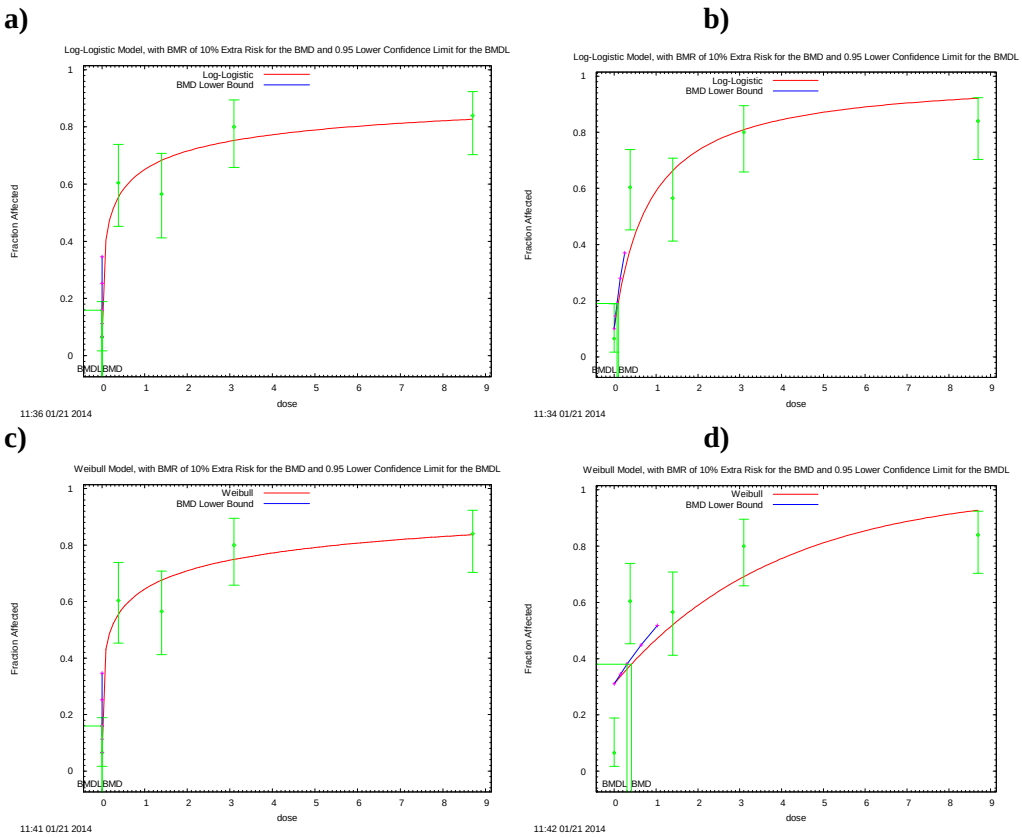


Figure J17: Fits of the unrestricted log-logistic model (a), restricted log-logistic model (b), unrestricted Weibull model (c) and restricted Weibull model (d) to the dose-response data on hystiocytic cellular infiltration in the mesenteric lymph node in female mice.

J 2.6. Hystiocytic cellular infiltration in liver in female mice.

For the dose-response analysis of the incidence of hystiocytic cellular infiltration in liver in female mice all non-restricted models, except the probit and logistic model showed an acceptable fit ($p > 0.05$) which resulted in $BMDL_{10}$ values ranging between 0.058 and 0.011 mg/kg b.w. per day.

The graphs in Figure J18 show the fit of the Gamma model, both for unrestricted and restricted modeling. The fit of the restricted model showed as expected higher $BMDL_{10}$ values, for the Gamma and the Weibull model the $BMDL_{10}$ values were 0.29 and 0.35 mg/kg b.w. per day, respectivele with acceptable fits ($p = 0.07$ and 0.08, respectively.

A $BMDL_{10} = 0.011$ mg/kg b.w. per day was used to characterize these data.

