Health Council of the Netherlands

Chromium VI compounds

Evaluation of the effects on reproduction, recommendation for classification

2016/04

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Gezondheidsraad
Health Council of the Netherlands

Aan de minister van Sociale Zaken en Werkgelegenheid

Onderwerp : aanbieding advies *Chromium VI compounds*
Uw kenmerk : DGV/BMO/U-932542
Ons kenmerk : U-963412/SV/jh/543-G16
Bijlagen : 1
Datum : 18 mei 2016

Geachte minister,

Graag bied ik u hierbij het advies aan over de effecten van een groep chroom VI-verbindingen op de vruchtbaarheid en de ontwikkeling van het nageslacht; het betreft ook effecten op de lactatie en via de moedermelk op de zuigeling.

Dit advies maakt deel uit van een uitgebreide reeks waarin voor de voortplanting giftige stoffen worden geclassificeerd volgens richtlijnen van de Europese Unie. Het gaat om stoffen waaraan mensen tijdens de beroepsuitoefening kunnen worden blootgesteld. Dit advies is een actualisatie van een advies dat in 2001 door de Gezondheidsraad is uitgebracht. De raad is gevraagd om deze actualisatie omdat de voorgestelde classificatie uit het eerdere advies afwijkt van de classificatie die op dit moment in de Europese Unie wordt gehanteerd.

Dit advies is opgesteld door een vaste commissie van de Gezondheidsraad, de Subcommissie Classificatie reproductietoxische stoffen. Het is vervolgens getoetst door de Beraadsgroep Volksgezondheid.

Ik heb dit advies vandaag ter kennisname toegezonden aan de minister van VWS en aan de staatssecretaris van IenM.

Met vriendelijke groet,

prof. dr. J.L. Severens,
vicevoorzitter

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Chromium VI compounds

Evaluation of the effects on reproduction,
recommendation for classification

Subcommittee on the Classification of Reproduction Toxic Substances,
a Committee of the Health Council of the Netherlands

to:

the Minister of Social Affairs and Employment

No. 2016/04, The Hague, May 18, 2016

The Health Council of the Netherlands, established in 1902, is an independent scientific advisory body. Its remit is "to advise the government and Parliament on the current level of knowledge with respect to public health issues and health (services) research..." (Section 22, Health Act).

The Health Council receives most requests for advice from the Ministers of Health, Welfare and Sport, Infrastructure and the Environment, Social Affairs and Employment, and Economic Affairs. The Council can publish advisory reports on its own initiative. It usually does this in order to ask attention for developments or trends that are thought to be relevant to government policy.

Most Health Council reports are prepared by multidisciplinary committees of Dutch or, sometimes, foreign experts, appointed in a personal capacity. The reports are available to the public.

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Contents

Samenvatting

In het voorliggende advies heeft de Gezondheidsraad chroomtrioxide, natriumchromaat, natriumdichromaat, kaliumdichromaat, chroomzuur, ammoniumchromaat, ammoniumdichromaat, calciumchromaat, kaliumchromaat, en dichroomtrischromaat onder de loep genomen. Deze chroom VI-verbindingen worden gebruikt in metaalafwerking, synthese van andere chroomverbindingen, houtverduurzamingsmiddelen, katalysatoren en pigmenten/kleurstoffen. Dit advies past in een reeks adviezen waarin de Gezondheidsraad op verzoek van de minister van Sociale Zaken en Werkgelegenheid de effecten van stoffen op de voortplanting beoordeelt. Het gaat vooral om stoffen waaraan mensen tijdens de beroepsuitoefening kunnen worden blootgesteld. De Subcommissie Classificatie reproductietoxische stoffen van de Commissie Gezondheid en beroepsmatige blootstelling aan stoffen (GBBS) van de raad, hierna aangeduid als de commissie, kijkt zowel naar effecten op de vruchtbaarheid van mannen en vrouwen als naar effecten op de ontwikkeling van het nageslacht. Daarnaast worden effecten op de lactatie en via de moedermelk op de zuigeling beoordeeld.

Op basis van Verordening (EG) 1272/2008 van de Europese Unie doet de commissie een voorstel voor classificatie. Voor de bovengenoemde chroom VI-verbindingen komt de commissie tot de volgende aanbevelingen:

- voor effecten op de fertiliteit adviseert de commissie ze te classificeren in categorie 1B *(stoffen waarvan verondersteld wordt dat zij toxisch zijn voor*

de menselijke voortplanting) en te kenmerken met H360F *(kan de vruchtbaarheid schaden)*

- voor effecten op de ontwikkeling adviseert de commissie ze te classificeren in categorie 1B *(stoffen waarvan verondersteld wordt dat zij toxisch zijn voor de menselijke voortplanting)* en te kenmerken met H360D *(kan het ongeboren kind schaden)*
- voor effecten tijdens de lactatie adviseert de commissie om ze te kenmerken met H362 *(kan schadelijk zijn via de borstvoeding)*.

Executive summary

In the present report the Health Council of the Netherlands reviewed chromium trioxide, sodium chromate, sodium dichromate, potassium dichromate, chromic acid, ammonium chromate, ammonium dichromate, calcium chromate, potassium chromate, and dichromium tris(chromate). These chromium VI compounds are used in metal finishing, manufacture of other chromium compounds, wood preservation products, catalysts, and pigments/dyes. This report is part of a series, in which the Health Council evaluates the effects of substances on reproduction, at the request of the Minister of Social Affairs and Employment. It mainly concerns substances to which man can be occupationally exposed. The Subcommittee on the Classification of Reproduction Toxic Substances of the Dutch Expert Committee on Occupational Safety (DECOS) of the Health Council, hereafter called the Committee, evaluates the effects on male and female fertility and on the development of the progeny. Moreover, the Committee considers the effects of a substance on lactation and on the progeny via lactation.

The Committee recommends classification according to Regulation (EC) 1272/2008 of the European Union. For the abovementioned chromium VI compounds, the Committee recommends:

- for effects on fertility, to classify these compounds in category 1B *(presumed human reproductive toxicant)*, and to label them with H360F *(may damage fertility)*

- for effects on development, to classify these compounds in category 1B *(presumed human reproductive toxicant)* and to label them with H360D *(may damage the unborn child)*
- for effects during lactation, to label these compounds with H362 *(may cause harm to breastfed babies)*.

Chapter 1

Scope

1.1 Background

As a result of the Dutch regulation on registration of compounds toxic to reproduction that came into force on 1 April 1995, the Minister of Social Affairs and Employment requested the Health Council of the Netherlands to classify compounds toxic to reproduction. This classification is performed by the Health Council's Subcommittee on the Classification of reproduction toxic substances of the Dutch Expert Committee on Occupational Safety (DECOS). The classification is performed according to European Union Regulation (EC) 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures. The CLP guideline is based on the Globally Harmonised System of Classification and Labelling of Chemicals (GHS). The Subcommittee's advice on the classification will be applied by the Ministry of Social Affairs and Employment to extend the existing list of compounds classified as reproductive toxicant (category 1A and 1B and 2) or compound with effects on or via lactation.

The ministry of Social Affairs and Employment asked the Health Council to update the evaluation and classification on reproduction toxicity of a series of substances. In this report, such an update was performed for a group of chromium VI compounds. For those specified in Chapter 2.1, this document replaces the recommendation regarding the classification for reproductive toxicity of chromium VI compounds that was published in 2001.[1]

1.2 Committee and procedure

This document contains the classification of a group of chromium VI compounds by the Health Council's Subcommittee on the Classification of Reproduction Toxic Substances, hereafter called the Committee. The members of the Committee are listed in Annex A. A submission letter (in English) to the Minister can be found in Annex B.

In 2015, the President of the Health Council released a draft of the report for public review. The individuals and organisations that commented on the draft report are listed in Annex C. The Committee has taken these comments into account in deciding on the final version of the report. The received comments, and the replies by the Committee, can be found on the website of the Health Council.

The classification is based on the evaluation of published human and animal studies concerning adverse effects with respect to fertility and development as well as lactation of the above mentioned compound.

Classification for reproduction (fertility (F) and development (D):	
Category 1	Known or presumed human reproductive toxicant (H360(F/D))
Category 1A	Known human reproductive toxicant
Category 1B	Presumed human reproductive toxicant
Category 2	Suspected human reproductive toxicant (H361(f/d))
No classification for effects on fertility or development	
Classification for lactation:	
	Effects on or via lactation (H362)
	No labelling for lactation

The classification and labelling of substances is performed according to the criteria of the European Union (Regulation (EC) 1272/2008) presented in Annex D. The classification of substances is ultimately dependent on an integrated assessment of the nature of all parental and developmental effects observed, their specificity and adversity, and the dosages at which the various effects occur. The criteria necessarily leave room for interpretation, dependent on the specific data set under consideration. In the process of using the regulation, the Committee has agreed upon a number of additional considerations (see Annex E).

1.3 Classification for lactation

The recommendation for classifying substances for effects on or via lactation is also based on Regulation (EC) 1272/2008. The criteria define that substances which are absorbed by women and have been shown to interfere with lactation or which may be present (including metabolites) in breast milk in amounts sufficient to cause concern for the health of a breastfed child, shall be classified and labelled. Unlike the classification of substances for fertility and developmental effects, which is based on hazard identification only (largely independent of dosage), the labelling for effects during lactation is based on a risk characterization and therefore, it also includes the consideration of the level of potential exposure of the breastfed child.

Consequently, a substance should be labelled for effects during lactation when it is likely that the substance would be present in breast milk at potentially toxic levels. The Committee considers a concentration of a substance as potentially toxic to the breastfed child when this concentration leads to exceeding the exposure limit for the general population, e.g. the acceptable daily intake (ADI).

1.4 Data

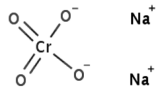
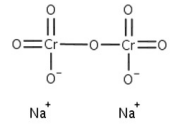
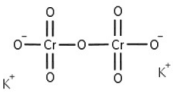
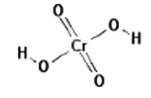
Literature searches were conducted in the on-line databases TOXLINE, MEDLINE and CAPLUS. A final search was performed in November 2015. Publications cited in the selected articles, but not selected during the primary search, were reviewed if considered appropriate. In addition, handbooks and most recent reviews were consulted.

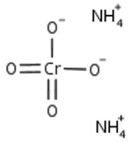
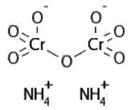
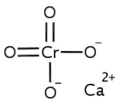
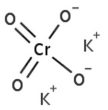
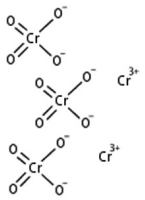
Chapter 2

Identity of the substance

2.1 Name and other identifiers of the substance

Data are derived from ECHA and HSDB.[2,3]

EC number	215-607-8	231-889-5	234-190-3	231-906-6	231-801-5
EC name	Chromium trioxide	Sodium chromate	Sodium dichromate	Potassium dichromate	Chromic acid
CAS number	1333-82-0	7775-11-3	10588-01-9	7778-50-9	7738-94-5
CAS name	Chromium oxide, (CrO_3)	Chromic acid, (H_2CrO_4), disodium salt	Chromic acid, ($H_2Cr_2O_7$), disodium salt	Chromic acid, ($H_2Cr_2O_7$), dipotassium salt	Chromic acid, (H_2CrO_4)
IUPAC name	Trioxochromium	Disodium dioxido(dioxo) chromium	Sodium dichromate	Dipotassium dichromate	dihydroxy(dioxo) chromium
CLP Annex VI Index number	024-001-00-0	024-018-00-3	024-004-00-7	024-002-00-6	-
Molecular formula	CrO_3	$Na_2CrO_4(\bullet 4H_2O)$	$Na_2Cr_2O_7(\bullet 2H_2O)$	$K_2Cr_2O_7$	CrH_2O_4
Molecular weight (g/mol)	99.99	161.99	261.96	294.22	118.0
Structural formula					

EC number	232-138-4	232-143-1	237-366-8	232-140-5	246-356-2
EC name	Ammonium chromate	Ammonium dichromate	Calcium chromate	Potassium chromate	Dichromium tris(chromate)
CAS number	7788-98-9	7789-09-5	13765-19-0	7789-00-6	24613-89-6
CAS name		Chromic acid, ($H_2Cr_2O_7$), diammonium salt		Chromic acid, (H_2CrO_4), dipotassium salt	Chromic acid (H_2CrO_4), chromium(3+) salt (3:2)
IUPAC name	Ammonium chromate	Diammonium dichromate	Calcium dioxido-(dioxo) chromium	Potassium chromate	Chromium (3+) dioxido (dioxo) chromium
CLP Annex VI Index number		024-003-00-1	024-008-00-9	024-006-00-8	024-010-00-X
Molecular formula	$(NH_4)_2CrO_4$	$(NH_4)_2Cr_2O_7$	$CaCrO_4$	K_2CrO_4	$CrH_2O_4.2/3Cr$
Molecular weight (g/mol)	152.07	252.06	156.09	194.20	451.97
Structural formula					

2.2 Composition of the substance

Not applicable.

2.3 Physico-chemical properties

Data are derived from EU RAR and ECHA.[2-4]

Property	Chromium trioxide	Sodium chromate	Sodium dichromate	Potassium dichromate	Chromic acid
State of the substance at normal temperature and pressure and 101,3 kPa	Dark red deliquescent crystals, flakes or powder	Yellow orthorhombic crystals	Reddish to bright orange deliquescent crystals in hydrated form usually dihydrated	Bright orange-red crystals - not hygroscopic or deliquescent	Dark purplish-red crystals
Melting/freezing point	196 °C	792 °C	Becomes anhydrous at 100 °C and melts at ~357 °C	~398 °C	196 °C
Relative density	~2.7	~2.4-2.7	~2.5	~2.7	1.67-2.82
Water solubility (at room temperature)	~1 667 g/L	~530 g/L	~2 355 g/L	~115 g/L	1 854 g/L

Autoignition and flammability	Decomposes at 250 °C to Cr_2O_3 and O_2	-	Decomposes above 400 °C	Decomposes above 500 °C	Decomposes at about 250 °C
Oxidizing properties	Violent oxidizing agent	Mildly oxidizing; strong oxidizer in acidic conditions	Strong oxidizing agent	Strong oxidizing agent	Strong oxidizing agent

Property	Ammonium chromate	Ammonium dichromate	Calcium chromate	Potassium chromate	Dichromium tris(chromate)
State of the substance at normal temperature and pressure and 101,3 kPa	Yellow crystals	Orange crystals	Yellow crystals	Yellow crystals	Dark purple/black granular solid, with an amorphous non-crystalline structure
Melting/freezing point	185 °C (decomposes)	180 °C (decomposes)	2,710 °C	968.3 °C	>300 °C
Relative density	~1.9 g/cm^3	~2.2 g/cm^3	~2.9 g/cm^3	~2.7 g/cm^3	~2.3 g/cm^3
Water solubility (at room temperature)	~405 g/L	~360 g/L	163 g/L	394 g/L	96.6 g/L
Autoignition and flammability	-	-	-	-	
Oxidizing properties	Oxidizing substance	Oxidizing substance	-	-	Oxidizing substance

2.4 International classifications

EU harmonised classification

Chromium trioxide	Sodium chromate	Sodium dichromate	Potassium dichromate	Chromic acid	Ammonium chromate	Ammonium dichromate	Calcium chromate	Potassium chromate	Dichromium tris(chromate)
Repr 2 (H361f)	Repr 1B (H360FD)	Repr 1B (H360FD)	Repr 1B (H360FD)	-	-	Repr 1B (H360FD)	-	-	-

In addition to classification for reproductive toxicity, chromium VI compounds have been classified for several other endpoints, including carcinogenicity and mutagenicity:

Chromium trioxide	Sodium chromate	Sodium dichromate	Potassium dichromate	Chromic acid	Ammonium chromate	Ammonium dichromate	Calcium chromate	Potassium chromate	Dichromium tris(chromate)
Carc. 1A (H350) Muta. 1B (H340)	Carc. 1B (H350) Muta. 1B (H340)	Carc. 1B (H350) Muta. 1B (H340)	Carc. 1B (H350) Muta. 1B (H340)	Carc. 1B (H350)	Carc. 1B (H350)	Carc. 1B (H350) Muta. 1B (H340)	Carc. 1B (H350)	Carc. 1B (H350) Muta. 1B (H340)	Carc. 1B (H350)

The classification of chromium VI compounds was last updated in the 29th ATP (2004), for which the proposal was discussed in 2002.

Chapter 3

Manufacture and uses

3.1 Manufacture

Not relevant for classification.

3.2 Identified uses

Main uses[2-4]

- *Chromium trioxide*: Used in metal finishing; for manufacturing of wood preservation products, catalysts, chromium dioxide and pigments
- *Sodium chromate*: Used for manufacturing of other chromium compounds
- *Sodium dichromate*: Used for manufacturing of other chromium compounds, wood preservative products, vitamin K and wax; as mordant in dyeing; in metal finishing
- *Potassium dichromate*: Used for manufacturing of pigments, wood preservation products, dyes, catalysts and chromium metal; as colouring agent in ceramics
- *Chromic acid*: Used in production of various chemicals (chromates, oxidizing agents, catalysts); as intermediate in chromium-plating, in ceramic glazes and colored glass. *Ammonium chromate*: Used as sensitiser for gelatin coatings used in photography; in textile printing pastes, and fixing chromate dyes on wool; as an analytical reagent, catalyst, and corrosion inhibitor

- *Ammonium dichromate*: Used as an intermediate and laboratory reagent
- *Calcium chromate*: Used as a pigment, a corrosion inhibitor; in electroplating, photochemical processing, and industrial waste treatment
- *Potassium chromate*: Used as a mordant in dyeing fabrics; as tanning agent in the leather industry, in bleach oils and waxes; as an oxidizing agent in organic synthesis
- *Dichromium tris(chromate)*: Used as corrosion inhibitor; as catalyst in the mordanting of yarns.

Chapter 4

Read-across and toxicokinetics (absorption, metabolism, distribution and elimination)

4.1 Read-across/grouping

Absorption of chromium VI compounds has been reported after inhalation, oral and dermal exposure, in both humans and animals, albeit at different degrees (see next sections). The absorption data have all been considered relevant for humans, including the oral absorption data. Importantly, in a species comparison in rats, mice and guinea pigs, it was concluded that the presence of a forestomach did not influence the toxicokinetics of sodium dichromate dehydrate.[5]

Toxicity data on chromium VI compounds mainly involve sodium chromate and potassium chromate. These data suggest that in addition to local toxicity, exposure can lead to systemic effects.