Using a different modelling approach not following EFSA (2009) ATSDR reported a $BMDL_{10}$ of 0.12 mg/kg b.w. per day (ATSDR, 2012).

Table J18: Incidence of liver histiocytic cellular infiltration in B6C3D1 female mice exposed to sodium dichromate dihydrate in drinking water for 2 years.

Data:					
Dose	0	0.38	1.4	3.1	8.7
Response	2/49	15/50	23/50	32/50	45/50

Models	Restriction	N of parameters	Minus Log-likelihood	P-value	Accepted	BMD_{10} (mg/kg b.w. per day)	$BMDL_{10}$ (mg/kg b.w. per day)
Full model	na	5	122.3	–	–	–	–
Null (reduced) model	na	1	172.1	-	-	-	-
Probit	na	2	132.3	0.0002	no	0.88	0.75
LogProbit	none	3	123.5	0.30	yes	0.16	0.058
Logistic	na	2	131.8	0.0003	no	0.85	0.70
LogLogistic	none	3	123.6	0.27	yes	0.15	0.050
Quantal-Linear (QL)	na	2	125.7	0.08	yes	0.35	0.28
Multistage Cancer	na	same	as	QL			
Multistage	none	3	124.3	0.14	yes	0.25	0.19
Weibull	none	3	122.7	0.70	yes	0.095	0.026
Gamma	**none**			**0.81**	**yes**	**0.067**	**0.011**

b.w.: body weight; na: not applicable; QL: Quantal linear.