A number of epidemiological studies have considered the association of exposure to chromium VI and systemic effects, as some evidence of kidney damage has been found among chromate production and chromium plating workers.[4,6]

In rats, adverse effects on liver function and the immune/lymphatic system have been reported after repeated inhalation exposure. After repeated oral exposure, haematoxicity, hepatotoxicity, and renal toxicity was observed in rats and mice.[4,6] In addition, reproductive toxicity has been reported for several species (see this report).

Although the exact mode of action is not known, chromium VI-induced toxicity has been attributed to the formation of reactive intermediates.

Information available indicates that most if not all chromium compounds specified in this document are either corrosive or irritating to skin. Under physiological conditions, chromium VI can be reduced intracellularly by e.g. hydrogen peroxide, glutathione reductase, ascorbic acid, to produce reactive intermediates, including chromium V, chromium IV, hydroxyl radicals, and ultimately, chromium III. These reactive intermediates can affect DNA, proteins, and membrane lipids, thereby disrupting cellular integrity and functions.[7] The reduction of chromium VI is therefore considered to serve as a detoxification process when it occurs at a distance from the target site. Reduction of chromium VI in or near the cell nucleus of target organs however, may be the cause of the toxicity that is observed.[8] If chromium VI is reduced to chromium III extracellularly, it is not readily transported into cells thereby limiting toxicity.

The Committee concludes that the available toxicokinetic, mechanistic and toxicity data suggest that the amount of systemically available chromium VI is responsible for the systemic effects observed after exposure to sodium chromate and potassium chromate. All the soluble chromium VI compounds specified in this report are, dependent on chromium ion concentration and pH, present as either chromium acid ($HCrO_4^-$), or chromate/dichromate anions (Figure 1). Overall, the data indicate that soluble chromium VI compounds can be present in different species, dependent on pH and irrespective of the form from which chromium VI enters solution.

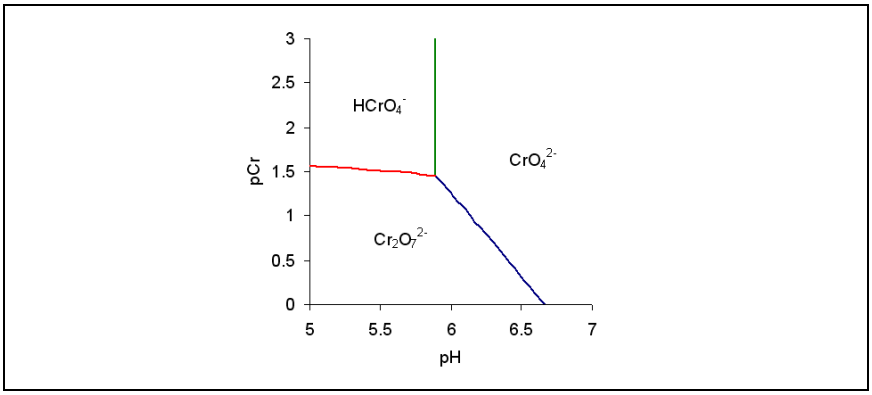


Figure 1 A predominance diagram showing the influence of concentration of chromium and pH on the predominance of chromium species in solution, in which pCr stands for minus the logarithm of the chromium concentration and pH stands for minus the logarithm of the hydrogen ion concentration. The lines on a predominance diagram indicate where adjacent species have the same concentration. As chromium VI is a strong oxidising agent, it only exists as oxygenated species in the environment. The actual species present in solution depends on the pH. At very low pH (e.g. near 0) the dominant species in solution will be the fully protonated form (H_2CrO_4). At pHs between 0 and 6-6.5, the dominant chromate species in solution would be $HCrO_4$-, and at pHs above 6-6.5 the main chromate species in solution would be CrO_4^{2-}.[4]

Therefore, the Committee assumes that a similar toxicity profile is likely for all soluble chromium VI compounds, although differences in potency may exist. Conform REACH Annex XI section 1.5, read-across may be applied for substances with 'common precursors and/or the likelihood of common breakdown products via physical and biological processes, which result in structurally similar chemicals'. Consequently, for the purpose of classification and labelling, the Committee applies read-across for all chromium VI compounds specified in this document.

The information provided in the next sections is summarised from the ATSDR[6] and EU RAR[4]. Additional sources are referenced separately.

4.2 Non-human studies

Absorption

Oral

Studies with ^{51}chromium in animals indicate that chromium and its compounds are poorly absorbed from the gastrointestinal tract after oral exposure. The absorption fraction of soluble chromium VI is higher than that of soluble chromium III. Absorption amounts of ≤1.4% of the administered oral dose have been reported for chromium VI in rats. When food was given ad libitum, only 1-3% of orally administered chromium VI was absorbed in rats and mice; the amount increased if food had been withdrawn for 16-48 hours. In contrast, one study reported at least 18% absorption in unstarved guinea pigs receiving potassium chromate orally. The question whether chromium VI absorption mainly occurs when the reducing capacity of the gastrointestinal tract is exhausted, has been noted as an issue to consider when evaluating and interpreting oral dosing bioassays.[6]

Inhalation

Following inhalation or intratracheal instillation of highly water-soluble chromium VI compounds, approximately 20-30% of the chromium dose has been reported to be rapidly absorbed into the bloodstream. Rats exposed for a single inhalation of chromium trioxide (3.18 mg chromiumVI/m^3) for 30 minutes rapidly absorbed chromium from the lungs. The kinetics of three chromium VI compounds, sodium chromate, zinc chromate, and lead chromate, have been

compared in rats in relation to their solubility. The results indicated that zinc chromate, which is ~1,000 times less soluble than sodium chromate, is more slowly absorbed from the lungs, but peak blood levels are higher than for sodium chromate. Lead chromate was more poorly and slowly absorbed, as indicated by very low levels in blood and other tissues, and greater retention in the lungs.

Dermal

Dermal absorption of highly water-soluble chromium VI compounds in guinea pigs varied between <1% and 4% of the applied aqueous dose, depending on the chromium concentration. The dermal absorption of sodium chromate (chromium VI) by guinea pigs was somewhat higher, though not statistically significantly, than that of chromium III trichloride.

Distribution

Oral

Several tissue distribution studies of chromium VI compounds have been described. In one experiment, 0-5.8 mg chromium VI/kg/day (administrated as sodium chromate in water by gavage) for 7 days. At low doses, chromium levels were comparable to controls whereas at 5.8 mg/kg/day, the largest amount of chromium (expressed as µg chromium/whole organ) was found in the liver (≈22 µg), followed by the kidney (≈7.5 µg), lung (≈4.5 µg), blood (≈2 µg), and spleen (≈1 µg).

In another experiment, rats were exposed by gavage to 7 mg chromium/kg/day for 7 days from various sources, including sodium chromate; calcium chromate; soil containing chromium (30% chromium VI). The highest levels of chromium were found in liver, spleen, kidney, lung, blood, brain, and testes after dosing with sodium chromate, but the relative levels in these tissues after the other treatments followed no consistent pattern.

The chromium content in major organs (heart, lung, kidney, liver, spleen, testes) of mice receiving drinking water that provided doses of 4.4, 5.0, or 14.2 mg chromium VI/kg/day as potassium dichromate was determined after 1 year of exposure. Accumulation was reported in all of the above organs, with the highest levels reported in the liver and spleen.

In rats exposed to 100 and 200 mg/L potassium chromate in the drinking water for 3 or 6 weeks, a general trend of increasing chromium concentration with time of exposure was apparent in the liver and kidneys, but only the kidneys

showed a difference in the concentration after exposure to 100 and 200 mg/L. Blood concentrations were almost saturated by 3 weeks with little further accumulation by 6 weeks. No chromium was detected in the lungs after drinking water exposure.

The distribution of potassium chromate was compared in male Fisher rats and C57BL/6J mice exposed either by drinking water (8 mg chromium VI/kg/day for 4 and 8 weeks). The concentrations of chromium (μg/g wet tissue) after drinking water exposures for 8 weeks in mice were: liver 13.83, kidney 4.72, spleen 10.09, femur 12.55, lung 1.08, heart 1.02, muscle 0.60, and blood 0.42. These concentrations were not markedly different than for 4-week exposures. For rats, the concentrations were: liver 3.59, kidney 9.49, spleen 4.38, femur 1.78, lung 0.67, heart 1.05, muscle 0.17, and blood 0.58. These results indicate that, although blood levels are comparable, considerable species differences exist between mice and rats.

A comparative absorption study of sodium dichromate dihydrate was performed by the National Toxicity Program (NTP) in F344/N rats, B6C3F1 mice and Hartley guinea pigs. prior to its repeated dose study.[5] Dose concentrations reported were 0, 2.87, 8.62, 28.7, 86.2, 287, and 862 mg chromium VI/L (equivalent to 0, 1, 3, 10, 30, 100, and 300 mg chromium/L, respectively. Chromium in blood and kidney increased with exposure concentration in all three species and although differences were seen in the absolute amounts of chromium in kidney and blood, uptake as a function of exposure concentration did not appear to differ qualitatively in guinea pigs when compared to rats and mice.

Twelve pregnant female albino rats (Druckrey strain) and 13 Swiss albino mice were exposed to 500 mg/L potassium dichromate in their drinking water (calculated to correspond to 11.9 and 3.6 mg chromium VI/day, respectively) during pregnancy up to 1 day before delivery. In untreated rats, concentrations of chromium were 0.067, 0.219, and 0.142 μg/g fresh weight in maternal blood, placenta, and foetuses, respectively, and 0.064, 0.304, and 0.366 μg/g fresh weight in mice, respectively. In treated rats, chromium levels were 3.2-fold higher in maternal blood, 3-fold higher in placenta, and 3.1-fold higher in foetal tissue when compared to control values. In treated mice, chromium levels were 2.5-fold higher in maternal blood, 3.2-fold higher in placenta, and 9.6-fold higher in foetuses when compared to control values. In treated mice, there was a significant elevation in chromium levels in placental and foetal tissues over maternal blood levels, and a significant increase in chromium levels in foetal tissue over placental concentrations when compared to controls.

Inhalation

Steady-state concentrations in the blood have been reported after ~4 days in rats exposed to 2.1 mg chromium VI/m^3 as zinc chromate for 6 hours/day.

Rats exposed for a single inhalation of chromium trioxide mist from electroplating (18 mg chromium VI/m^3) for 30 minutes rapidly absorbed chromium from the lungs. The content of chromium in the lungs declined from 13.0 mg immediately after exposure to 1.1 mg at 4 weeks.

Dermal

Chromium compounds are absorbed after dermal administration by guinea pigs. Measurement of ^{51}chromium in the organs and body fluids revealed distribution, due to dermal absorption of chromium VI compounds, to the blood, spleen, bone marrow, lymph glands, urine, and kidneys. Absorption was greater for chromium VI than for chromium III.

Metabolism

Chromium VI is unstable inside the body and is ultimately reduced to chromium III, which is essential to physiological processes, including glucose, protein, and fat metabolism and intracellular reduction and oxidation reactions.

In vivo and in vitro experiments in rats indicated that, in the lungs, chromium VI can be reduced to chromium III by ascorbate. When ascorbate is depleted from the lungs, chromium VI can also be reduced by glutathione. The reduction of chromium VI by glutathione is slower and results in greater residence time of chromium in the lungs, compared to reduction by ascorbate. Because chromium III does not readily enter cells, these data suggest that reduction of chromium VI by the epithelial lining fluid may constitute the first line of defense against toxicity of inhaled chromium compounds. Furthermore, uptake and reduction of chromium compounds by the pulmonary alveolar macrophages may constitute a second line of defense against pulmonary toxicity of chromium VI compounds.

The first defense against chromium VI after oral exposure is the reduction of chromium VI to chromium III in the gastric environment where gastric juice and ascorbate play important roles.

In addition to the reduction of chromium VI by ascorbate or glutathione, in vitro studies have demonstrated reduction of chromium VI by microsomal enzymes. Microsomal reduction of chromium VI occurs in the lungs mainly as it does in the liver, involving cytochrome P450, NADPH-dependent-cytochrome

P450 reductase, and to a lesser extent cytochrome b5 and NADH-dependent-cytochrome b5 reductase.

Elimination

Inhalation

It is generally believed that that absorbed chromium VI compounds may be excreted more slowly than absorbed chromium III compounds due to a higher ability to enter cells. Inhaled or intratracheal instilled chromium VI is excreted in urine and faeces in similar amounts (in the range 20-70% of administered dose). Peak urinary chromium concentrations were observed at 6 hours (the first time point examined) in rats exposed intratracheally to chromium VI as sodium dichromate. Chromium urinary concentrations decreased rapidly, falling from 2,947 µg chromium/g creatinine at 6 hours to 339 µg chromium/g at 72 hours.

Elimination of chromium was very slow in rats exposed to 2.1 mg chromium VI/m^3 as zinc chromate 6 hours/day for 4 days. Urinary levels of chromium remained almost constant for 4 days after exposure and then decreased, from which it has been concluded that chromium bound inside the erythrocyte is released slowly.

Oral

Given the low absorption of chromium compounds by the oral route, the major pathway of excretion after oral exposure is through the faeces. Chromium in urine and faeces is in the form of chromium III complexes, with glutathione for example.

Rats given 18 mg chromium VI/kg as potassium dichromate by gavage excreted about 25 µg chromium in the first 24 hours after dosing and appr. 10 µg chromium in each of the next 24-hour periods.

In rats and hamsters fed chromium compounds, faecal excretion of chromium varied slightly from 97 to 99% of the administered dose. Urinary excretion of chromium varied from 0.6 to 1.4% of the dose administered as either chromium VI or chromium III compounds.

Dermal

^{51}Chromium was detected in the urine of guinea pigs after a radiolabeled sodium chromate solution was placed over skin depots that were monitored by scintillation counting to determine the dermal absorption.

4.3 Human studies

Absorption

Oral

Gastrointestinal absorption of chromium in humans is estimated to be <10% of the ingested dose, with the absorption fraction of soluble chromium compounds being higher than that of insoluble forms. The absorption of chromium VI was measured in four male and two female volunteers (ages ranging from 25 to 39 years) treated orally with potassium chromate (chromium VI) in capsules at doses of 0.005 mg/kg/day. Subjects were exposed for 3 days. Based on urinary excretion data, the mean absorption of potassium chromate was 3.4% (range 0.69-11.9%).

In a follow-up study by the same group, five male volunteers ingested in total a litre of deionised water containing chromium VI concentrations ranging from 0.1 to 10.0 mg/L (approximately 0.001-0.1 mg chromium VI/kg/day) for 3 days. A dose-related increase in chromium levels was seen in urine, plasma, and erythrocytes. The percentage of the dose excreted in urine ranged from <2 to 8%.

In a repeated dose study, three healthy adults ingested chromium VI (as $K_2Cr_2O_7$) in water at 5 mg chromium/day for 3 consecutive days. Three divided doses were taken at approximately 6-hour intervals over a 5-15-minute period. After at least 2 days without dosing, the 3-day exposure regimen was repeated at 10 mg chromium/day. Estimated doses based on body weight were 0.05 and 0.1 mg/kg/day, respectively. Bioavailability based on 4-day urinary excretion was 1.7% (range 0.5-2.7%) at 0.05 mg chromium VI/kg/day and 3.4% (range 0.8-8.0%) at 0.1 mg chromium VI/kg/day. Absorption of 0.05 mg chromium VI/kg appeared to be somewhat lower when given as three divided doses rather than when given as a single bolus dose (1.7 versus 5.7%).

Uptake of potassium dichromate was determined in a man who was given 0.8 mg of chromium VI in drinking water 5 times each day for 17 days. Steady-state concentrations of chromium in blood were attained after 7 days. Red blood cell and plasma levels returned to background levels within a few days after exposure

was stopped. The data are consistent with a bioavailability of 2% and a plasma elimination half-life of 36 hours.

The average absorption fractions that were determined from cumulative urinary excretion in 8 healthy adults, who ingested 5 mg chromium (in 10 mg chromium/L drinking water as $K_2Cr_2O_7$), amounted to 6.9% (± 3.7).

Experimental studies in humans did not find evidence for an effect of conditions that limit the reduction of chromium on the absorption of chromium. The range of doses of chromium administered to humans in these different studies was considerable and demonstrated oral bioavailability at all doses.

Inhalation

The absorption of inhaled chromium compounds depends on a number of factors, including physical and chemical properties of the particles (oxidation state, size, solubility) and the activity of alveolar macrophages. The identification of chromium in urine, serum and tissues of humans occupationally exposed to soluble chromium VI compounds in air indicates that chromium can be absorbed from the lungs. In most cases, chromium VI compounds were found to be more readily absorbed from the lungs than chromium III compounds, in part due to differences in the capacity to penetrate biological membranes.

Dermal

Chromium VI can penetrate human skin to some extent, especially if the skin is damaged. Fourteen days after a salve containing potassium chromate was applied to the skin of an individual to treat scabies, appreciable amounts of chromium were found in the blood, urine, feces, and stomach contents. Potassium dichromate has also been reported to penetrate the excised intact epidermis of humans.

An average rate of systemic uptake of chromium in four volunteers submersed up to the shoulders in a tub of chlorinated water containing a 22 mg chromium VI/L solution of potassium dichromate for 3 hours was measured to be $3.3x10^{-5}$-$4.1x10^{-4}$ µg Cr/cm^2-hour (based on urinary excretion of total chromium).

The influence of solvent on the cutaneous penetration of potassium dichromate by humans has been studied. Results with dichromate in water and in petrolatum revealed that about 10 times more chromium penetrated when potassium dichromate was applied in petrolatum than when applied in water.

About 5 times more chromium penetrated when potassium dichromate was applied than when a chromium trichloride glycine complex was applied.

Distribution

Oral

The distribution of chromium in human body tissue after acute oral exposure was determined in the case of a 14-year-old boy who ingested 7.5 mg chromium VI/kg as potassium dichromate. Despite extensive treatment by dialysis and chelation, the boy died 8 days after admission to the hospital. Upon autopsy, the chromium concentrations were as follows: liver, 2.94 mg/100 cc (normal, 0.016 mg/100 cc); kidneys, 0.64 and 0.82 mg/100 cc (normal, 0.06 mg/100 cc); and brain, 0.06 mg/100 cc (normal, 0.002 mg/100 cc).

Inhalation

Examination of tissues from Japanese chrome platers and chromate refining workers at autopsy revealed higher chromium levels in the hilar lymph node, lung, spleen, liver, kidney, and heart, compared to normal healthy males.

Chromium concentrations have been measured in organs and tissues at autopsy of a man who died of lung cancer, 10 years after his retirement from working in a chromate producing plant for 30 years and exposed mainly to chromium VI. This analysis revealed measurable levels of chromium in the brain, pharyngeal wall, lung, liver, aorta, kidney, abdominal rectal muscle, suprarenal gland, sternal bone marrow, and abdominal skin. These levels were higher than in five controls without occupational exposure to chromium.