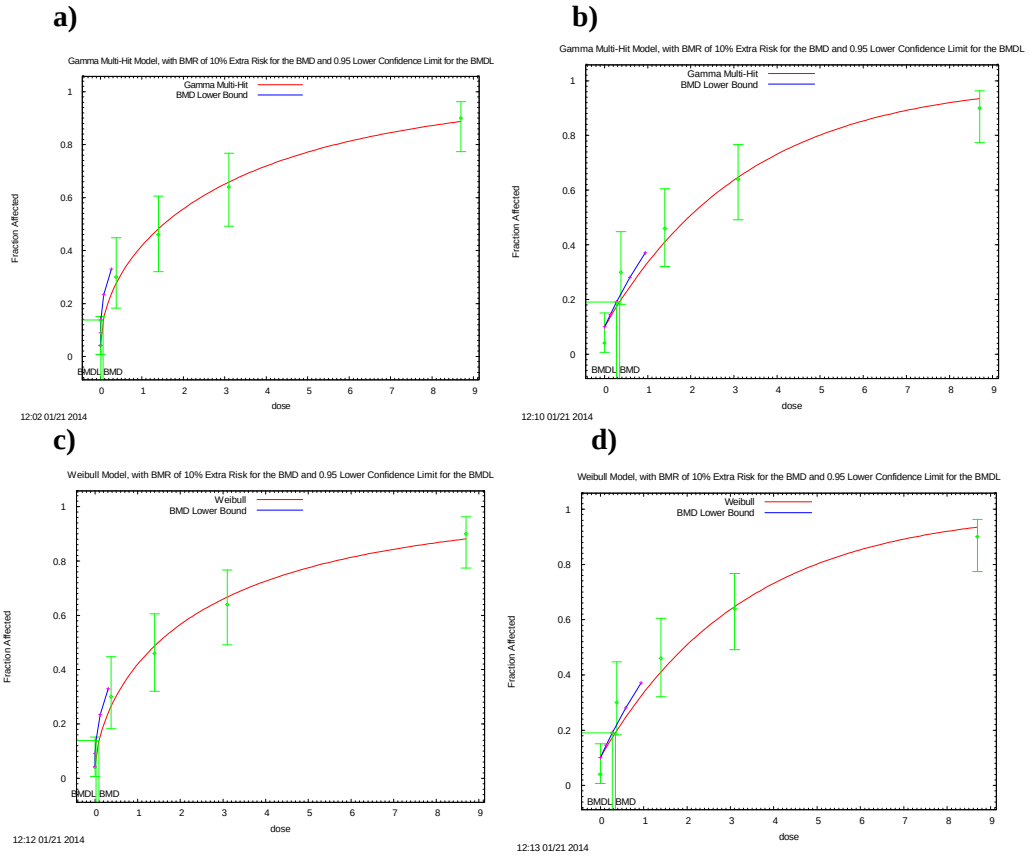


Figure J18: Fits of the unrestricted gamma model (a), restricted gamma model (b), unrestricted Weibull model (c) and restricted Weibull model (d) to the dose-response data on hystiocytic cellular infiltration in the liver in female mice.

J 2.7. Acinus, cytoplasmic alteration in pancreas female mice

For the dose-response analysis of the incidence of **acinus,cytoplasmic alteration in pancreas female mice** all non-restricted models, except the probit and logistic model showed an acceptable fit ($p > 0.05$) which resulted in $BMDL_{10}$ values ranging between 0.72 and 0.26 mg/kg b.w. per day.

The graphs in Figure J19 show the fit of the Gamma and the Weibull model. A $BMDL_{10}$ = 0.26 mg/kg b.w. per day was used to characterize these data.

Using a different modelling approach not following EFSA (2009) ADTSR reported a $BMDL_{10}$ of 0.52 mg/kg b.w. per day.

Table J19: Incidence of pancreas acinus cytoplasmic alteration in B6C3D1 female mice exposed to SDD in drinking water for 2 years. The benchmark dose (BMD_{10}), the 95 % benchmark dose lower confidence limit (BMDL-10) values for a BMR of 10 % extra risk with characteristics of the model fit.

Data	Dose	0	0.38	1.4	3.1	8.7
	Response	0/48	6/50	6/49	14/50	32/50

Models	**Restriction**	**N of parameters**	**Minus Log-likelihood**	**P-value**	**Accepted**	**BMD_{10} (mg/kg b.w. per day)**	**$BMDL_{10}$ (mg/kg b.w. per day)**
Full model	na	5	99.0	–	–	–	–
Null (reduced) model	na	1	134.9	-	-	-	-
Probit	na	2	103.6	0.03	no	2.24	1.89
LogProbit	none	3	102.2	0.08	yes	0.60	0.30
Logistic	na	2	103.9	0.02	no	2.44	2.03
LogLogistic	none	3	101.6	0.14	yes	0.64	0.31
Quantal-Linear /QL)	na	2	101.4	0.17	yes	0.92	0.72
Multistage Cancer	na	same	as	QL			
Multistage	none	3	101.4	0.08	yes	0.89	0.57
Weibull	none	2	100.8	0.25	yes	0.64	0.30
Gamma	**none**	**2**	**100.7**	**0.30**	**yes**	**0.61**	**0.26**

b.w.: body weight; na: not applicable; if: invalid fit.

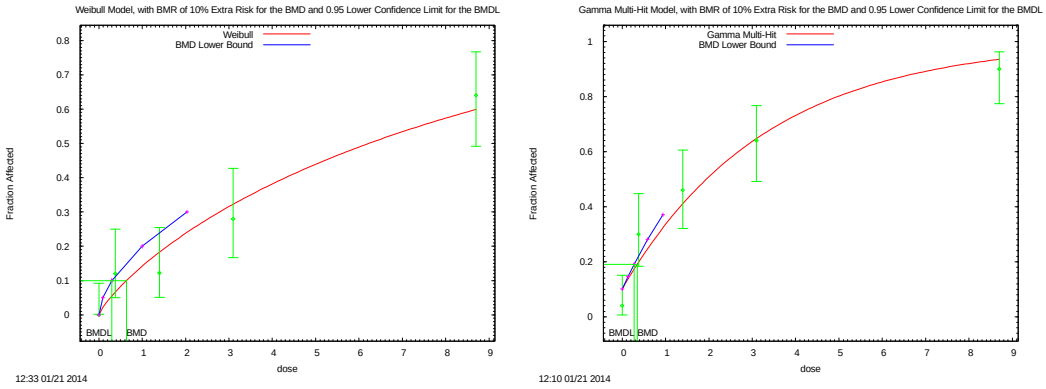


Figure J19: Fits of the Weibull model (left) and gamma model (right) to the dose-response data on acinus, cytoplasmic alteration in pancreas in female mice.

J.3. Chromium (VI): haematological effects in male F/344 rats exposed to sodium dichromate dihydrateSDD in drinking water for 22 days

Table J20: Haematocrit

The benchmark dose (BMD_{05}) and the 95 % benchmark dose lower confidence limit ($BMDL_{05}$) values are given for a BMR of 5 % decrease of weights (mg/10 g b.w.) relative to control with characteristics of the model fit. The model with lowest $BMDL_{05}$ is highlighted in bold

Models	Converged	N of parameters	Log-likelihood	BMD_{05} (mg/kg b.w. per day)	$BMDL_{05}$ (mg/kg b.w. per day)
EXPONENTIAL MODELS					
full	1	6	74.49		
m1	1	2	31.89		
m2	1	3	71.02		
m3	1	4	73.65		
m4	1	4	74.08		
m5	**1**	**5**	**74.11**	**0.64**	**0.21**
HILL MODELS					
full	na	6	74.49		
m1	1	2	31.89		
m2	**1**	**3**	**72.17**	**0.85**	**0.74**
m3	1	4	73.77		
m4	1	4	74.01		
m5	1	5	74.06		

b.w.: body weight; na: not applicable.

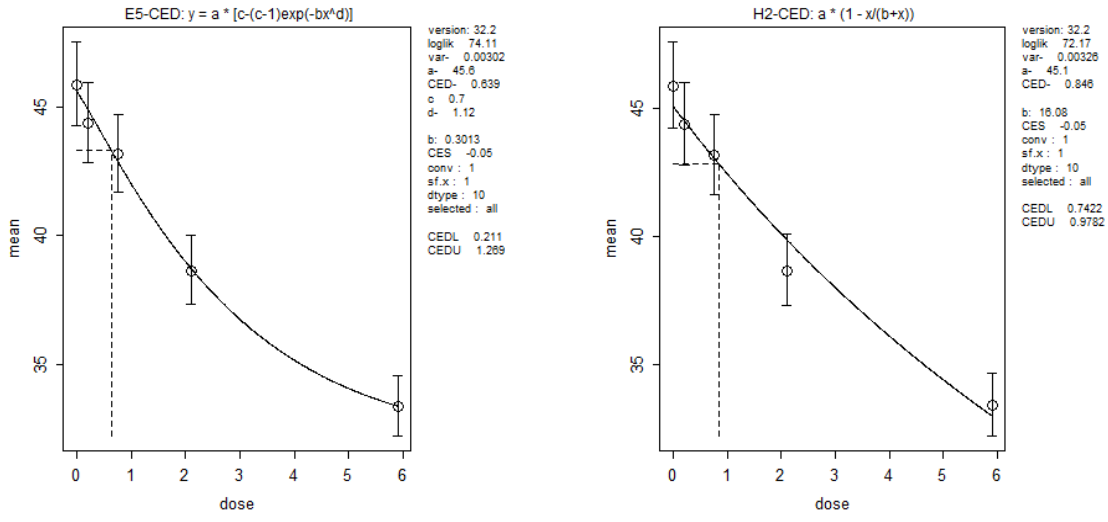


Figure J20: Fit of Model fit for the Exponential model (left) and the Hill (right) model