The levels of chromium were higher in the lungs, but not in the liver or kidneys, of autopsy specimens from 21 smeltery and refinery workers in North Sweden compared with that for a control group of 8 individuals.

In tissues from three individuals having lung cancer who were industrially exposed to chromium, cumulative chromium exposures amounted to 3.45, 4.59, and 11.38 mg/m^3 years. All tissues from the three workers had elevated levels of chromium with the possible exception of neural tissues. Levels were orders of magnitude higher in lungs than in other tissues. The highest lung level reported was 456 mg/10 g tissue in the first worker, 178 in the second worker, and 1,920 for the third worker. Significant chromium levels were found in the tissue of the second worker even though he had not been exposed to chromium for 18 years.

Dermal

The findings of toxic effects in the heart, stomach, blood, muscles, and kidneys of humans who were dermally exposed to chromium compounds are suggestive of distribution to these organs. Fourteen days after a salve containing potassium chromate was applied to the skin of an individual to treat scabies, appreciable amounts of chromium were found in the blood (2-5 mg/100 mL), urine (8 mg/L), feces (0.61 mg/100 g), and stomach contents (0.63 mg/100 mL).

A transient increase in the levels of total chromium in erythrocytes and plasma was observed in subjects immersed in a tank of chlorinated water containing potassium dichromate.

Metabolism

No data are available.

Elimination

Oral

Given the low absorption of chromium compounds by the oral route, the major pathway of excretion after oral exposure is through the faeces. Urinary excretion of inorganic chromium VI compounds has been reported to be higher than that of inorganic chromium III compounds, indicating a higher absorption.

An amount of 89.4% of an acute, oral dose of chromium VI as sodium chromate, was retrieved in a 6-day fecal collection, whereas 2.1% of the dose was found in a 24-hour urine collection. In subjects drinking increasing doses of chromium (0.001-0.1 mg chromium VI/kg/day as potassium chromate in water for 3 days), increasing doses ranging from < 2 to 8% of the dose were excreted in the urine.

After ingestion of 0.05 mg chromium VI/kg, approximately 76-82% of the 14-day total amount of chromium in the urine was excreted within the first 4 days (mean peak concentration 209 µg chromium/g creatinine; range 29-585 µg chromium/g creatinine). The average urinary excretion half-life for four of the volunteers was 39 hours at this dose. All subjects had returned to background concentrations (0.5-2.0 µg chromium/g creatinine) by 14 days post-dosing.

Uptake of potassium dichromate was determined in a man who was given 0.8 mg of chromium VI in drinking water 5 times each day for 17 days. Steady-state

concentrations of chromium in blood were attained after 7 days and a plasma elimination half-life of 36 hours was estimated.

Inhalation

In workers exposed to chromium VI as chromium trioxide in the chrome plating industry, an association was observed between exposure concentrations and post-shift urinary chromium levels. Workers mainly exposed to chromium VI compounds have been reported to have higher urinary chromium levels than workers primarily exposed to chromium III compounds.

Dermal

Fourteen days after application of a salve containing potassium chromate VI, which resulted in skin necrosis and sloughing at the application site, chromium was found at 8 mg/L in the urine and 0.61 mg/100 g in the faeces of one individual.

A slight increase (over background levels) in urinary chromium levels was observed in four subjects submersed in a tub of chlorinated water containing 22 mg chromium VI/L as potassium dichromate for 3 hours. For three of the four subjects, the increase in urinary chromium excretion was <1 µg/day over the 5-day collection period.

4.4 Summary and discussion on toxicokinetics

There is a reasonably good database available on the toxicokinetics of the chromium VI compounds, although there are relatively few human data. For all routes, it is generally assumed that chromium VI is better absorbed than chromium III. It is further assumed that absorption is higher for soluble chromium compounds than for insoluble.

Chromium VI is poorly absorbed after oral administration, with absorption fractions reported from 1-3% (in rats and mice) to 18% in guinea pigs. For humans, gastrointestinal absorption is estimated to be <10% of the administered dose. Amounts of 20-30% absorption have been reported in animals after inhalation or intratracheal instillation. Systemic exposure after inhalation exposure has been reported in humans, but no quantitative studies are available. Dermal absorption of highly water-soluble chromium VI compounds varied between 1-4% in guinea pigs. Chromium VI can penetrate human skin to some extent, especially if the skin is damaged.

Results of animal testing indicate that chromium can remain in the lungs for several weeks after inhalation exposure and also becomes bound to haemoglobin in erythrocytes for the lifespan of the cells. In the body, chromium VI is reduced to chromium III, for example by glutathione. The observed distribution appears widespread, even after a single dose, and includes transfer of across the placenta and accumulation in foetal tissue.

Ingested chromium is excreted primarily in the faeces, whereas absorbed chromium appears to be primarily excreted in the urine. Repeated exposure leads to accumulation of chromium in several tissues, particularly the spleen because of uptake of senescent erythrocytes.

The available data indicate that generally the chromium VI compounds are likely to behave in a similar manner in respect of toxicokinetics, and that the kinetic behaviour of these substances would be similar in those species studied, including humans.

Chapter 5

Toxicity for reproduction

5.1 Effects on fertility

5.1.1 *Non-human information*

Animal fertility studies are summarised in the next table and described in the following text. Changes and differences mentioned are statistically significant unless stated otherwise.

Table 1 Summary table of fertility studies in animals.

authors	species	experimental period/design	dose/route	general toxicity	effects on reproduction
Glaser et al., 1984	Rat, males and female, Wistar; n = 8-11	130 days per generation for 3 generations (time/d not indicated)	0 or 200 µg Cr/m^3 as sodium dichromate (0 or 0.2 mg Cr/kg bw/d (assuming a breathing volume of 200 ml/min))/inhalation	No clinical signs; no effect on food- and water consumption NOAEL=0.3 mg Cr/kg bw/d	No effect on reproduction NOAEL= 0.3 mg Cr/kg bw/d
Subramanian et al., 2006	Monkey; Macaca radiata; n = not specified; males; 7-9 kg	During 6 months; highest dose 6 m-recovery; monthly semen sampling	0, 50, 100, 200 or 400 mg Cr as potassium dichromate/L drinking water (0, 3, 6, 11 or 23 mg Cr/kg bw/d)/oral	Not reported NOAEL=23 mg Cr/kg bw/d	6 mg/kg bw and higher: decreased sperm count, decreased sperm forward motility, decreased activity of superoxide dismutase and catalase, and decreased concentration of reduced glutathione in both seminal plasma and sperm NOAEL=3 mg Cr/kg bw/d
Aruldhas et al., 2005	Monkey; Macaca radiata; n = 3/dose; males; 7-8 kg	During 6 months; 3/dose 6 m-recovery; ultrastructrural and biochemical analysis of testis	0, 100, 200 or 400 mg Cr as potassium dichromate/L drinking water (0, 6, 11 or 23 mg Cr/kg bw/d)/oral	Not reported NOAEL= 23 mg Cr/kg bw/d	All doses: decreased testis weight; disrupted spermatogenesis leading to accumulation of prematurely released spermatocytes, spermatids and uni- and multinucleate giant cells in the lumen of seminiferous tubules LOAEL=6 mg Cr/kg bw/d

Li et al., 2001	Rat; Wistar; males; n = 8-11	For 6 days; sacrificed after 6 weeks	0, 10 or 20 mg chromium trioxide/kg bw/d by gavage (0, 5 or 10 mg Cr/kg bw/d)/ oral	Not reported NOAEL=10 mg Cr/kg bw/d	Both levels: decreased sperm count increased sperm abnormality; decreased diameter of seminiferous tubules and germ cell rearrangement within tubules LOAEL=5 mg Cr/kg bw/d
Kanojia et al., 1996	Rat; Swiss albino; females; n = 10	During 20 days (one folliculogenesis cycle) prior to gestation	0, 250, 500 or 750 mg Cr/L as potassium dichromate in drinking water (0, 31, 59 and 76 mg/kg bw/d)/oral	No mortality or change in clinical signs; decreased water consumption; decreased body weight gain in all doses LOAEL=31 mg Cr/kg bw/d	All doses: reduced mating index and fertility index; persistent diestrus phase At the two highest doses: increase in estrus cycle, decrease in the number of corpora lutea and implantations LOAEL=31 mg Cr/kg bw/d
Kanojia et al., 1998	Rat; Druckrey; females; n = 10	during 3 months prior to gestation	0, 250, 500 or 750 mg Cr/L as potassium dichromate in drinking water (0, 45, 89 and 124 mg/kg bw/d); drinking water	89 and 124 mg/kg bw: 15% and 10% of animals, resp., died within 14 days during treatment NOAEL=45 mg Cr/kg bw/d	At the end of treatment, all exposed animals showed persistent dioestrous phase; oestrous cycle became regular again within 15-20 days and they began to mate LOAEL=45 mg Cr/kg bw/d
Chowdhury and Mitra, 1995	Rat; Charles Foster; males; n = 10	during 90 days	rats 0, 20, 40 or 60 mg/kg bw/d of sodium dichromate by gavage (0, 8, 16 or 24 mg Cr/kg bw/d)/oral	16 and 24 mg: decreased body weight gain NOAEL=8 mg Cr/kg bw/d	All doses: decreased testicular protein and serum testosterone, decreased activity of 3β-Δ^5-hydroxysteroid dehydrogenase 16 and 24 mg/kg: decreased testis weight, number of resting spermatocytes (high dose), number of pachytene spermatocytes and stage-7 spermatids, population of Leydig cells, seminiferous tubular diameter, and DNA and RNA and succinic dehydrogenase; increased testicular cholesterol Testicular ascorbic acid: increased at 8 and 16 mg/kg and decreased at 24 mg/kg LOAEL=8 mg Cr/kg bw/d
NTP, 1996a	Rat; Sprague-Dawley; n = 24 males and 48 females	Daily for 9 weeks; 8 weeks recovery; necropsy after 3, 6, 9 and 17 weeks	0, 15, 50, 100, and 400 mg potassium dichromate/kg diet (0, 0.4, 1, 2 and 9 mg Cr/ kg bw/d)(mean doses of males and females combined)/oral	Mean corpuscular volume and haemoglobin values in high-dose group were decreased for both males and females (reversible), no other treatment related findings were observed NOAEL=2 mg Cr/kg bw/d	No effect observed on testicular cell counts for Sertoli nuclei and preleptotene spermatocyte in Stage X and XI, or with microscopy of ovaries NOAEL=9 mg Cr/kg bw/d
NTP, 1996b	Mouse, BALB/c, n = 24 males and 48 females	Daily for 9 weeks; 8 weeks recovery; necropsy after 3, 6, 9 and 17 weeks	0, 15, 50, 100, and 400 mg potassium dichromate/kg diet (0, 1, 5, 10 and 41 mg Cr/ kg bw/d) (mean doses of males and females combined)/oral	Decrease in mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) in males and females in the highest dose group. Cytoplasmic vacuolisation in hepatocytes was noted in the 5, 10, and 41 mg/kg bw/d in males and females. High dose group: loss of body weight (10%) for females, decrease of absolute liver weight for males and females (10%) NOAEL=1 mg Cr/kg bw/d	Testicular cell counts for Sertoli nuclei and preleptotene spermatocyte counts in Stage X and XI did not reveal any differences compared to controls NOAEL=41 mg Cr/kg bw/d

NTP, 1997	Mouse, BALB/c, n = 10-20/sex	F_0: 1 week prior to mating and 12 week mating period (continuous breeding phase); F_1: after weaning until 74 +/- 10 days of age continued with 1 week mating period	0, 100, 200, and 400 mg/potassium/kg diet dichromate (F0: 0, 7, 14 and 30 mg Cr/kg bw/d; F_1: 0, 8, 16 and 37 mg Cr/kg bw/d)/ oral	Slight decrease in body weights, increased feed consumption, and small decreases in MCV, MCH, and haemoglobin (F1 animals only). NOAEL F0=30 mg Cr/kg bw/d LOAEL F1=8 mg Cr/kg bw/d	F_0: no effect on pregnancy index and number of litters per pair; no effect on epididymal sperm density, percent abnormal sperm and testicular sperm count; no effect on gonadal organ weights NOAEL F0=30 mg Cr/kg bw/d F_1: no effect on mating index, pregnancy index, fertility index or gestation length, oestrous cycle and sperm parameters NOAEL F1=37 mg Cr/kg bw/d
NTP, 2007	Mouse; B6C3F1, BALB/c, and am3-C57BL/ 6 males; n = 5-10	For 3 m; sacrificed 2w later	0, 62.5, 125, or 250 mg sodium dichromate dihydrate/ L in drinking water (3, 5 or 9 mg Cr/kg bw, as reported by authors)/ oral	Decreased body weight in: -5 and 9 mg/kg bw/d (B6C3F1 and BALB/C) -all treatment groups (C5BL/6); conc. dep. decrease in mean red cell volume and haemoglobin (all strains); Increase in erythrocyte counts (B6C3F1 and BALB/C) LOAEL=3 mg/kg bw/d	No effects on sperm parameters reported. NOAEL= 9 mg/kg bw/d
Trivedi et al., 1989	Mouse, females, Swiss; n = 10-13	entire gestation period, sacrifice GD 19	0, 250, 500 or 1000 mg potassium dichromate/L in drinking water (0, 59, 120 and 234 mg Cr/kg bw/d)	Reduction in body weight gain NOAEL=59 mg Cr/kg bw/d	Pre-implantation loss at 120 and 234 mg/kg NOAEL=59 mg Cr/kg bw/d
Zahid et al., 1990	Mouse; males, Balb-C Swiss, n =7	from weaning during 7 weeks; testis and epididymis	0, 100, 200 or 400 mg potassium dichromate/kg diet (0, 11, 21 or 46 mg Cr/kg bw/d)/oral	Body weight gain and food consumption not affected NOAEL=46 mg Cr/kg bw/d	All doses: slight, but significantly increased number of seminiferous tubules degenerated; increased number of intact tubules without spermatogonia; decreased number of spermatogonia/tubules and increased number of resting and pachytene spermatocytes 21 and 46 mg Cr/kg bw/d: decreased sperm count and increased percentage of abnormal sperms LOAEL=11 mg Cr/kg bw/d
Murthy et al., 1996	Mouse, Swiss, females, n = 10/subgroup	During 20 days; subgroups: 1. follicle counting; 2. ova counting; 3. measuring estrous cycle	0, 250, 500 or 750 mg Cr/L as potassium dichromate in drinking water (0, 54, 109 or 163 mg Cr/kg bw/d)/oral	Not reported NOAEL=163 mg Cr/kg bw/d	Decrease in number of follicles at different stages of maturation at all doses; 109 and 163 mg/kg: decreased number of ova; proliferated, dilated, and congested blood vessels, pyknotic nuclei in follicular cells, and atretic follicles; 163 mg/kg: increased length of oestrous cycle LOAEL=54 mg Cr/kg bw/d
Murthy et al., 1996	Mouse, Swiss, females, n = 10/subgroup	During 90 days; electron microscopic study of ovaries	0, 0.05, 0.5 or 5 mg Cr/L as potassium dichromate in drinking water (0, 0.01, 0.1 or 1 mg/kg bw/d)/oral	Not reported NOAEL=1 mg Cr/kg bw/d	1 mg/kg: disintegrated cell membranes of two layered follicular cells and altered villiform mitochondria in thecal cells NOAEL=0.1 mg Cr/kg bw/d

Elbetieha and Al-Hamood, 1997	Mouse, males, Swiss; n = 9-20	12 weeks prior to mating; body weight and rel. organ weight only studied at 2000 and 5000 mg/L	0, 1000, 2000, 4000 or 5000 mg potassium dichromate/L drinking water (0, 74, 148, 296, 370 mg Cr/kg bw/d)/ oral	148 and 370 mg: decreased body weight; NOAEL=74 mg Cr/kg bw/d	148 mgc: increased relative testis weight; reduced number of implantations and viable foetuses 296 mg/kg: decreased number of implantations and viable foetuses 370 mg/kg: increased relative testis weight; decreased weight of seminal vesicles and preputial gland; increased total number of resorptions and dead foetuses NOAEL=74 mg Cr/kg bw/d
	Mouse, females, Swiss, n = 11-18	12 weeks prior to mating	0, 2000 or 5000 mg potassium dichromate/L drinking water (0, 153 or 383 mg Cr/kg bw/d)	NOAEL=383 mg Cr/kg bw/d	383 mg/kg: increased ovary weight; in both groups no effect on fertility or on uterine weight; decreased number of implantations and live foetuses NOAEL=153 mg Cr/kg bw/d
Yousef et al., 2006	Rabbit; New Zealand White; males; n = 6	Daily during 10 w; semen collected weekly; blood every other week; sacrificed after 10 w	0 or 4 mg Cr/kg bw/d as potassium dichromate by gavage/oral	No adverse clinical signs; no effect on food consumption; decrease body weight ($p<0.05$); NOAEL=4 mg Cr/kg bw/d	Decrease in relative weight of testes and epididymis and plasma testosterone concentration; decrease in various sperm parameters, initial fructose and libido (by decreasing reaction time); increase in percentage of dead sperm and initial pH; no effect on semen ejaculate volume LOAEL=4 mg Cr/kg bw/d
Ernst, 1990	Rat, males, Wistar, n = 8	5 consecutive days, sacrifice at 7 or 60 days after the last dose	0, 1, 2 or 4 mg Cr/kg bw/d as sodium chromate/ip	Decreased body weight gain at all doses; no mortality or clinical signs; no effect on food and water intake LOAEL=1 mg Cr/kg bw/d	1, 2 and 4 mg/kg: no effect 7 days after last administration 60 d: dose dependent reduction in relative testis weight; increased number of atrophic seminiferous tubules with loss of spermiogenic epithelium; decreased number of epididymal spermatozoa; additionally at 4 mg/kg: testis, loss of cellular organisation; complete degeneration of seminiferous tubules and atrophy of Leydig cells LOAEL=1 mg Cr/kg bw/d
Ernst and Bonde, 1992	Rat, Wistar, males, n = 10	5 d/w for 8 w; recovery group 8 w	0 or 0.5 mg Cr/kg bw/d as sodium chromate/ip	No clinical signs; no effect on body weight, food- and water consumption NOAEL=0.5 mg Cr/kg bw/d	Decreased sperm motility and serum testosterone (T), increase in follicle stimulating hormone (FSH) and luteinizing hormone (LH); after recovery period sperm motility, T and FSH returned to normal levels, LH level remained decreased LOAEL=0.5 mg/kg bw/d
Behari et al., 1978	Rabbit, male, ITRC colony, n = 8-10	daily injection for 3 or 6 w, sacrifice 72 h after last injection	0 or 2 mg/kg bw/d potassium dichromate (0 or 0.7 mg Cr/kg bw/d)/ ip	No mortality or morbidity NOAEL=0.7 mg Cr/kg bw/d	0.7 mg/kg (3w): testis, decreased level of in succinic dehydrogenase and mild oedema of interstitial tissue 0.7 mg/kg (6w): testis, decreased level of succinic dehydrogenase and adenosine triphosphate, marked oedema of interstitial tissue and no spermatocytes in seminiferous tubules LOAEL=0.7 mg Cr/kg bw/d

Abbrevations used: GD = gestation day; iv = intravenous; ip = intraperitoneal; PND = post-natal day; P1/2/3 = first/second/third parental generation. If applicable, doses were converted to amount of chromium based on molecular weight. Conversion of doses to mg/kg bw/d, if applicable, was performed using the general conversion factor for body weight to food or water consumption (assuming dry diet), and the default values for body weights (if not specified by the authors) as specified in the Technical Guidance on Risk Assessment, Part 1, Appendix 6. The Committee notes that this conversion does not specifically take into account pregnant animals.