Table J21: Haemoglobin

The benchmark dose (BMD_{05}) and the 95 % benchmark dose lower confidence limit ($BMDL_{05}$) values are given for a BMR of 5 % decrease of weights (mg/10 g b.w.) relative to control with characteristics of the model fit. The model with lowest $BMDL_{05}$ is highlighted in bold

Models	Converged	N of parameters	Log-likelihood	BMD_{05} (mg/kg b.w. per day)	$BMDL_{05}$ (mg/kg b.w. per day)
ANALYSIS WITH EXPONENTIAL MODELS					
full	1	6	72.89		
m1	1	2	17.31		
m2	1	3	62.92		
m3	1	4	69.37		
m4	**1**	**4**	**72.18**	**0.34**	**0.27**
m5	1	5	72.82		
HILL MODELS					
full	na	6	72.89		
m1	1	2	17.31		
m2	1	3	66.17		
m3	1	4	70.07		
m4	**1**	**4**	**71.7**	**0.31**	**0.23**
m5	1	5	72.75		

b.w.: body weight.

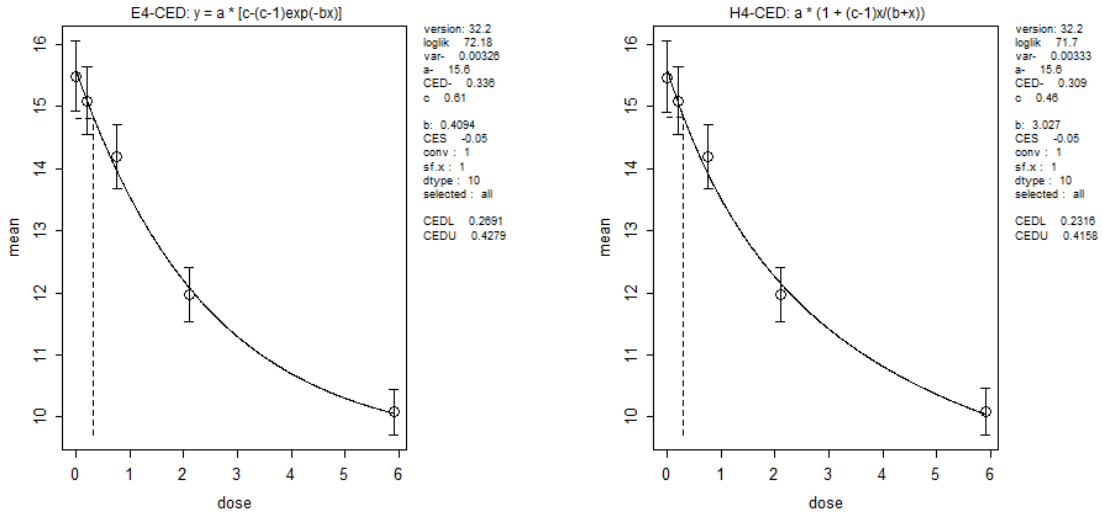


Figure J21: Fit of Model fit for the Exponential (left) and the Hill (right) models

Table J.22: MCV

The benchmark dose (BMD_{05}) and the 95 % benchmark dose lower confidence limit ($BMDL_{05}$) values are given for a BMR of 5 % decrease of weights (mg/10 g b.w.) relative to control with characteristics of the model fit. The model with lowest $BMDL_{05}$ is highlighted in bold.

Models	Converged	N of parameters	Log-likelihood	BMD_{05} (mg/kg b.w. per day)	$BMDL_{05}$ (mg/kg b.w. per day)
EXPONENTIAL MODELS					
full	1	6	103.7		
m1	1	2	36.22		
m2	1	3	71.72		
m3	1	4	86.01		
m4	1	4	99.07		
m5	**1**	**5**	**103.67**	**0.55**	**0.41**
HILL MODELS					
full	na	6	103.07		
m1	1	2	36.22		
m2	1	3	74.33		
m3	1	4	87.01		
m4	1	4	95.51		
m5	**1**	**5**	**103.43**	**0.61**	**0.47**

b.w.: body weight; na: not applicable.

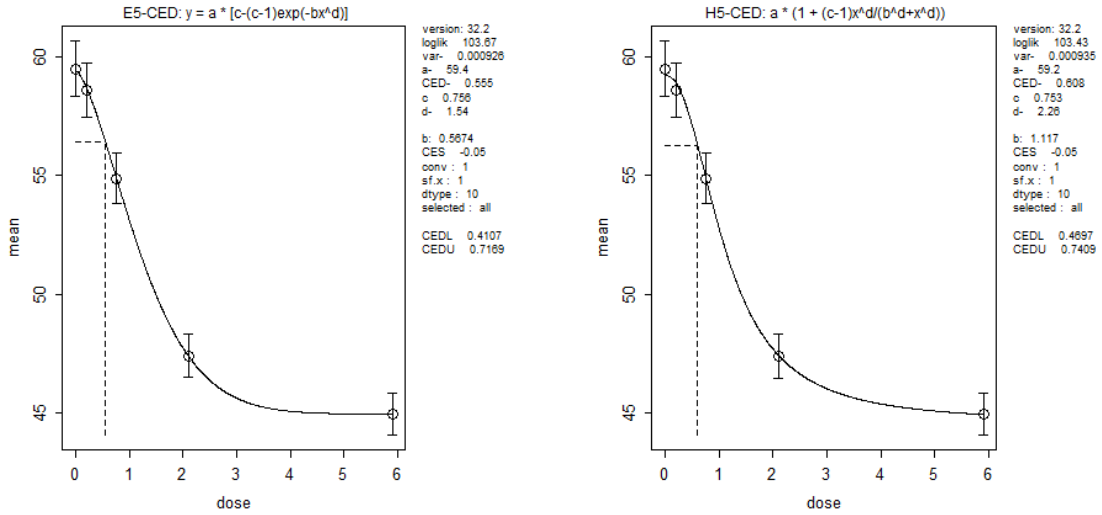


Figure J22: Fit of Model fit for the Exponential (left) and the Hill (right) model

Table J23: MCH

The benchmark dose (BMD_{-5}), the 95 % benchmark dose lower confidence limit ($BMDL_{-5}$) values for a BMR of 5 % decrease of weights (mg/10 g b.w.) relative to control for the selected model with characteristics of the model fit using PROAST. Selected model for dose-response analysis in bold.

Models	Converged	N of parameters	Log-likelihood	BMD_{05} (mg/kg b.w. per day)	$BMDL_{05}$ (mg/kg b.w. per day)
EXPONENTIAL MODELS					
full	1	6	79.05		
m1	1	2	34.57		
m2	1	3	44.81		
m3	1	4	59.94		
m4	1	4	66.51		
m5	**1**	**5**	**71.51**	**0.53**	**0.33**
HILL MODELS					
full	na	6	79.05		
m1	1	2	34.57		
m2	1	3	45.55		
m3	1	4	55.26		
m4	1	4	62.03		
m5	**1**	**5**	**71.15**	**0.62**	**0.49**

b.w.: body weight; na: not applicable.