Inhalation

Glaser et al. studied the effects of continuous inhalation of 200 µg Cr/m^3 as sodium dichromate on the reproduction and development of offspring in 3 generations of Wistar rats (n = 8-11).[9] Pregnant F0 females were exposed directly after giving birth together with their offspring till day 25 and then sacrificed. Their offspring was further continuously exposed. No effects on reproductive parameters were observed, including number of pregnancies, implantations and foetuses. From generation to generation, a decrease in serum-immunoglobulin level and increased relative lung weight was noted.

The Committee notes that the exposure concentrations of 200 µg chromium VI/m^3 applied in this study correspond to an equivalent oral dose of 0.3 mg Cr/kg bw, respectively (assuming a breathing volume of 200 mL/min).

Oral

Subramanian et al. collected monthly semen samples from adult monkeys (Macaca radiata; number not specified) exposed to 0, 50, 100, 200 or 400 mg chromium VI/L (equivalent to 0, 3, 6, 11 or 23 mg Cr/kg bw/d) as potassium dichromate for 6 months via drinking water.[10] No effects were observed at 50 mg/L. A reduction of sperm concentration and motility was observed at 100, 200 and 400 mg/L. Activities of superoxide dismutase and catalase, and glutathione concentration were decreased and hydrogen peroxide concentration increased in seminal plasma and sperm. All the effects were dependent on dose and duration: the higher the dose the earlier the effect was observed and the increase/decrease was larger with time exposed. All observed effects were restored with 3-6 months of a chromium-free exposure period. Vitamin C co-administration of 0.5, 1 or 2 g/L prevented the above effects.

In an earlier histological investigation, Aruldhas et al. had shown that exposure of Macaca monkeys (n = 3/group) to 100, 200 or 400 mg chromiumVI/L (equivalent to 0, 6, 11 or 23 mg Cr/kg bw/d) for 6 months via the drinking water resulted in decreased testis weight, disrupted spermatogenesis, leading to accumulation of prematurely released spermatocytes, spermatids and uni- and multinucleate giant cells in the lumen of seminiferous tubules.[11] Withdrawal of chromium treatment for 6 months normalized plasma chromium concentrations, spermatogenesis and the status of pro- and antioxidants in the testis.

Li et al.[12] treated male Wistar rats (n = 8-11/group) with 0, 10 or 20 mg chromium trioxide/kg 'by oral feeding' (it is not clear whether this dose was administered by gavage or via the diet; equivalent to 5 or 10 mg Cr/kg bw/d by gavage) for 6 days. Rats were sacrificed after 6 weeks. Sperm count was decreased and sperm abnormality increased in both exposed groups. Histopathological evaluation of the testes showed a decreased diameter of the seminiferous tubules and germ cell rearrangement within the tubules.

Table 2 Summary of sperm analysis data of Li et al., 2001.

	Control	5 mg/kg bw/d	10 mg/kg bw/d
Sperm count × 10^6/g epididymis	87.40 ± 3.85	21.4 ± 1.2*	17.48 ± 1.04*
% abnormal sperm	2.75 ± 0.06	6.68 ± 0.32**	7.6 ± 0.15**

* $p<0.05$; ** $p<0.01$

Kanojia et al. exposed Swiss albino rats (n = 10/group) with levels of 0, 250, 500 or 750 mg CrVI/L in the drinking water (equivalent to 31, 59 or 76 mg Cr/kg bw/d) for 20 days prior to gestation. Dams were sacrificed on gestation day 19. Kanojia et al. also investigated these exposure levels in drinking water in female Druckrey rats (corresponding to 45, 89 or 124 mg Cr/kg bw/d), for 3 months prior to gestation.[13,14] A reduced body weight gain was noted for both strains. All CrVI rats from both strains showed a reduced mating index, a reduced fertility index, an increased oestrous cycle length and a reduction in the number of corpora lutea and number of implantations. The 3-month exposure of Druckrey

Table 3 Summary of fertility effects reported by Kanojia et al., 1996, 1998.

Swiss albino rats (Kanojia et al. 1996)	Control	31 mg/kg bw/d	59 mg/kg bw/d	76 mg/kg bw/d
Maternal weight gain (g)	70.50 ± 5.19	65.02 ± 3.17[a]	60.92 ± 2.13[ab]	55.5 ± 3.01[abc]
Mating index (%)	100	80	70	40
Fertility index (%)	96	75	57	31
Number of corpora lutea	10.02 ± 0.91	9.81 ± 0.95	7.13 ± 0.61[ab]	4.43 ± 0.50[abc]
Number of implantations	9.51 ± 0.96	9.61 ± 0.83	5.91 ± 0.39[ab]	2.27 ± 0.36 [abc]
Pre-implantation loss	5.08 ± 0.65	2.03 ± 0.31	17.11 ± 2.13[ab]	48.75 ± 5.81[abc]
Druckrey rats (Kanojia et al. 1998)	Control	54 mg/kg bw/d	89 mg/kg bw/d	124 mg/kg bw/d
Maternal weight gain (g)	69.16 ± 3.66	61.66 ± 4.23	57.50 ± 2.82[a]	54.16 ± 3.11 [a]
Mating index (%)	100	70	60	40
Fertility index (%)	98	67	58	50
Number of corpora lutea	10.50 ± 0.56	9.56 ± 0.73	8.33 ± 0.49[a]	7.13 ± 0.60[ab]
Number of implantations	9.83 ± 0.92	8.32 ± 0.82	6.86 ± 0.42[a]	5.70 ± 0.72[ab]
Pre-implantation loss (%)	6.38 ± 0.63	12.97 ± 0.75[a]	17.64 ± 0.45[ab]	20.05 ± 0.45[abc]

Values represent mean ± S.E. of 10 rats in each group. The significance of the difference among various groups was evaluated by applying one-way ANOVA ($p<0.05$). Comparison between two groups: [a]vs control, [b]vs 31 mg/kg bw, [c]vs 59 mg kg/bw.

rats resulted in a persistent diestrous phase. The oestrous cycle became regular again within 15-20 days when animals began to mate. This recovery period was not investigated after 20-day exposure where they were allowed to mate for only one night.

Chowdhury and Mitra[15] administered male Charles Foster rats (n = 10/group) 0, 20, 40 or 60 mg/kg bw/d sodium dichromate (equivalent to 0, 8, 16 or 24 mg Cr/kg bw/d) for 90 days by gavage. Body weight gain was lower at 16 and 24 mg/kg bw. Testis weight was decreased at these doses compared to controls. Spermatogenesis was inhibited as shown by a decreased count of resting spermatocytes (high dose), pachytene spermatocytes and stage-7 spermatids at 40 and 60 mg/kg bw/d. At these dose levels also the population of Leydig cells, seminiferous tubular diameter, DNA and RNA were reduced compared to control. Testicular protein and serum testosterone were reduced at all dose levels, and testicular cholesterol was increased at 40 and 60 mg/kg bw/d indicating steroidogenic impairment, which was confirmed by inhibition of 3β-Δ^5-hydroxysteroid dehydrogenase at all dose levels.

In a study of the National Toxicology Program (NTP) 0, 15, 50, 100 or 400 mg potassium dichromate/kg diet was administered to Sprague Dawley rats (24 males and 48 females/group) (equivalent to mean doses of 0, 0.4, 1, 2, or 9 mg Cr/kg bw/d for males and females combined) for 9 weeks with a recovery period of 8 weeks.[16] Interim necropsies were performed after 3, 6 and 9 weeks of administration and a terminal necropsy after week 17. No treatment-related findings were observed on body weight, water and food consumption, organ weights, macroscopy, and microscopy of the liver and kidneys, and haematological parameters, except for a decrease in mean corpuscular volume and mean corpuscular haemoglobin at the highest dose in both sexes, which had disappeared after the recovery period. Testicular cell counts for Sertoli nuclei and preleptotene spermatocyte counts investigated in Stage X and XI did not reveal any differences between the treated groups and control, nor were any treatment related changes in microscopical lesions found in ovaries.

Table 4 Summary of testicular cell counts in rats reported by NTP, 1996.

Weeks of interim kill	Control	9 mg Cr/kg bw/d
3	2.401 ± 0.274 (6)	2.800 ± 0.235 (6)
6	3.053 ± 0.190 (6)	2.710 ± 0.101 (6)
9	2.825 ± 0.486 (6)	2.891 ± 0.380 (6)

Note: Ratio = number of germ cells / number of Sertoli cells.

The same study design was used to expose Balb/C mice to potassium (equivalent doses of 0, 1, 5, 10 and 41 mg Cr/kg bw/d, for males and females combined).[17] No treatment-related findings were observed on clinical signs, and macroscopic evaluation. Body weight of males in the highest dose group was decreased during the dosing period. Food consumption was generally increased in both sexes, especially at 41 mg/kg bw/d. Water consumption was decreased (8-9%) in the highest dose group in both sexes during the first half of the dosing period. Mean corpuscular volume and mean corpuscular haemoglobin at 41 mg/kg bw/d were decreased in both sexes, and returned to normal in both sexes during recovery. Cytoplasmic vacuolization in hepatocytes was noted at 1 mg/kg bw/d in females and in both sexes at 5 and 41 mg/kg bw/d. Testicular cell counts for Sertoli nuclei and preleptotene spermatocyte counts in Stage X and XI did not reveal any difference compared to controls.

Table 5 Summary of testicular cell counts in mice reported by NTP, 1996.

Weeks of interim kill	Control	41 mg Cr/kg bw/d
3	5.79 ± 0.62 (5)	5.71 ± 0.46 (6)
6	6.00 ± 1.06 (6)	5.73 ± 0.49 (6)
9	5.44 ± 0.68 (6)	5.37 ± 0.79 (6)

Note: Ratio = number of germ cells / number of Sertoli cells.

Subsequently, the NTP investigated reproduction and sperm parameters in a continuous breeding study with BALB/c mice dosed via the diet with 0, 100, 200 and 400 mg/kg diet potassium dichromate.[18] F_0 animals (20 mice/sex/group) received 0, 7, 14, or 30 mg Cr/kg bw/d for 7 days pre-mating and 12 weeks during cohabitation. Litters produced during cohabitation were counted and weighed by sex and sacrificed on postnatal day 1. F_0 pairs were separated and any litter born after the continuous breeding phase were reared and weaned on postnatal day 21. F_0 animals were sacrificed and examined. In each dose group 20 weanlings/sex/group were selected, received diets containing 0, 100, 200 or 400 mg/kg diet potassium dichromate (equivalent to 8, 16 and 37 mg/kg bw/d) and were cohabitated on postnatal day 74 +/-10 for 7 days and separated. Offspring was counted and weighed by sex on postnatal day 1. At necropsy, F_1 terminal body weight and organ weight were obtained, sperm analysis and macroscopy performed and histopathology of liver and kidney.

F_0 fertility was not affected. No treatment-related findings on pregnancy index and number of litters per pair were observed. No effect on epididymal sperm density, percent abnormal sperm and testicular sperm count was noted. No effect on gonadal organ weights was seen. In week 14 and during lactation and at necropsy, body weight of F_0 dams at the two highest doses was decreased at

delivery of one or more litters. No treatment-related effect was observed on mortality, body weight of sires, clinical signs, food consumption, macroscopy or microscopy. Liver weight was decreased at the highest dose level in both sexes.

Also in the F_1-generation no effect was found on mating index, pregnancy index, fertility index or gestation length. The oestrous cycle and sperm parameters were not affected. Again no treatment-related mortality, clinical signs, gross or microscopic lesions were observed in F_1 animals. Adult body weights were decreased at the highest dose level (body weights were already decreased at weaning; see developmental toxicity), whereas food consumption was increased. At necropsy, mean corpuscular volume was decreased at the two highest dose groups in males and all dose groups in females. Mean corpuscular haemoglobin was decreased in males and mean haemoglobin was decreased in females in the highest dose group. To further evaluate the decreased mean corpuscular volume, erythrocyte morphology was investigated and found to be not affected. Developmental details are described under 'developmental toxicity'.

Table 6 Summary of pregnancy index[a] of breeding pairs reported by NTP, 1997.

Litter	Control	7 mg Cr/kg bw/d	14 mg Cr/kg bw/d	30 mg Cr/kg bw/d	Trend[b]
1	20/20 (100)	20/20 (100)	20/20 (100)	20/20 (100)	p=0.500
2	20/20 (100)	20/20 (100)	20/20 (100)	20/20 (100)	p=0.500
3	19/20 (95)	20/20 (100)	17/20 (85)	19/20 (95)	p=0.241
4	15/20 (75)	18/20 (90)	13/20 (65)	14/20 (70)	p=0.147
5[c]	2/20 (10)	3/20 (15)	4/20 (20)	2/20 (10)	p=0.392

[a] Number of females delivering / number of cohabiting pairs (percent pregnant).

[b] P-value, from Cochran-Armitage trend test for decrease in pregnancies. Each dose group is compared to the control group with a chi-square test (*=p<0.05).

[c] The cohabitation period for this study was reduced from the standard 4 weeks to 12 weeks so only four litters would be produced during this study. However, eleven animals produced five litters.

Table 7 Summary of number of live pups[a] reported by NTP, 1997.

Litter	Control	7 mg Cr/kg bw/d	14 mg Cr/kg bw/d	30 mg Cr/kg bw/d	Trend[b]
1	5.2 ± 0.5 (20)	6.6 ± 0.5 (20)	5.3 ± 0.5 (20)	4.5 ± 0.5 (20)	p=0.151
2	6.0 ± 0.5 (20)	6.1 ± 0.6 (20)	6.4 ± 0.7 (20)	5.9 ± 0.7 (20)	p=0.795
3	7.7 ± 0.5 (19)	7.6 ± 0.5 (20)	7.1 ± 0.9 (17)	6.5 ± 0.4 (10)	p=0.127
4	7.6 ± 0.9 (15)	6.4 ± 0.5 (18)	7.4 ± 0.7 (13)	6.7 ± 0.9 (14)	p=0.680
5	9.0 ± 1.0 (2)	6.3 ± 2.0 (3)	9.0 ± 0.9 (4)	5.5 ± 1.5 (2)	p=0.562
Combined[c]	6.6 ± 0.3 (20)	6.7 ± 0.2 (20)	6.5 ± 0.3 (20)	5.9 ± 0.3 (20)	p=0.081

[a] Mean ± standard error (number of pairs producing live pups).

[b] Each dose group is compared to the control group with Shirley's test when a trend is present (p<0.01 from Jonckheere's trend test), otherwise Dunn's test is applied (*=p<0.05).

[c] Mean of the average number of live pups produced by each fertile pair ± standard error (number of fertile pairs).

Table 8 Summary of sperm analysis data[a] reported by NTP, 1997.

Endpoint	Control	7 mg Cr/kg bw/d	14 mg Cr/kg bw/d	30 mg Cr/kg bw/d	Trend[b]
Computed assisted sperm analysis					
Total cells analyzed	204.00 ± 5.26 (20)	-	-	201 ± 5.59 (10)	p=0.201
Sperm motility, % motile	75.89 ± 1.09 (20)	-	-	73.00 ± 1.45 (10)	p=0.065
Velocity (µm/s)	121.45 ± 1.95 (20)			119.48 ± 2.34 (10)	p=0.538
Linearity	6.07 ± 0.11 (20)	-	-	6.04 ± 0.15 (10)	p=0.628
ALH max (µm)	6.40 ± 0.17 (20)	-	-	6.36 ± 0.26 (10)	p=0.725
ALH mean (µm)	5.44 ± 0.15 (20)	-	-	5.40 ± 0.20 (10)	p=0.775
Beat/cross frequency (hz/sec)	13.98 ± 0.27 (20)	-	-	13.92 ± 0.29 (10)	p=0.660
Average radius (µm)	33.72 ± 1.18 (20)	-	-	34.56 ± 1.92 (10)	p=0.895
Circular cells	22.70 ± 1.21 (20)	-	-	22.70 ± 1.98 (10)	p=0.895
Circular over all cells (%)	11.16 ± 0.54 (54)	-	-	11.36 ± 0.98 (10)	p=0.860
Circular over motile cells (%)	14.74 ± 0.72 (20)	-	-	15.57 ± 1.32 (10)	p=0.524
Epididymal sperm density[c]	582.66 ± 50.31 (19)	530.68 ± 79.36 (10)		540.94 ± 44.29 (10)	p=0.343
Epid. Sperm morphology, % abnormal[d]	26.93 ± 0.69 (20)	-		24.65 ± 1.55 (10)	p=0.098
Spermatids/mg testis[e]	174.46 ± 7.06 (20)	193.55 ± 713.79 (10)	212.36 ± 12.06 (10)	184.79 ± 6.39 (10)	p=0.122
Total spermatids/testis[f]	15.30 ± 0.84 (20)	18.11 ± 1.30 (10)	17.55 ± 1.04 (10)	15.47 ± 1.16 (10)	p=0.516

[a] Endpoint mean ± standard error (number of animals).

[b] When only two dose groups are present, the dosed group is compared to the control group using wilcoxon's test. However, when more than two groups are present, each dose group is compared with the control group by Shirley's test if $p<0.01$ from Jonckheere's trend test; otherwise Dunn's test is applied (*=$p<0.05$).

[c] Sperm density, expressed as 1,000 sperm per mg right caudal tissue.

[d] Dose group means and standard errors are computed only from samples with at least 200 sperm.

[e] Spermatid heads per mg testis (x 1,000) = (total number of spermatid heads per right testis) / (right testis weight in mg).

[f] Total number of spermatid heads (x 1,000,000) = (average number of spermatid heads x 2.5 x 100) / 0.0001.