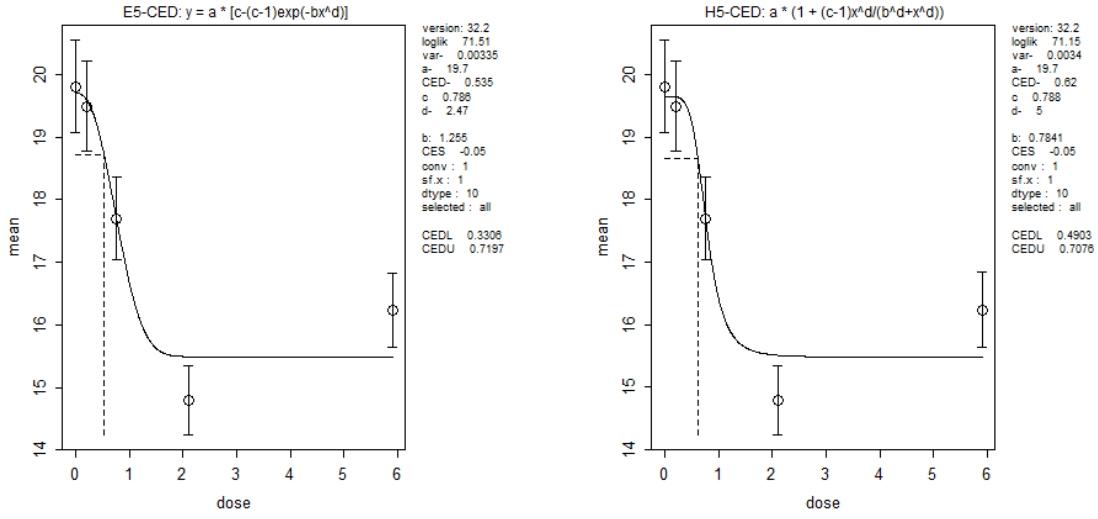


Figure J23: Fit of Model fit for the Exponential (left) and the Hill (right) models

Abbreviations

8-OHdG	8-hydroxy-2'-deoxyguanosine
AAS	Atomic absorption spectrometry
AFC Panel	EFSA Panel on Food Additives Flavourings, Processing Aids and Materials in Contact with Food
AI	Adequate intake
ANS Panel	EFSA Panel on Food Additives and Nutrient Sources added to Food
ALT	Alanine aminotransferase;
AST	Aspartate aminotransferase
ATSDR	Agency for Toxic Substances and Disease Registry
BCF	Bioconcentration factor
BE	Belgium
BEC	Background equivalent concentration
BG	Bulgaria
Bipea	Bureau Interprofessionnel d'Etudes Analytiques
BMD	Benchmark dose
$BMDL_{05}$	Lower 95 % confidence limit for a benchmark dose at 5 % extra risk
$BMDL_{10}$	Lower 95 % confidence limit for a benchmark response at 10 % extra risk
BP	Boiling point
BSO	Buthionine sulfoximine
b.w.	Body weight
CAdSV	Catalytic adsorptive stripping voltammetry
CCA	Chromated copper arsenate
CCT	Collision/reaction cell technology
CDPH	California Department of Public Health (former California Department of Health Services, CDHS)
CFA	Continuous flow analysis
CHO	Chinese hamster ovary
CICAD	Concise International Chemical Assessment Document
CNS	Central nervous system
COM	Committee on Mutagenicity of Chemicals in Food (UK)
COMA	Committee on Medical Aspects of Food Policy (UK)
CONTAM Panel	EFSA Panel on Contaminants in the Food Chain
Cr	Chromium
$Cr_2O_7^{2-}$	Dichromate ions
Cr(III)	Trivalent chromium
$Cr(OH)_3$	Chromium trihydroxide

CrO_4^{2-}	Chromate ion
Cr(VI)	Hexavalent chromium
CRL	Crown-rump length
CRM	Certified reference material
CY	Cyprus
CZ	The Czech Republic
DCM	Dietary and Chemical Monitoring unit
DE	Germany
DK	Denmark
DMSO	Dimethyl sulfoxide
DPAdSV	Differential pulse adsorptive stripping voltammetry
DPC	DNA-protein cross-links
DR	Dose-response
d.w.	Dry weight
EFSA	European Food Safety Authority
EFET	Hellenic Food Authority
EL	Greece
EPA	Environmental Protection Agency (U.S.)
ES	Spain
ETAAS	Electrothermal atomic absorption spectrometry
EVM	Expert group on Vitamins and Minerals (UK)
EWG	Environmental Working Group (U.S.)
F	Female
FAAS	Flame atomic absorption spectrometry
FAPAS	Food Analysis Performance Assessment Scheme
FCM	Food Contact Materials
FEEDAP Panel	EFSA Panel on Additives and Products or Substances used in Animal Feed
FEP	Perfluoro ethylene/propylene
FI	Finland
FIA	Flow injection analysis
FR	France
FSA	Food Standard Agency (UK)
GC	Gas chromatography
GD	Gestation day
GI	Gastrointestinal
GFAAS	Graphite furnace atomic absorption spectrometry
GSH	Glutathione