Note: Computer assisted sperm analysis and sperm morphology were not required for the low and mid-dose groups.

The NTP also conducted two more recent studies in rats and mice.[5] In the first study, groups of male and female F344/N rats and B6C3F1 mice (n = 10) were given drinking water containing 0, 62.5, 125, 250, 500, or 1,000 mg sodium dichromate dihydrate/L for 3 months (equivalent to approximately 2, 4, 6, 11, and 21 mg hexavalent chromium/kg body weight per day to rats and 3, 5, 9, 16, and 28 mg/kg per day to mice). No histological findings on reproductive organs were reported.

In the second study, sodium dichromate dihydrate was administered in drinking water to male B6C3F1 (n = 10), BALB/c (n = 10), and am3-C57BL/6 (n = 5) mice for 3 months at exposure concentrations of 0, 62.5, 125, or 250 mg/L

(equivalent to 3, 5, or 9 mg Cr/kg bw/d) to B6C3F1, BALB/c, and am3-C57BL/6 mice. No effects on sperm count and motility were observed.

Table 9 Summary of sperm analysis data[a] reported by NTP, 2007.

	Control	3 mg Cr/kg bw/d	5 mg Cr/kg bw/d	9 mg Cr/kg bw/d
B6C3F1				
Weights (g)				
Necropsy bw	46.8 ± 0.8	46.7 ± 0.6	42.7 ± 1.0**	36.4 ± 0.5**
L Cauda epididymis	0.0142 ± 0.0005	0.0140 ± 0.0005	0.0150 ± 0.0004	0.0146 ± 0.0005
L. Epididymis	0.0457 ± 0.0008	0.0446 ± 0.0010	0.0439 ± 0.0006	0.0430 ± 0.0009
L. Testis	0.1192 ± 0.0020	0.1230 ± 0.0016	0.1176 ± 0.0010	0.1183 ± 0.0016
Spermatid measurements				
Spermatid heads (10^7/g testis)	19.52 ± 0.42	20.31 ± 0.68	20.08 ± 0.62	20.72 ± 0.33
Spermatid heads (10^7/testis)	2.19 ± 0.07	2.33 ± 0.07	2.19 ± 0.07	2.32 ± 0.05
Epididymal spermatozoal measurements				
Sperm heads (10^7/g cauda testis)	87.02 ± 8.41	103.26 ± 6.69	87.04 ± 7.05	102.93 ± 11.18
Sperm heads (10^7/cauda testis)	1.21 ± 0.09	1.45 ± 0.11	1.26 ± 0.11	1.48 ± 0.14
Sperm motility (%)	87.67 ± 0.89	88.05 ± 0.65	88.61 ± 0.83	87.63 ± 1.19
BALB/c				
Weights (g)				
Necropsy bw	28.8 ± 0.5	28.2 ± 0.6	27.0 ± 0.3**	25.7 ± 0.4**
L Cauda epididymis	0.0101 ± 0.0006	0.0109 ± 0.0003	0.0097 ± 0.0004	0.0101 ± 0.0005
L. Epididymis	0.0351 ± 0.0006	0.0355 ± 0.0009	0.0349 ± 0.0006	0.0333 ± 0.0007
L. Testis	0.0973 ± 0.0029	0.0962 ± 0.0021	0.0928 ± 0.0017	0.0929 ± 0.0022
Spermatid measurements				
Spermatid heads (10^7/g testis)	14.07 ± 1.05	14.90 ± 0.45	14.72 ± 0.29	15.87 ± 0.57
Spermatid heads (10^7/testis)	1.29 ± 0.12	1.35 ± 0.06	1.31 ± 0.04	1.35 ± 0.06
Epididymal spermatozoal measurements				
Sperm heads (10^7/g cauda testis)	111.07 ± 7.40	110.40 ± 5.50	120.70 ± 5.70	114.90 ± 11.60
Sperm heads (10^7/cauda testis)	1.10 ± 0.03	1.20 ± 0.05	1.17 ± 0.07	1.15 ± 0.11
Sperm motility (%)	91.42 ± 0.40	89.75 ± 0.37	90.54 ± 0.43	91.28 ± 0.46
Am3-C57BL/6				
Weights (g)				
Necropsy bw	42.6 ± 1.5	36.0 ± 2.6*	35.4 ± 1.3**	27.4 ± 0.2**
L Cauda epididymis	0.0131 ± 0.0003	0.0114 ± 0.0007	0.0175 ± 0.0040	0.0119 ± 0.0009
L. Epididymis	0.0426 ± 0.0021	0.0387 ± 0.0016	0.0471 ± 0.0062	0.0429 ± 0.0053
L. Testis	0.1124 ± 0.0019	0.1061 ± 0.0038	0.1098 ± 0.0024	0.0996 ± 0.0015**
Spermatid measurements				
Spermatid heads (10^7/g testis)	20.91 ± 1.41	19.55 ± 0.33	19.60 ± 0.65	20.04 ± 0.72
Spermatid heads (10^7/testis)	2.24 ± 0.16	2.01 ± 0.09	2.06 ± 0.03	1.93 ± 0.08

Epididymal spermatozoal measurements				
Sperm heads (10^7/g cauda testis)	123.00 ± 11.20	114.20 ± 9.80	108.70 ± 18.10	123.30 ± 8.40
Sperm heads (10^7/cauda testis)	1.61 ± 0.15	1.30 ± 0.14	1.66 ± 0.15	1.47 ± 0.16
Sperm motility (%)	89.90 ± 0.53	90.28 ± 0.17	90.22 ± 0.92	90.10 ± 0.59

* Significantly different (P≤0.05) from the control group by Dunn's test.
** Significantly different (P≤0.01) from the control group by Williams' test.
[a] Data are presented as mean ± standard error. Differences from the control group are not significant by Dunnett's test (tissue weights) or Dunn's test (spermatid and epididymal spermatozoal measurements).

In the developmental toxicity study (more details provided in section developmental toxicity) with mice (n = 10-13) from Trivedi et al., an increased pre-implantation loss was noted at 500 mg/L drinking water (120 mg Cr/kg bw/d) and no implantations at all were seen at 1,000 mg/L (234 mg Cr/kg bw/d).[19]

Table 10 Developmental effects reported by Trivedi et al., 1989.

Exposure group	Control	59 mg/kg bw/d	120 mg/kg bw/d	234 mg/kg bw/d
Corpora lutea	6.6 ± 0.72	5.0 ± 0.71	8.0 ± 0.54	No implantation
Preimplantation loss (%) (number of incidences)	3.60 ± 2.60 (2)	7.88 ± 3.36 (3)	26.19 ± 1.54***(6)	No implantation

*** p<0.001

Zahid et al. fed male Balb/C mice (5-7 animals/group) 0, 100, 200 or 400 mg potassium dichromate/kg diet (equivalent to 0, 11, 21 or 46 mg Cr/kg bw/d) in the diet for 7 weeks.[20] Body weight and food consumption were not affected as were testis and epididymis weight. A slight, but significant increase of the number of degenerated seminiferous tubules was observed at all dose levels (dose-related). The number of spermatogonia per tubule was decreased related to the dose with an increase of the number of resting and pachyteen spermatocytes at all dose levels. At 200 and 400 mg/kg diet, sperm count was decreased without a dose relation, whereas the number of abnormal sperm cells increased dose-dependently.

Table 11 Results of sperm analysis data reported by Zahid et al., 1990.

	Control	11 mg Cr/kg bw/d	21 mg Cr/kg bw/d	46 mg Cr/kg bw/d
Degenerated tubules	0/1400 (0%)	21/1774* (1.2%)	26/1129 *(2.3%)	33/1372 *(2.4%)
Ungenerated tubules without spermatogonia	18/90 (20.0%)	45/90 (50%)**	54/90 (60%)*	81/90 (90%)*
Spermatogonia	11.1 ± 6.4	2.2 ± 3.8*	1.2 ± 1.7*	0.6 ± 0.3*
Resting spermatocytes	7.8 ± 11.7	20.2 ± 16.9***	19.6 ± 16.3***	22.8 ± 19.2*
Sperm count (x10^5/mm^3)	10.2 ± 1.3	5.7 ± 1.1	4.5 ± 1.1*	4.4 ± 0.9*
Abnormal sperm	385/3193 (12.1%)	165/1242 (13.3%)	304/1607 (18.9%)*	318/1296 (24.5)*

* p<0.001; ** p<0.01; *** p<0.05

Murthy et al. investigated the ovarian effects in Swiss mice (n = 10/group) exposed to 0, 250, 500 or 750 mg CrVI/L drinking water (equivalent to 0, 54, 109 or 163 mg Cr/kg bw/d) for 20 days.[21] A reduction in the number of follicles at different stages of maturation was seen at all dose levels in a dose-dependent manner. The number of ova was decreased at 109 and 163 mg/kg bw and histopathological examination revealed proliferated, dilated, and congested blood vessels, pyknotic nuclei in follicular cells, and atretic follicles to be present. The oestrous cycle was extended at 163 mg/kg bw.

Table 12 Results of female reproductive toxicity data reported by Murthy et al., 1996.

	Control	54 mg/kg bw/d	109 mg/kg bw/d	163 mg/kg bw/d
No. small follicles	39.4 ± 0.5	36.2 ± 0.4	34.0 ± 0.3[a]	25.2 ± 0.4[abc]
No. med follicles	9.8 ± 0.4	7.6 ± 0.2[a]	6.2 ± 0.3[ab]	4.6 ± 0.2[abc]
No. large follicles	7.6 ± 0.2	6.6 ± 0.2[a]	5.2 ± 0.2[ab]	2.4 ± 0.24[abc]
Estrous cycle length (days)	4.4 ± 0.6	4.7 ± 0.6	5.8 ± 0.4	7.7 ± 0.8[ab]
No. ova/mouse	26.2 ± 1.1	25.4 ± 0.5	18.4 ± 1.2[ab]	2.4 ± 0.9[abc]

The values represent mean +/- S.E. of 10 mice per group. The significance of the differences was evaluated using one-way ANOVA ([abc]$p<0.05$; [a]vs control, [b]vs54 mg/kg bw/d, [c]vs109 mg/kg bw/d).

They also administered another group of Swiss mice (n = 10/group) 0, 0.05, 0.5 or 5 mg CrVI/L for 90 days (equivalent to 0, 0.01, 0.1 and 1 mg Cr/kg bw/d) and examined the ovaries with electron microscopy. At the highest dose level disintegrated cell membranes of two layered follicular cells and altered villiform mitochondria in thecal cells were observed.

Elbetieha and Al-Hamood studied the effects on fertility in male Swiss (n = 9-20/ group) mice after exposure to 1,000, 2,000, 4,000 and 5,000 mg potassium dichromate/L in the drinking water (equivalent to 74, 148, 296 and 370 mg Cr/kg bw/d) during 12 weeks.[22] In the male mice exposed to the two highest doses, body weight, testis, seminal vesicles and preputial gland of the parental animals were weighed at necropsy after 12 weeks of exposure. In both exposure groups, body weight was decreased and relative testis weight was increased. Furthermore, in the highest dose group, relative seminal vesicle and preputial gland weights were decreased. In female mice mated with male mice exposed to 296 and 370 mg Cr/kg bw/d, the number of implantations and the number of viable foetuses were reduced. In the female mice mated with male mice from the highest dose group, these reductions were not statistically significant.

Table 13 Results of male reproductive toxicity data reported by Elbetieha and Al-Hamood, 1997.

	0 mg/L	74 mg Cr/kg bw/d	148 Cr/kg bw/d	296 Cr/kg bw/d	370 Cr/kg bw/d
Number of pregnant females (%)	33/40 (82.5)	33/38 (86.8)	20/22 (90.9)	16/18 (88.8)	19/26 (73)
Mean number of implantations	8.18 ± 1.59 (33)	7.84 ± 1.56 (33)	6.33 ± 2.79 (20)**	6.86 ± 1.88 (16)*	7.84 ± 2.73 (16)
Mean number of viable foetuses	8.18 ± 1.59 (33)	7.75 ± 1.80 (33)	6.33 ± 2.79 (20)**	6.86 ± 1.88 (16)*	7.15 ± 2.98 (19)
Total number of resorptions and dead foetuses	0	3 resorptions	0	0	6 resorptions, 6 dead foetuses

* p<0.05, ** p<0.01; Significance when compared to control value (Student's t test).

In addition, Elbetieha and Al-Hamood studied the effects of chromium VI in female mice (n = 15 and 11) after exposure to 2,000 and 5,000 mg potassium dichromate/L in the drinking water (equivalent to 153 and 384 mg Cr/kg bw/d) during 12 weeks, after which the treated females were mated with untreated males for 10 days. No effect on body weight was observed, but the relative ovary weight was increased at 384 mg Cr/kg bw/d. No effect on the number of pregnant females was noted. However, the number of implantations and the number of viable foetuses were reduced in both groups of chromium VI treated female mice mated with untreated male mice.

Table 14 Results of female reproductive toxicity data reported by Elbetieha and Al-Hamood, 1997.

	Control	153 Cr/kg bw/d	384 Cr/kg bw/d
Number of pregnant females	17/18	14/15	9/11
Mean number of implantations	9.00 ± 1.36 (17)	7.35 ± 1.54 (14)**	7.44 ± 1.50 (9)*
Mean number of viable foetuses	8.76 ± 1.39 (17)	6.55 ± 2.18 (9)*	5.88 ± 2.47 (9)**
Number of mice with resorptions	2/18 (11)	8/15 (53)***	7/11 (63)****
Total number of resorptions	4	37	14

* p<0.05, ** p<0.01; Student's t-test; *** p<0.01, **** p<0.005; Chi-square test; Significance when compared to control value.

Yousef et al. administered 0 or 4 mg Cr/kg bw as potassium dichromate to male rabbits (n = 6/group) by gavage daily during ten weeks. Semen was collected weekly, blood every other week and rabbits were sacrificed at the end of the exposure period.[23] Rabbits showed no adverse clinical signs. Body weight, relative weight of testes and epididymis and plasma testosterone concentration were decreased in chromium VI-exposed rabbits. Packed sperm volume, sperm concentration, total sperm output, sperm motility, total motile sperm per ejaculate, total functional sperm fraction, normal sperm, initial fructose and libido (by decreasing reaction time) were decreased compared to controls, while dead sperm and initial pH were increased. No effect on semen ejaculate volume

was noted. In seminal plasma of chromium VI-treated rabbits, the concentration of thiobarbituric acid-reactive substances was elevated and activities of glutathione S-transferase, aspartate aminotransferase and acid phosphatase were decreased. Seminal plasma lipids, glucose and urea were increased in chromium VI-treated rabbits, while total cholesterol, low-density-lipoprotein and high-density-lipoprotein cholesterol were decreased. No effect on triglycerides, total protein and albumin in seminal plasma was noted.

Table 15 Results of male reproductive toxicity data reported by Yousef, 2006.

	Control	4 mg Cr/kg bw/d
Ejaculate volume (mL)	0.69 ± 0.01	0.66 ± 0.01
pH	7.4 ± 0.04	7.7 ± 0.05*
Reaction time (s)	10.5 ± 0.3*	22.0 ± 0.9*
Packed sperm volume (%)	16.2 ± 0.47	14.6 ± 0.33*
Sperm concentration (x10^6/mL)	266 ± 6.08	218 ± 7.60*
Total sperm output (x10^6)	185 ± 4.17	137 ± 3.96
Sperm motility (%)	66.9 ± 0.90	63.8 ± 1.30*
Total motile sperm (x10^6)	137 ±1.8	90 ± 2.9*
Dead sperm (%)	24.7 ± 0.78	30.6 ± 0.79*
Normal sperm (%)	85 ± 0.4	82 ± 0.6*
Total functional sperm fraction (x10^6)	116 ± 1.66	73 ± 2.53*
Initial fructose (mg/dL)	140 ± 0.95	134 ± 0.89*

* $p<0.05$

Intraperitoneal

As the Committee considers the intraperitoneal route of administration of limited relevance for classification, no summarising tables are provided for these studies.

Ernst treated male Wistar rats intraperitoneally for 5 consecutive days with 0, 1, 2 or 4 mg Cr/kg bw/d (as sodium chromate) (n = 8/group). At all dose levels no testicular toxicity was observed 7 days after the last administration.[24] Sixty days after the last administration, a dose-dependent reduction in relative testis weight, an increase in the number of atrophic seminiferous tubules with a loss of spermiogenic epithelium and a reduced number of epididymal spermatozoa were observed in all chromium treated groups. In the highest dose group, the cellular organisation of the testis was lost; complete degeneration was observed in almost every seminiferous tubule and the Leydig cells appeared to be atrophic. In all CrVI dose groups a dose-related decreased weight gain was observed, not accompanied by decreased food or water consumption.

Ernst and Bonde studied in male Wistar rats (n = 10) the effects of subchronic intraperitoneal treatment (5 days/week during 8 weeks) to 0.5 mg Cr/kg bw/d (as sodium chromate).[25] No clinical signs or effect on body weight, food or water consumption were noted. At the end of the exposure period, a reduction in epididymal sperm motility, a decrease in serum testosterone (T) and an increase in follicle stimulating hormone (FSH) and in luteinizing hormone (LH) were observed. After a recovery period of 8 weeks sperm motility and T and FSH levels were normal again, whereas LH level was decreased.

Behari et al. studied male rabbits (n = 8-10/group) after a daily intraperitoneal injection of 0 or 2 mg potassium dichromate/kg bw for 3 or 6 weeks(equivalent to 0 or 0.7 mg Cr/kg bw/d).[26] The animals were sacrificed 72 hours after the last injection. After 3 weeks of exposure, a decrease in succinic dehydrogenase activity and mild oedema of interstitial tissue in the testis were observed in the CrVI treated group. After 6 weeks of dosing, a decrease in succinic dehydrogenase and adenosine triphosphatase activities, and marked oedema of the interstitial tissue were observed. Furthermore, no spermatocytes were observed in the seminiferous tubules of the testis after 6 weeks.

5.1.2 Human studies

Most studies pertain to chromium and other metals present in welding fumes, but exposure levels were not determined.

In a case-control study, Rachootin and Olson studied among others the associations between chromium VI during welding and male sperm abnormalities, female hormonal disturbances and infertility.[27] All cases (n = 927) and controls (n = 3,728) were identified at Odense University Hospital in Denmark during the period 1977-1980. No statistically significant associations were observed between welding of stainless steel (containing chromium VI) and male sperm abnormalities, female hormonal disturbances, delayed conception or idiopathic infertility.

Jelnes and Knudsen found that the semen quality of stainless steel welders (n = 75-77) was similar to that of non-welders within the same plant in the period January to July 1987, when matched with respect to age and smoking habits.[28] The semen quality was also comparable to that of referents not working in the metal industry.

Mortensen studied the semen quality of workers visiting fertility clinics in Denmark.[29] Based on information from questionnaires, workers could be placed in one of the following groups: welders, metal workers not exposed to welding, other industrial workers and unexposed workers (not exposed to chemical or physical agents suspected of influencing sperm quality). Cases were defined as having at least one of the following: sperm concentration less than 20 million/ mL, less than 50% of sperm cells motile or less than 50% of sperm cells with a normal morphological appearance. Men with sperm that did not fulfill any of the criteria were considered to have normal sperm and were classified as controls. The odds ratio of reduced sperm quality among welders (27 cases; 28 controls) was 2.00 (95% CI 1.16-3.45). The odds ratio was even higher when stainless steel welders were separated from nonstainless steel welders: 2.34 (95% CI 0.95-5.73). Potential confounders, such as living quarters, age, smoking habits, alcohol intake, medication use, and earlier diagnosis of mumps with or without orchitis were not associated with the occupation of welding.

Bonde and Ernst examined the associations of chromium VI with sex hormones and semen quality in welders in Denmark.[30] The study included 30 tungsten inert gas stainless steel welders, 30 mild steel welders and 47 non-welding workers from six different plants. Participants were classified into three groups according to the concentration of chromium in their urine defined by the median and the 75 percentile of the distribution of urine chromium concentration: <1.07, 1.07-1.78 and >1.78 nmol/mmol creatinine. All groups had subjects in all three classes, but the number of stainless steel welders increased with increasing concentration, while for mild steel workers and non-welding metal workers most subjects were in the lowest class. For electricians, all subjects were in the lowest class. None of the sperm parameters and serum concentrations of sexual hormones showed an inverse association with chromium concentration in urine.

In a review of 1993, Bonde reported on several studies on male metal workers, including results from the above-mentioned field study, but with an increased group of mild steel welders (46) and non-welding referents (54).[31] All workers were employed on 1 December 1986 at one of six plants in Aalborg, Denmark. While the sperm concentration in seminal plasma was not reduced among welders and no increased rate of subjects with oligospermia was found, sperm count and degree of sperm motility were decreased in stainless steel and mild steel workers. The linear penetration rate in egg white and the proportion of normal sperm forms were decreased in mild steel welders only. Serum testosterone was lower in stainless steel workers compared to referents, whereas

no difference in the level of follicle stimulating hormone was observed. Among 538 questionnaire respondents working at the six plants, an increased risk of infertility (inability to conceive within 2 years) was noted with an odds ratio of 2.2 (95% CI 1.1-4.6) when compared to age-matched non-welding referents. In a nationwide cohort of metalworkers (n = 3,507), no association was found between fertility and welding exposure.

Li et al. investigated the semen status of 21 workers exposed to chromium VI in an electroplating factory in Henan, China for 1-15 years.[12] A control group of workers not exposed to any harmful chemicals was recruited from the same factory. The average age was similar in both groups. In exposed workers, semen samples were collected preferably 5 days after sexual abstinence. Sperm counts and motility were reduced to 53 and 85% of those in the control group, respectively. Seminal volume and liquefaction time were not affected. Serum follicle stimulating hormone was increased compared to controls (7.34 and 2.41 IU/mL in exposed and control groups, respectively; $p<0.01$), while serum luteinizing hormone was not affected. The chromium concentration was 1.40 and 1.26 µmol/mL in serum and 7.55 and 6.38 µmol/mL in seminal fluid in exposed and control group, respectively; these differences were not statistically significant. The zinc concentration (role in oxidant defense system) and activity of lactate dehydrogenase and lactate dehydrogenase C4 isoenzyme (specific for seminal fluid of mature testicles) in seminal fluid were decreased in exposed workers to 26, 60 and 70% of those in the control group.

Kumar et al. reported a cross-sectional study on 61 workers exposed to chromium during chromium sulphate manufacturing and 15 controls from a nearby industry not involved in the manufacturing of chromium compounds. Lifestyle differences between groups included differences in smoking, drinking, and chewing habits. Chromium blood levels were higher in exposed workers compared to control subjects (63.7 ± 50.4 µg/L versus 22.8 ± 17.7 µg/L). A higher number of morphologically abnormal sperm cells were reported in the exposure group, and a statistically significant correlation (r=301) was found for this parameter and blood chromium levels. However, no significant differences were found in semen volume, liquefaction time, mean pH value, and sperm viability, concentration or motility.

5.2 Developmental toxicity

5.2.1 *Non-human information*

Animal developmental toxicity studies are summarised in Table 16 and described below. Changes and differences mentioned are statistically significant unless stated otherwise.

Table 16 Summary table of developmental toxicity studies in animals.

authors	species	experimental period/ design	dose/route	general toxicity	developmental toxicity
Glaser et al., 1984	Rat; Wistar; n = 10-12	Daily during gestation (time/d not indicated); sacrificed on GD 21	0, 50, 100 or 200 µg Cr/m^3 as sodium dichromate (0, 0.1, 0.2 and 0.3 mg Cr/kg bw/d (assuming a breathing volume of 200 ml/min))/ inhalation	No mortality or clinical signs; increased relative organ weight, particularly lung. Hyperplasia at the highest concentration LOAEL=0.3 mg Cr/kg bw/d	No developmental effects observed (macroscopy, number of foetuses, foetal weight and placental weight) NOAEL=0.3 mg Cr/kg bw/d
Kanojia et al., 1996	Rat; Swiss albino; n = 10	During 20 days (one folliculogenesis cycle) prior to gestation; sacrificed on GD 19; 1/3 foetuses visceral abnormalities, remaining external and skeletal	0, 250, 500 or 750 mg Cr/L as potassium dichromate in drinking water (31, 59 and 76 mg Cr/kg bw/d)/oral	No mortality or change in clinical signs; decreased water consumption; decreased weight gain in all doses LOAEL=31 mg Cr/kg bw/d	All doses: decreased number of live foetuses, number of resorptions, and post-implantation loss; 59 and 76 mg/kg: decreased placental weight; non-significant decrease foetal weight and crown-rump length; subdermal haemorrhagic patches, short tail, and reduced caudal ossification 76 mg/kg: kinky tail, reduced parietal and interparietal ossification LOAEL=31 mg Cr/kg bw/d
Kanojia et al., 1998	Rat; Druckrey; n = 10	During 3 months prior to gestation; sacrificed on GD 19; 1/3 foetuses visceral abnormalities, remaining external and skeletal	0, 250, 500 or 750 mg Cr/L as potassium dichromate in drinking water (0, 45, 89 or 124 mg/ kg bw/d)/oral	Gestational weight gain decrease in all dose groups; 89 and 124 mg/kg: 15% and 10% of animals, resp., died within 14 days; liver and kidney; reduced water consumption LOAEL=45 mg Cr/kg bw/d	All dose levels: decreased number of live foetuses/litter, foetal weight and crown-rump length; increased number of resorptions; increased pre- and post implantation loss; decreased placental weight at 89 and 124 mg/kg; drooping wrist and subdermal haemorrhagic patches on thoracic and abdominal region at all doses; kinky and/or short tail at 89 and 124 mg/kg; no visceral abnormalities; reduced caudal ossification at all doses, and reduced parietal and interparietal ossification at 89 and 124 mg/kg; increased Cr level in placenta and foetus LOAEL=45 mg Cr/kg bw/d
Elsaieed and Nada, 2002	Rat; Wistar; n = 10	Gestation day 6-15; sacrifice GD 20; 1/3 visceral, remainder skeletal examination	0 or 50 mg/L Cr as potassium dichromate in drinking water (7 mg Cr/kg bw/d)/ oral	No adverse clinical signs; no effect on food intake and water consumption; decrease gestational weight (40%) LOAEL=7 mg Cr/kg bw/d	Increase of pre- and post-implantation losses, number of resorbed and dead foetuses per litter; decreased foetal weight, number of visceral (renal pelvis dilatation) and skeletal (incomplete ossification of skull bone) anomalies per litter LOAEL=7 mg Cr/kg bw/d

Soudani et al., 2011a,b,c	Rat; Wistar; n = 6	on GD 14 to postnatal day 14; biochemical parameters, lipid peroxidation, oxidative stress and histopathology in kidney of dams and offspring (culled to 4/ sex); 24h-urine sample before sacrifice; blood sample after sacrifice	0 or 700 mg /L potassium dichromate in drinking water (0 or 24 mg Cr/kg bw/d)/oral	No mortality or clinical signs; slight decrease in food and water consumption; decrease in body weight. Changes of biochemical parameters in treated dams and histopathological examination indicated damage to kidney Haemorrhage, leukocyte infiltration and necrosis in liver (more pronounced than in pups) LOAEL=24 mg Cr/kg bw/d	Changes of biochemical parameters in treated dams and histopathological examination indicated damage to kidney of pups. Also reduced femur weight and length were reported in pups, and changes in bone mineralization, observed after biochemical and histological analysis Haemorrhage, leukocyte infiltration and necrosis in liver (less pronounced than in dams) LOAEL=24 mg Cr/kg bw/d
NTP, 1997	Mouse; BALB/c; n = 20/sex	F_0: 1 week prior to mating and 12-week mating period (continuous breeding phase; intermediate litters evaluated on PND 1); F_1: after weaning until 74 +/- 10 days of age continued with 1 week mating period	0, 100, 200 or 400 mg potassium dichromate/kg diet (F_0: 0, 7, 14 or 30 mg Cr/kg bw/d; F_1: 0, 8, 16 or 37 mg Cr/kg bw/d)/oral	F_0 : decreased body weight dams at mid and high dose at delivery of one or more litters, in week 14, during lactation and at necropsy; no treatment-related effect on mortality, body weight of sires, clinical signs, food consumption, macroscopy or microscopy; decreased absolute liver weight at 30 mg Cr/kg bw/d (M/F) NOAEL F_0=7 mg Cr/kg bw/d F_1: no treatment-related mortality, clinical signs, gross or microscopic lesions; decreased body weight and increased food consumption at 37 mg/kg; at necropsy, decreased MCV at 16 and 37 (M/F) and at 8 mg/kg (F); decreased MCH (M) and mean haemoglobin (F) at 37 mg/kg; no effect on erythrocyte morphology LOAEL F_1=8 mg Cr/kg bw/d	F_1: no influence on number of live pups per litter, proportion of pups born alive, sex ratio and live pup weight; decreased body weight at PND 21 (M) at 37 mg/kg; NOAEL F_0=30 mg Cr/kg bw/d F_2: no influence on number of live pups per litter, proportion of pups born alive, sex ratio and adjusted live pup weight NOAEL F_1=37 mg Cr/kg bw/d
Trivedi et al., 1989	Mouse; ITCR;females; n = 10-13	During gestation; sacrifice on GD 19; 1/ 3 of foetuses visceral abnormalities; remainder skeletal abnormalities	0, 250, 500 or 1,000 mg potassium dichromate/L in drinking water (0, 59, 120 and 234 mg Cr/kg bw/d)/ oral	No effect on clinical signs or placental weight; 234 mg/kg: severe effect on body weight NOAEL=120 mg Cr/kg bw/d	59 mg/kg: increased number of resorptions and postimplantation loss; decreased litter size; decrease foetal weight and length; retarded ossification of skull bones; additionally, at 120 mg/kg: increased in external abnormalities (kinking of tail, subdermal haemorrhagic patches; retarded ossification of fore- and hindlimb phalanges, vertebrae and sternebrae; accumulation of Cr in foetus at 120 mg/kg LOAEL=59 mg Cr/kg bw/d
De Flora et al.. 2006	Mouse; Swiss; n = 5	During gestation; sacrifice GD 18; number of foetuses/ litter and foetal weight and micronuclei in foetal liver and blood	0, 5 or 10 mg Cr/ L (0, 1, or 2 mg Cr/kg bw/d); or 10 mg Cr/L drinking water as potassium dichromate (2 mg Cr/kg bw/d)/oral.	No effect on frequency of micronuclei in polychromatic erythrocytes and PCE/NCE ratio in bone marrow NOAEL=2 mg Cr/kg bw/d (repeated dose)	No effect on number of foetuses/litter, foetal weight, frequency of micronuclei in polychromatic erythrocytes and PCE/NCE ratio in foetal liver and peripheral blood NOAEL=2 mg Cr/kg bw/d (repeated dose)
			50 mg Cr/kg bw once, as sodium dichromate dehydrate or potassium dichromate/ip	NOAEL=50 mg Cr/kg bw (single dose)	NOAEL=50 mg Cr/kg bw (single dose)

Junaid et al., 1996	Mouse; Swiss; 30 g bw; n = 10	GD 6-14; sacrificed on GD 19; 1/3 of foetuses visceral abnormalities; remainder first external then skeletal deformities	0, 250, 500 or 750 mg Cr/L as potassium dichromate in drinking water (0, 67, 125 and 182 mg Cr/kg bw/d)/ oral	No clinical signs; 125 and 182 mg/kg: reduced body weight gain (8 and 30% reduction, respectively) NOAEL=67 mg Cr/kg bw/d	67 mg/kg: increased number of resorptions and post-implantation loss; 125 and 182 mg/kg: decreased number of foetuses, increased number of dead foetuses, resorptions and post-implantation loss; decreased foetal weight; no effect on crown-rump length; Gross and skeletal defects: 125 mg/kg: increased incidence of reduced caudal ossification;182 mg/kg: increased incidence of drooping wrist and subdermal haemorrhagic patches; increased incidence of reduced ossification (nasal, frontal, parietal, interparietal, caudal, tarsal); Cr foetal levels increased at all doses LOAEL=67 mg Cr/kg bw/d
Elbetieha and Al-Hamood, 1997	Mouse, females, Swiss; n = 9-20	12 weeks prior to mating; body weight and relative organ weight only studied at 2000 and 5,000 mg/L	0, 2,000, or 5,000 mg potassium dichromate/L drinking water (153 or 384 mg Cr/kg bw/d)/oral	153 and 384 mg/kg: decrease body weight; LOAEL=153 mg Cr/kg bw/d	153 and 384 mg/kg: reduced number of viable foetuses; increase in total number of pregnant mice with resorptions LOAEL=153 mg Cr/kg bw/d
Al-Hamood et al., 1998	Mouse; Swiss; n =25/ group	From GD 12 to PND 20; mating of treated M/F with untreated pups at PND 60 for 10 d; sacrificed 1w after removal of male (fertility parameters); other group sacrificed at PND 50 (body and organ weight)	0 or 1000 mg Cr potassium dichromate/L as in drinking water (0 or 71 mg Cr/kg bw/d)/oral	no effect on water consumption; no further effects reported NOAEL=71 mg Cr/kg bw/d	No effect on pup weight, weight of ovaries, uterus, testes, seminal vesicles and preputial glands; no effect on male fertility (as measured by the number of pregnant females, number of implantations and number of viable foetuses); delayed vaginal opening (27.1 d compared to 24.6 d in controls); female fertility was affected: decreased number of pregnant females, implantations and viable foetuses, and increased number of resorptions LOAEL=71 mg Cr/kg bw/d
Sivakumar et al., 2014	Rat; n = 5	From GD 9.5-14.5. At PND 60, F1 female pups (n-15) were allowed to mate with non-exposed males.	0 and 25 mg/L potassium dichromate in drinking water (0 and 1 mg Cr/kg bw/d)/oral	Not reported	Increased germ cell apoptosis; up-regulation p53, p27, Bax, Caspase-3; increase p53 and SOD-2 co-localisation; accelerated germ cell cyst breakdown; advanced primordial follicle assembly and primary follicle transition; down regulation of p-AKT,p-ERK and XIAP; induction of early reproductive senescence and decrease in litter size in F1 female progeny LOAEL=1 mg Cr/kg bw/d
Marouani et al., 2010	Rat; Wistar; n = 8	GD 6-15; killed on GD 19	0, 1 or 2 mg Cr/kg bw/d as potassium dichromate/ip	No mortality or changes in clinical signs; reduction in body weight gain (to 71 and 40% of control at 1 and 2 mg/kg, resp.) and relative weight of the uterus (to 79 and 70% of control at 1 and 2 mg/kg, resp.) NOAEL=2 mg Cr/kg bw/d	At 1 and 2 mg/kg: Reduced foetal bw, retarded foetal development, reduced number of foetuses per mother and high incidences in dead foetuses and resorptions; gross morphological abnormalities; incomplete ossification in nasal, cranium, abdominal or caudal bones. At 2 mg/kg: absence of sacral vertebrae; histological changes with atrophy of vital organs LOAEL=1 mg Cr/kg bw/d
Endo and Watanabe 1988	Mouse; Jcl:ICR; n = 15-17	Single injection on GD 9; sacrifice GD 17; foetuses sexed, examined externally and 1/3 for skeletal abnormalities	0 or 15 mg chromium trioxide/kg bw (8 mg Cr/kg bw)/ ip	Stated to be observed daily for signs of toxicity (no results reported); no effect on body weight NOAEL=8 mg Cr/kg bw/d	No effect on live, dead or resorbed foetuses, foetal weight (male/female) or number of malformations NOAEL=8 mg Cr/kg bw/d

Gale 1978	Hamster, Golden (LAK: LVG(SYR, n = 4-21	Single injection GD 8; sacrifice GD 12, 14 or 15	0, 5, 7.5, 10 or 15 mg chromium trioxide/kg bw (0, 3, 4, 5 or 8 mg Cr/ kg bw/d)/iv	3 mg/kg: no effects; 4 and 5 mg/kg: most lost weight; urinary bladders distended with clear urine (control milky urine); mottled kidneys, gall bladders abnormally distended with bile (histologically normal); clear vacuoles in periportal hepatocytes; moderate to extensive tubular necrosis with hyaline casts in kidney; 8 mg/kg: 3 out of 4 died shortly after treatment NOAEL=3 mg Cr/kg bw/d	3, 4 and 5 mg/kg: increased number of resorptions; increased number of malformations (cleft palate, hydrocephalus), retarded ossification (vertebral column and sternum (skull at 4 and 5), hyoid bone, hind limb, fore limb (5 and 8) LOAEL=3 mg Cr/kg bw/d
Gale and Bunch 1979	Hamster; Golden (LAK: LVG (SYR)); n = 6 (control 3)	Single injection GD 7, 8, 9, 10 or 11; sacrifice GD 12, 14 or 15	0 or 8 mg chromium trioxide/kg bw (0 or 4 mg Cr/kg bw)/iv	Weight loss; mottled kidneys with varying degrees of tubular necrosis; some gall bladders (GD 8 and 9) were distended with bile (histologically normal) LOAEL=4 mg Cr/kg bw/d	GD 7, 8, 9, 10: decreased crown-rump length GD 7,8,9: increased number of resorptions, increased number of live foetuses with external defects and cleft palate; some kidneys small or absent at GD 7 and 8; GD 11: no effects LOAEL=4 mg Cr/kg bw/d

Abbrevations used: GD = gestation day; iv = intravenous; ip = intraperitoneal; PND = post-natal day; P1/2/3 = first/second/third parental generation. If applicable, doses were converted to amount of chromium based on molecular weight. Conversion of doses to mg/kg bw/d, if applicable, was performed using the general conversion factor for body weight to food or water consumption (assuming dry diet), and the default values for body weights (if not specified by the authors) as specified in the Technical Guidance on Risk Assessment, Part 1, Appendix 6. The Committee notes that this conversion does not specifically take into account pregnant animals.