GSH/GSSG:	Reduced-to-oxidized glutathione ratio
HBGV	Health-based guidance value
HD	Highest dose
HFC	Human diploid fibroblasts
HPLC	High performance liquid chromatography
HPRT	Hypoxanthine phosphoribosyltransferase
HU	Hungary
IARC	International Agency for Research on Cancer
IC	Ion chromatography
ICP-AES	Inductively coupled plasma atomic emission spectrometry
ICP-MS	Inductively coupled plasma mass spectrometry
ICP-OES	Inductively coupled plasma optical emission spectrometry
IE	Ireland
IOM	Institute of Medicine
IPCS	International Programme on Chemical Safety
IT	Italy
LB	Lower bound
LMWCr	Low-molecular-weight chromium-binding substance
LOAEL	Lowest-observed-adverse-effect level
LOD	Limit of detection
LOQ	Limit of quantification
LV	Latvia
M	Male
MCH	Mean corpuscular haemoglobin
MCL	Maximum contaminant limit
MCV	Mean corpuscular volume
MDA	Malondialdehyde
MLs	Maximum levels
MMA	Manual metal arc
MMR	Mismatch repair
MOE	Margin of exposure
MP	Melting point
MRL	Minimal risk level
MS	Member State
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	Molecular weight
na	Not applicable

NAA	Neutron Activation Analysis
nd	non detected
NER	Nucleotide excision repair
Ni	Nickel
ni	not indicated
NIOSH	National Institute for Occupational Safety and Health (U.S.)
NL	The Netherlands
NOAEL	No-observed-adverse-effect level
NRC	National Research Council
NTP	National Toxicology Programme
OR	Odds ratio
PAHs	Polycyclic aromatic hydrocarbons
PARNUTS	Foodstuffs for particular nutritional use
PBK	Physiologically based kinetic
PE	Polyethylene
PE-HD	Polyethylene high density
PFA	Perfluoroalkoxy polymer
PHA	Phytohemagglutinin
PND	Postnatal day
PP	Polypropylene
PTS	Proficiency testing schemes
PTFE	Polytetrafluoroethylene
PTQA	2-(a-pyridyl)thioquinaldinamide
QL	Quantal Linear
RBCs	Red blood cells
RfD	Reference dose
ROS	Reactive Oxygen Species
RP	Reference point
SCF	Scientific Committee on Food
SDD	Sodium dichromate dihydrate
SE	Sweden
SID	Speciated isotope dilution
SID-HPLC-ICP-MS	Speciated isotope-dilution high performance liquid chromatography hyphenated to ICP-MS detection
SIDMS	Speciated isotope-dilution mass spectrometry
SISE-EAUX	French Health & Environment Information System on Water database
SMR	Standardised mortality ratio

SOD	Superoxide dismutase
SPE/DRC-ICP-MS	Solid-phase extraction/dynamic reaction cell inductively coupled to plasma mass spectrometry
SRM	Standard reference material
SDD	Sodium dichromate dihydrate
SOD	Superoxide dismutase
TDI	Tolerable daily intake
TDS	Total diet study
UB	Upper bound
UCMR	Unregulated chemicals for which monitoring is required
UHT	Ultra High Treatment
UK	The United Kingdom
UL	Upper level
UV	Ultraviolet
WHO	World Health Organization