Inhalation

Glaser et al. treated pregnant Wistar rats with 0, 50, 100 or 200 µg chromium VI/m^3 as sodium dichromate by inhalation for 21 days after which animals were sacrificed and foetuses were weighed.[9] The only effects observed on dams was an increased relative lung weight at all dose levels and increased relative liver weight at 100 and 200 µg/m^3. The number of foetuses, foetal weight or placental weight were not affected. In foetuses at 200 µg/m^3 the lymphocyte count was decreased, plasma levels of total bilirubin were increased, whereas triglycerides were decreased. The latter was also decreased at 100 µg/m^3.

Glaser et al. also studied the effects of 200 µg chromium VI/m^3 on the reproduction and development of offspring in 3 generations of rats (see details under fertility). Effects on parents included haematological, clinical biochemistry effects, decreased serum-immunoglobulin levels and increased lung weights. No effects on number of foetuses, live foetuses at postnatal day 25, foetal weight or skeletal development were noted.

The Committee notes that the equivalent oral doses corresponding to the exposure concentrations of 50, 100, and 200 µg chromium VI/m^3 applied in this study are rather low, i.e. 0.1, 0.2 and 0.3 mg Cr/kg bw/d, respectively (assuming a breathing volume of 200 mL/min).

Oral

In the first study by Kanojia et al.[13] with rats (for details see section 'effects on fertility'), dams showed a decreased weight gain at all doses (i.e. 250, 500 and 750 mg Cr/L, equivalent to 31, 59 and 76 mg Cr/kg bw/d). The number of live foetuses was dose-related decreased and the number of resorptions (not dose-related) and post-implantation loss (dose-related) was increased at all doses. At the two highest dose levels decreases were seen in the foetal and placental weights, and increased incidences of subdermal haemorrhagic patches, short tail, and reduced caudal ossification. A kinky tail and reduced parietal and interparietal ossification was observed in foetuses at 76 mg/kg bw/d only. No visceral abnormalities were noted.

Table 17 Results as reported by Kanojia et al., 1996.

	Control	31 mg/kg bw/d	59 mg/kg bw/d	76 mg/kg bw/d
Weight gain in mothers (g)	70.50 ± 5.19	65 ± 3.17[a]	60.92 ± 2.13[ab]	55.5 ± 3.01[abc]
Number of live foetuses	9.11 ± 0.87	8.29 ± 0.93[a]	4.12 ± 0.51[ab]	1.21 ± 0.13[abc]
Number of resorptions	0.40 ± 0.24	1.09 ± 0.34[a]	1.72 ± 0.23[a]	1.03 ± 0.29[a]
Post-implantation loss	4.20 ± 0.41	13.73 ± 1.57[a]	30.28 ± 4.19[ab]	46.69 ± 5.21[abc]
Foetal weight (g)	3.54 ± 41	3.46 ± 0.29	3.08 ± 0.37	2.53 ± 0.31
Placental weight (g)	0.67 ± 0.08	0.71 ± 0.09[a]	0.79 ± 0.19[a]	0.86 ± 0.12[a]
Crown-rump length (cm)	3.18 ± 0.19	3.01 ± 0.27	2.78 ± 0.31	2.61 ± 0.23

Values represent mean ± S.E. of 10 rats in each group. The significance of the difference among various groups was evaluated by applying one-way ANOVA ($p<0.05$). Comparison between two groups: [a]vs control, [b]vs 31 mg/kg bw/d, [c]vs 59 mg/kg bw/d.

Table 18 Results as reported by Kanojia et al., 1996.

	Control	31 mg/kg bw/d	59 mg/kg bw/d	76 mg/kg bw/d
Gross abnormalities				
Number of pups/litter observed	72/10	70/10	51/10	19/10
Drooping wrist	0	0	0	6/4 (32)
Sub-dermal hemorrhagic patches	0	0	8/6 (16)	8/4 (42)[a]
Kinky tail	0	0	0	8/6 (42)[a]
Short tail	0	0	4/4	10/4 (53)[a]
Skeletal abnormalities				
Number of foetuses/litter observed	48/10	45/10	34/10	19/10
Reduced parietal ossification	0	0	0	12/10 (63)[a]
Reduced inter-parietal ossification	0	0	0	10/10 (53)[a]
Reduced caudal ossfication	6/4 (12)	8/5 (18)	18/8 (53)[a]	18/10 (95)[a]

Gross and skeletal abnormalities are represented as number of abnormal pups/litter observed; percentage in parentheses calculated by the total number of pups observed. Statistical significance evaluated by Fisher's Exact test; comparison between two groups: [a]vs. Control ($p<0.05$).

In another study using the same exposure levels of chromium in drinking water, Kanojia et al.[14] (for details see section 'effects on fertility') reported that at 500 and 750 mg/L (equivalent to 89 and 124 mg/kg bw/d) 15% and 10% of animals, respectively, died within 14 days of start of exposure. Gestational weight gain of dams was decreased in all dose groups. The number of live foetuses/litter, foetal weight and crown-rump length were decreased in all treated groups in a dose-related manner. The number of resorptions, and pre- and post-implantation loss were increased at all chromium VI dose levels with a dose-response relationship. Placental weight was decreased at 500 and 750 mg/L. In foetuses subdermal haemorrhagic patches on the thoracic and abdominal region were noted in all dose groups with a dose-relationship compared to none in controls. No visceral abnormalities were seen. A dose-dependent reduced ossification of caudal bones

Table 19 Results as reported by Kanojia et al., 1998.

	Control	45 mg/kg bw/d	89 mg/kg bw/d	124 mg/kg bw/d
Body weight (g)	182 ± 10	168 ± 14	150 ± 16	138 ± 15[a]
Weight gain in mothers (g)	69.16 ± 3.66	61.66 ± 4.23	57.50 ± 2.82[a]	54.16 ± 3.11[a]
Number of live foetuses per litter	9.30 ± 0.92	7.30 ± 0.53	5.45 ± 0.42[ab]	4.16 ± 0.31[abc]
Number of resorptions per litter	0.53 ± 0.25	1.02 ± 0.30	1.41 ± 0.37[a]	1.67 ± 0.34[a]
Pre-implantation loss (%)	6.38 ± 0.63	12.97 ± 0.75[a]	17.64 ± 0.45[ab]	20.05 ± 0.45[abc]
Post-implantation loss (%)	5.39 ± 0.35	12.25 ± 0.63[a]	20.25 ± 0.54[ab]	22.80 ± 0.91[abc]
Foetal weight (g)	4.36 ± 0.28	3.44 ± 0.22[a]	3.07 ± 0.16[a]	2.75 ± 0.22[a]
Placental weight (g)	0.72 ± 0.06	0.67 ± 0.07	0.52 ± 0.05[a]	0.37 ± 0.08[ab]
Crown-rump length (cm)	3.68 ± 0.16	3.15 ± 0.13	2.76 ± 0.09[a]	2.65 ± 0.16[ab]

Values represent mean ± S.E. of 10 rats in each group. The significance of the difference among various groups was evaluated by applying one-way ANOVA ($p<0.05$). Comparison between two groups: [a]vs control, [b]vs 89 mg/kg bw/d, [c]vs 124 mg/kg bw/d.

Table 20 Results as reported by Kanojia et al., 1998.

	Control	45 mg/kg bw/d	89 mg/kg bw/d	124 mg/kg bw/d
Gross abnormalities				
Number of pups/litter observed	77/10	63/10	46/10	25/10
Drooping wrist	0	3/3 (5)[a]	7/5 (15)[ab]	7/5 (25)[ab]
Sub-dermal hemorrhagic patches	0	5/4 (8)[a]	8/6 (17)[ab]	6/6 (24)[ab]
Kinky tail	0	0	5/3 (11)[ab]	5/3 (20)[ab]
Short tail	0	0	6/4 (13)[ab]	12/7 (48)[abc]
Skeletal abnormalities				
Number of foetuses/litter observed	42/10	35/10	29/10	22/10
Reduced parietal ossification	0	0	7/6 (24)[ab]	22/9 (50)[abc]
Reduced inter-parietal ossification	0	0	8/6 (28)[ab]	10/8 (45)[abc]
Reduced caudal ossfication	5/3 (12)	9/6 (25)[a]	17/7 (59)[ab]	20/9 (91)[abc]

Gross and skeletal abnormalities are represented as number of abnormal pups/litter observed; percentage in parentheses calculated by the total number of pups observed. Statistical significance evaluated by Fisher's Exact test ($p<0.05$); comparison between two groups: [a]vs. Control, [b]vs 250 mg/L, [c]vs 500 mg/L.

was observed in all dose groups, while reduced ossification of parietal and interparietal bones was seen at 500 and 750 mg/L only.

Elsaieed and Nada administered 0 or 50 mg chromium VI/L as potassium dichromate in their drinking water (equivalent to 7 mg/kg bw/d) to pregnant Wistar rats during gestation day 6-15.[32] Dams were sacrificed on gestation day 20. Gestational weight gain was decreased 40% relative to control in chromium VI treated dams, which may be related to the foetal toxicity observed. Pre- and post-implantation losses and the number of resorbed and dead foetuses per litter were increased compared to controls. Foetal weight was decreased by 33% and the number of visceral (renal pelvis dilatation) and skeletal (incomplete ossification of skull bone) anomalies per litter were increased. Exposed dams showed an increase of chromium levels in blood, placental and foetal tissues.

Table 21 Results as reported by Elsaieed and Nada, 2002.

	Control	7 mg kg/bw/d
Gestation weight gain (g)	23.6 ± 1.3	14.2 ± 1.7[a]
Number of corpora lutea/litter	7 ± 0.49	7.1 ± 0.48[a]
Number of pre-implantation loss/litter	0	2.1 ± 0.36[a]
Number of post-implantation loss/litter	0	1.5 ± 0.34[a]
Number of resorbed foetuses/litter	0	1.2 ± 0.13[a]
Number of dead foetuses/litter	0.1 ± 0.099	1.2 ± 0.24[a]
Number of live foetuses/litter	6.8 ± 0.44	1.5 ± 0.29[a]
Foetal weight (g)	3.9 ± 0.42	2.6 ± 0.23[a]
Number of visceral anomalies/litter	0	2.1 ± 0.39[a]
Number of skeletal anomalies/litter	0	1.0 ± 0.34[a]

[a] significantly different at $p<0.05$

Soudani et al. studied the effects of potassium dichromate on liver and kidney function of female rats and their progeny and on the bones of offspring.[33-35] Female Wistar rats (6 animals/group) received 700 mg/L potassium dichromate in their drinking water (equivalent to 24 mg Cr/kg bw/d) from gestation day 14 to postnatal day 14. Pups were culled to 4/sex after parturition. No mortality or clinical signs were observed in the treated group. A reduction in body weight was observed in both mothers and offspring. An increase of relative liver and kidney weight was observed in the pups of exposed dams. Renal and hepatic toxicity was measured in both mothers and pups by biochemical, urinary and histological analysis.

In pups, the femur weight and length was decreased compared to controls. Bone calcium and phosphorus levels of pups were decreased. Calcium was

increased in plasma and decreased in urine, while phosphorus was decreased in plasma and increased in urine. Impairment of bone function was confirmed histologically.

Potassium dichromate administered at 0, 100, 200 and 400 mg/kg diet (equivalent to 0, 7, 14 and 30 mg Cr/kg bw/d) to BALB/c mice in a NTP RACB (Reproductive Assessment by Continuous Breeding)-study did not influence the number of live pups per litter, proportion of pups born alive, sex ratio and live pup weight (for additional data see under fertility).[18] For the last litter produced in the continuous breeding phase – treated with potassium dichromate from birth (via lactation) and after weaning via the diet – litter parameters were investigated. Mean average pup weights of the parents (F1) were slightly less (9-16%; not statistically significant) than controls on postpartum day 14 and 21 for both sexes. At 21 days postpartum after culling, males of the high dose group had lower body weights. At 74 days postpartum at the start of mating, males of the high dose group and females of the mid and high dose group had lower body

Table 22 Results as reported by the NTP, 1997.

	Control	7 mg Cr/kg bw/d	14 mg Cr/kg bw/d	30 mg Cr/kg bw/d	Trend
Average litters per pair[abd]	3.8 ± 0.2 (20)	4.1 ± 0.1 (20)	3.7 ± 0.2 (20)	3.8 ± 0.2 (20)	p=0.554
Live pups per litter[cd]					
Male	3.0 ± 0.2 (20)	3.0 ± 0.2 (20)	2.8 ± 0.2 (20)	2.7 ± 0.2 (20)	p=0.191
Female	3.6 ± 0.2 (20)	3.8 ± 0.1 (20)	3.7 ± 0.2 (20)	3.2 ± 0.2 (20)	p=0.150
Combined	6.6 ± 0.3 (20)	6.7 ± 0.2 (20)	6.5 ± 0.3 (20)	5.9 ± 0.3 (20)	p=0.081
Proportion of pups born alive[cd]	0.99 ± 0.00 (20)	0.96 ± 0.01 (20)*	0.96 ± 0.02 (20)	0.96 ± 0.01 (20)	p=0.393
Sex of pups born alive[ed]	0.45 ± 0.02 (20)	0.44 ± 0.02 (20)	0.43 ± 0.02 (20)	0.46 ± 0.03 (20)	p=0.966
Live pups weight (g)[cd]					
Male	1.52 ± 0.03 (20)	1.50 ± 0.02 (20)	1.45 ± 0.02 (20)	1.53 ± 0.03 (20)	p=0.928
Female	1.56 ± 0.08 (20)	1.50 ± 0.03 (20)	1.44 ± 0.02 (20)	1.49 ± 0.03 (20)	p=0.305
Combined	1.51 ± 0.02 (20)	1.49 ± 0.02 (20)	1.44 ± 0.02 (20))	1.52 ± 0.03 (20)	p=0.630
Adjusted live pup weight (g)[fg]					Overall
Male	1.52 ± 0.02 (20)	1.51 ± 0.02 (20)	1.46 ± 0.02 (20)	1.50 ± 0.02 (20)	p=0.214
Female	1.56 ± 0.04 (20)	1.52 ± 0.05 (20)	1.44 ± 0.04 (20)	1.46 ± 0.05 (20)	p=0.246
Combined	1.51 ± 0.02 (20)	1.51 ± 0.02 (20)	1.45 ± 0.02 (20)	1.49 ± 0.02 (20)	p=0.090

*p<0.05

a Only pairs surviving to the end of task 2 were included for statistical analysis of data.

b Mean ± standard error (number of cohabited pairs).

c Mean ± standard error (number of fertile pairs).

d Each dose group is compared to the control group with Shirley's test when a trend is present (p<0.01 from Jonckeere's trend test), otherwise Dunn's test is used.

e Mean ± standard error (number of fertile pairs producing live pups).

f Least squares estimate of the mean of the average pup weight from each fertile pair, adjusted for average litter size ± standard error (number of fertile pairs producing live pups).

g Overall p-values from an F-test for equality of group means. Dunnett's test was used to compared each dosed group to the control group.

weights, whereas feed consumption was increased for all dose-groups. Body weights of males stayed lower than controls for the final 4 weeks of the test. Three blood parameters (mean corpuscular volume, mean corpuscular haemoglobin and mean haemoglobin) were decreased in all dose groups at necropsy. For the F_2 generation no differences were observed in number of live pups per litter, proportion of pups born alive, sex ratio and adjusted live pup weight.

Trivedi et al. administered 0, 250, 500 and 1,000 mg/L in drinking water (probably specified as doses chromium and administered as potassium dichromate, equivalent to 0, 59, 120 and 234 mg Cr/kg bw/d) to ITRC mice (n = 10-13) during the entire gestation period.[19] Dams were sacrificed on gestation day 19 and foetuses were examined. Dams at 234 mg/kg bw showed a body weight loss, while dams at 120 mg/kg bw showed a reduced body weight gain. No effects on clinical signs were noted. At the highest dose, no implantations were observed. In the 59 and 120 mg/kg bw group an increased number of resorptions and post-implantation loss, a decreased litter size, foetal weight and length were observed with a dose dependence. Furthermore, a retarded ossification of skull bones was observed. Additionally, in the 120 mg/kg bw group an increase in external abnormalities (kinking of tail, subdermal haemorrhagic patches), retarded ossification of fore- and hindlimb phalanges, vertebrae and sternebrae was noted.

Table 23 Results reported by Trivedi et al.,1989.

	Control	59 mg/kg bw/d	120 mg/kg bw/d	234 mg/kg bw/d
Number of litters (number of females)	10 (10)	10 (13)	10 (12)	0 (10)
Body weight gain (g)	16.43 ± 2.10	13.5 ± 1.94	13.0 ± 0.65[a]*	-1.19 ± 2.8[abc]***
Litter size	6.3 ± 0.61	3.8 ± 0.73	3.5 ± 0.57[a]*	No implantation
Placental weight (mg)	134.0 ± 5.00	123.0 ± 4.37	126.0 ± 4.30	No implantation
Sex ratio (M/F)	2.40/4.00	2.25/1.50	1.50/2.00	No implantation
Foetal weight (g)	1.95 ± 0.08	1.32 ± 0.05 ***	1.09 ± 0.03[a]*** [b]**	No implantation
Crown-rump length (CRL) (cm)	2.87 ± 0.12	2.38 ± 0.08[a]***	2.05 ± 0.12 [a]*** [b]**	No implantation
Incidences of resorptions (%)	1 (10.0)	9 (32.69)[a]***	7 (51.70)[a]**	No implantation
Postimplantation loss (%) (number of incidences)	1.66 ± 1.25 (1)	26.48 ± 1.46[a]*** (3)	88.09 ± 2.12[ab]*** (7)	No implantation

The values represent means +/- SEM of 10 mice/group. The significance of the difference among various groups was evaulated by applying one-way ANOVA, except for incidence of resorptions, where Fisher Exact Test was used for comparison. Comparison between two groups: [a]vs control; [b]vs 250 mg/L, [c]vs 500 mg/L.*p<0.05; **p<0.01, ***p<0.001.

As part of a genotoxicity study, timed-pregnant Swiss albino mice (n = 5) were given sodium dichromate dihydrate or potassium dichromate up to a concentration of 10 mg CrVI/L drinking water throughout gestation.[36] All dams were sacrificed on gestation day 18. Neither litter size nor foetal weights were affected by treatment (no further details were provided).

Junaid et al. administered potassium dichromate at levels of 0, 250, 500 and 750 mg Cr/L in drinking water (equivalent to 67, 125 and 182 mg Cr/kg bw/d) to Swiss mice (n = 10/group) during gestation days 6-14.[37] No effect on clinical signs or behaviour of dams was noted. At the two highest dose levels, a reduced body weight gain was observed; no effect on placental weight was seen. In the 67 mg/kg bw group an increased number of resorptions ($p<0.05$) and post-implantation loss (not significant) was noted. In the 125 mg/kg bw group a reduced number of foetuses, a reduced foetal weight, an increased number of resorptions, an increased post-implantation loss and an increased number of foetuses with reduced caudal ossification were observed. In addition, at 182 mg/kg bw an increased number of foetuses with reduced ossification of several bones (nasal, frontal, parietal, interparietal, caudal, tarsal) and an increased number of foetuses with external abnormalities (drooping wrist and subdermal haemorrhagic patches) were observed. No effect on crown-rump length was noted.

Table 24 Results reported by Junaid et al.,1996.

	Control	67 mg/kg bw/d	125 mg/kg bw/d	182 mg/kg bw/d
Weight gain in mothers (g)	15.57 ± 0.20	15.21 ± 0.31	14.29 ± 0.54[a,b]	11.79 ± 0.49[a,b,c]
Number of corpora lutea	9.5 ± 0.42	9.80 ± 0.24	9.20 ± 0.40	9.80 ± 0.24
Number of foetuses (dead and live)/litter	8.8 ± 0.29	8.20 ± 0.20	7.00 ± 0.36[ab]	7.20 ± 0.24[ab]
Number of dead foetuses (number of litters)	All alive	All alive	3 (2)	12 (7)
Foetal weight (g)	1.31 ± 0.41	1.27 ± 0.03	1.14 ± 0.03[ab]	1.06 ± 0.029[ab]
Placental weight (g)	0.15 ± 0.007	0.15 ± 0.005	0.16 ± 0.006	0.15 ± 0.010
Crown-rump length (cm)	2.82 ± 0.38	2.75 ± 0.03	2.67 ± 0.04	2.60 ± 0.044
Number of resorption sites	0.30 ± 0.21	1.00 ± 0.21[a]	1.70 ± 0.3[a,b]	2.30 ± 0.273[abc]
Post-implantation loss (%)	4.32 ± 2.34	10.60 ± 2.11	21.93 ± 3.96[a,b]	34.60 ± 2.54[abc]

Values represent mean ± S.E. of 10 rats in each group. The significance of the difference among various groups was evaluated by applying one-way ANOVA ($p<0.05$). Comparison between two groups: [a]vs control, [b]vs 67mg/kg bw/d, [c]vs 125 mg/kg bw/d.

Table 25 Results reported by Junaid et al.,1996.

	Control	67 mg/kg bw/d	125 mg/kg bw/d	182 mg/kg bw/d
Number of pups/litter observed	40/10	40/10	30/10	25/10
Drooping wrist	0	3/2 (7.5)	3/2 (10)	4/3 (16)[a]
Sub-dermal hemorrhagic patches	0	0	3/2 (10)	4/3 (16)[a]
Kinky tail	0	0	2/1 (6.66)	3/3 (12)
Short tail	0	0	0	2/1 (8)
Skeletal abnormalities				
Number of pups/litter observed	40/10	40/10	30/10	25/10
Reduced nasal ossfication	0	0	0	16/8 (32)[a]
Reduced frontal ossification	0	0	0	10/6 (40)[a]
Reduced parietal ossification	0	0	0	8/5 (32)[a]
Reduced inter-parietal ossification	0	0	0	10/7 (40)[a]
Reduced caudal ossification	1/1 (2.5)	3/1 (7.5)	14/5 (46.6)[a]	21/7 (84)[a]
Reduced carpals ossification	0	0	0	0
Reduced metacarpals ossification	0	0	0	0
Reduced tarsals ossification	0	0	0	19/8 (76)[a]
Reduced claws ossification	0	0	0	0

Gross and skeletal abnormalities are represented as number of abnormal pups/litter observed; percentage in parentheses calculated by the total number of pups observed. Statistical significance evaluated by Fisher's Exact test ($p<0.05$); comparison between two groups: [a]vs. Control.

Elbetieha and Al-Hamood studied the effects of CrVI in female mice (n = 11-15) after exposure to 2000 and 5000 mg potassium dichromate/L in the drinking water (equivalent to 153 and 384 mg Cr/kg bw/d) during 12 weeks (see also under fertility).[22] A reduced water consumption was noted for the highest dose group. The relative ovary weight was significantly increased at the highest dose group. No effect on the number of pregnant females was noted. However, the number of implantations and the number of viable foetuses were significantly reduced in the chromium VI treated female mice mated with untreated male mice. The number of mice with resorptions was significantly increased in both dose groups compared to the control group.

Table 26 Developmental effects reported by Elbetieha and Al-Hamood, 1997.

	Control	153 mg/kg bw/d	384 mg/kg bw/d
Number of females	18	15	11
Number of pregnant females	17/18	14/15	9/11
Number of implantations	9.00 ± 1.36 (17)	7.35 ± 1.54 (14)**	7.44 ± 1.50 (9)
Number of viable foetusesa	8.76 ± 1.39 (17)	6.55 ± 2.18 (9)*	5.88 ± 2.47 (9)**
Number of mice with resorptions	2/18 (11%)	8/15 (53%)***	7/11 (63%)****
Total number of resorptions	4	37	14

* $p<0.05$, ** $p<0.01$ (compared to control value, Student's t-test); *** $p<0.01$, **** $p<0.005$ (compared to control value, Chi-square test)

Al-Hamood et al. exposed female Swiss mice from day 12 of gestation till day 20 of lactation to 0 or 1000 mg/L of potassium dichromate in their drinking water (equivalent to 71 mg Cr/kg bw/d).[38] Pups were culled to 8/litter at birth and weaned on postnatal day 21. At postnatal day 60 treated male offspring was mated with untreated females (1:2) and vice versa. Male fertility was not affected as measured by the number of pregnant females; the number of viable foetuses in females impregnated by exposed males was not affected. Female fertility was affected as shown by a reduced number of pregnant females, implantations and viable foetuses and increased number of resorptions. Vaginal opening was delayed. Another group of pups sacrificed at postnatal day 50 showed no effect on body weight, and weight of ovaries, uterus, testes, seminal vesicles and preputial glands.

Table 27 Developmental effects reported by Al-Hamood et al., 1998.

	Control	71 mg/kg bw/d
Number of animals	12	22
Number of pregnant animals	12/12 (100%)	14/22 (63.6%)
Number of implantations	10.25 ± 1.48	9.07 ± 1.14*
Number of viable foetuses	10.25 ± 1.48	8.85 ± 1.56*
Total number of resorptions	0	3

* $p<0.05$ Student's t-test

In a recent study, Sivakumar et al. exposed pregnant rats (n = 5) to 25 mg/L potassium dichromate in drinking water (equivalent to 1 mg Cr/kg bw/d) from gestational day 9.5 to 14.5.[51] Ovaries were removed from the F1 offspring on post natal day 1 and analyses were performed. Increased germ cell apoptosis was observed and was correlated with up-regulating of proteins associated with apoptosis. Also accelerated germ cell cyst breakdown was shown, together with advanced primordial follicle assembly and primary follicle transition and downregulation of survival pathway proteins. According to the authors, these event led to early reproductive senescence and a decrease in litters size in F1 female progeny.

Intraperitoneal or intravenous administration

As the Committee considers the intraperitoneal and intravenous route of administration of limited relevance for classification, no summarising tables are provided for these studies.

Marouani et al. have investigated the embryo- and foetotoxicity of potassium dichromate in rats.[39] Female Wistar rats (n = 8) were administered 0, 1 or 2 mg Cr/kg bw/d as potassium dichromate in saline intraperitoneally from day 6 to15 of gestation. Treated dams showed no mortality or changes in clinical signs, but a significant reduction in body weight gain (to 71 and 40% of control at 1 and 2 mg/kg bw/d, respectively) and relative weight of the uterus (79 and 70% of control at 1 and 2 mg/kg bw/d, respectively) was seen. A significantly reduced number of foetuses (dead and alive)/litter, increased number of dead foetuses and litters with dead foetuses, reduced foetal weight and crown rump length were noted at both dose levels. The number of resorptions was increased at both doses. Consequently, post implantation loss had significantly increased in a dose-dependent manner. Placenta weight was also significantly reduced at both dose levels. External examination of the foetuses revealed oedema, and subdermal haemorrhagic patches on the thoracic and abdominal regions at both doses. At 2 mg/kg bw/d effects were more severe and also facial defect, lack of tail and hypotrophy were observed. Skeletal abnormalities observed by radiography at 1 mg/kg bw/d included incomplete ossification in nasal, cranium, abdominal or caudal bones, while at 2 mg/kg bw/d also absence of ossification of the sacral vertebrae was noted. Microscopic inspection revealed atrophy of organs such as liver, lung, heart and sacral vertebrae at 2 mg/kg bw/d. Histopathological examination of the placenta showed dose-dependent atrophy of decidual cells, degeneration of chorionic villi and hypertrophy of blood lacuna.

Endo and Watanabe dosed Jcl:ICR mice intraperitoneally on gestation day 9 with 0 or 15 mg chromium trioxide/kg bw (equivalent to 0 or 8 mg Cr/kg bw).[40] Dams were killed on gestation day 17 and foetuses were examined for external malformations, sexed and about one third of the foetuses were examined for skeletal malformations and variations. No effect was observed on number of live, dead or resorbed foetuses, male and female foetal weight or number of malformations.

Gale treated golden hamsters (LAK:LVG(SYR) intravenously with 0, 5, 7.5, 10 and 15 mg chromium trioxide/kg bw (equivalent to 0, 3, 4, 5 or 8 mg Cr/kg bw) on day 8 of gestation.[41] Dams were killed on gestation day 12, 14 or 15 and all foetuses were examined for external malformations. Of the 15-day-old foetuses approximately half were examined for visceral abnormalities and half for skeletal abnormalities. Some of the 14- and 15-day-old foetuses were examined by dissection of the abdominal, thoracic and cephalic region. The high dose induced severe maternal mortality shortly after treatment; 3 out of 4 females

died. At 4 and 5 mg/kg bw weight loss, mottled kidneys with extensive tubular necrosis and hyaline casts was described; some livers exhibited clear vacuoles in some of the periportal hepatocytes. A dose-related increased number of resorptions and foetal abnormalities, including cleft palates and skeletal defects, and hydrocephalus was observed. At all dose levels retarded ossification of the vertebral column and sternum, skull (4 and 5 mg/kg bw), hyoid bone, hind limb, fore limb (4 and 5 mg/kg bw) was noted.

Gale and Bunch dosed hamsters intravenously with a single injection of 0 and 8 mg chromium trioxide/kg bw (equivalent to 4 mg Cr/kg bw) on gestation days 7, 8, 9, 10 or 11.[42] Dams were killed on gestation day 15 and foetuses were examined for external and internal malformations. All chromium-treated dams lost weight with the most severe effect in dams dosed on gestation day 7. The kidneys of most treated dams were mottled with varying degrees of tubular necrosis. Examination of pups showed after dosing on gestation days 7, 8, 9 and 10 a decreased average crown-rump length compared to controls. From dams treated on gestation day 7, 8 and 9, an increased number of live foetuses with external defects and cleft palates were observed. Based on the average crown-rump length, the chromium-exposed foetuses with cleft palates are smaller than the chromium-treated foetuses with normal palates. Internal defects observed were small or absent kidney in one and four foetuses after dosing on gestation day 7 and 8, respectively, compared to none in the control groups. No effects were observed after dosing on gestation days 11.

5.2.2 Human studies

In the human studies on exposure to chromium and other metals present in welding fumes, exposure levels were not determined.

Bonde investigated the effects on offspring among male welders in a nationwide Danish cohort consisting of male workers employed for at least 1 year at 79 Danish stainless steel or mild steel manufacturing companies from April 1964 through December 1984 (n = 10,059), who fathered 3569 children in 1973 through 1986.[31] Occurrences of low birth weight, pre-term delivery, infant mortality and congenital malformations were not increased among children at risk from either paternal stainless steel or mild steel welding. Increased occurrences of any organ-specific congenital malformation were not observed either.

Hjollund et al. recruited a cohort of first-pregnancy planners from members of the union of metal workers and three other trade unions in Denmark.[43] The cohort (n = 406) was followed for 6 menstrual cycles from cessation of contraceptive use. In total, 280 pregnancies were conceived, of which 203 had a paternal background without welding exposure, 54 with mild steel welding (but not stainless steel) and 23 with stainless steel welding. Of the spontaneous abortions, 36 were clinically diagnosed and 35 were detected by human chorionic gonadotrophic hormone analysis but did not survive to a clinically recognized pregnancy. The relative risk for spontaneous abortion from paternal exposure to stainless steel welding (10 cases) was increased in comparison with pregnancies without paternal welding exposure (RR 3.5, 95% CI 1.3-9.1), adjusted for the confounders centre, female age, female body mass index, menstrual cycle length, male and female smoking, caffeine and alcohol consumption, and reproductive disease. Separate analysis of early pregnancy loss and loss of clinically recognized pregnancies also showed increased risks (RR 3.0, 95% CI 1.1-8.0, and RR 3.2, 95% CI 1.1-9.8, respectively). All spontaneous abortions in spouses of stainless steel workers happened before the 10th gestational week. The risk of pregnancy loss increased with the number of years of stainless-steel welding, being 2.6 (95% CI 1.1-6.1) after 5 years. Mild steel welding, which does not involve exposure to hexavalent chromium, was not associated with an increased risk of pregnancy loss.

Hjollund et al. also investigated the association between welding exposure and survival of pregnancies after in vitro fertilization (IVF).[44] From the Danish IVF register, couples with the first treatment after 1 January 1996 were selected after completing a questionnaire (n = 4,007). Excluded were treatment with donated or thawed eggs, fertilization with donor sperm and treatment cycles ending with ectopic pregnancies, mola or induced abortion. A subgroup of male metal workers received a second questionnaire on exposure to welding (n = 319) and was classified as stainless steel welder (n = 91), mild steel welder (n = 128), other welder or non-welder (n = 100). The proportion of miscarriages before the 28th gestational week was 17.6% (16 out of 91) in pregnancies where the father welded stainless steel compared to 25.0% (32 out of 128) for mild steel welding and 28.4% in the group of unexposed reference pregnancies (830 out of 2,925). The risk ratios for miscarriages from pregnancies with paternal exposure to stainless steel and mild steel welding were 0.6 (95% CI 0.4-1.0) and 0.95 (95% CI 0.7-1.4), respectively, after adjustment for IVF center, male and female smoking, coffee and alcohol consumption, age and number of transferred embryos. Number of hours with stainless steel welding or number of years with

stainless steel welding had no effect on the estimates. Risk estimates were similar when based on analyses with spontaneous abortion defined as foetal death before gestational week 20. According to the authors, this result should not be extrapolated to natural pregnancies as the cohort was preselected by their reproductive problems and spontaneous abortion in IVF is only registered in case of death of all implanted embryos (usually the two most viable ones) resulting in an overestimation of foetal survival.

Aschengrau et al., investigated potential associations between levels of drinking water contaminants, including chromium, and the occurrence of late adverse pregnancy outcomes in a case-control study.[45] Trace element levels were gathered from routine analyses of public water supplies from the residing communities and compared between 1039 congenital anomaly cases, 77 stillbirth cases, 55 neonatal death cases, and 1177 controls. No differences were found between women residing in areas with detectable chromium levels, and those who resided in areas with undetectable chromium levels. The Committee notes that this study provides no information on actual chromium VI exposure.

5.3 Other relevant information

Lactation

Animal studies

Table 28 Developmental toxicity studies in animals after lactational exposure.

authors	species	experimental period/ design	dose/route	general toxicity	developmental toxicity
Banu et al., 2008	Rat; Wistar; n not specified	Postnatal day 1-20; pilot study	0, 50, 100, 200 or 400 mg Cr/L as potassium dichromate in drinking water (6, 12, 25 or 50 mg Cr/kg bw/d)/ oral	50 mg/kg: weight loss; weakness; decrease intake of food and water; decrease uterus and ovary weights; 25 mg/kg: similar symptoms but less severe NOAEL=12 mg Cr/kg bw/d	25 and 50 mg/kg: decreased pup weight; follicle number, increased follicular atresia NOAEL=12 mg Cr/kg bw/d

Banu et al., 2008	Rat; Wistar; n = 18	Litters culled to 4 female pups; dams exposed on postnatal day 1-21; groups of 6 dams (= 24 pups) sacrifice on PND 21, 45 and 65; blood and ovary parameters; onset of puberty; follicle number	0 or 200 mg Cr/L as potassium dichromate in drinking water (0 or 25 mg Cr/kg bw/d)/oral	No maternal toxicity for main study described	Delay in vaginal opening, extended dioestrous phase of the oestrous cycle, At different days after partus: impaired follicle development, decreased plasma levels of of oestradiol, testosterone and progesterone; decreased growth hormone and prolactin plasma levels and increased follicle stimulating hormone LOAEL= 25 mg Cr/kg bw/d
Samuel et al., 2011	Rat; Wistar; n = 12	During PND 1-21; at birth litters were culled to 4 female pups/dam; pups sacrificed on PND 42 and 65	0, 50 or 200 mg Cr/L as potassium dichromate in drinking water (6 or 25 mg Cr/kg bw/d)/oral	Decreased body weight at both doses, severely at 25 mg/kg NOAEL=6 mg Cr/kg bw/d	Decreased uterus weight at 200 mg; delayed puberty at both doses (dose-related); extended oestrous cycle (dioestrous stage) at both doses; also extended metoestrous stage at 200 mg; decreased uterine activity of antioxidants and serum testosterone and progesterone at both doses (dose-related); increased H2O2 and lipid peroxidation, and FSH at both doses (dose-related) LOAEL=6 mg Cr/kg bw/d
Stanley et al., 2013	Rat; SD; n = 5	During PND 1-21; at birth litters were culled to 4 female pups/dam; pups sacrificed on PND 25, 45 and 65	0, 50 100, or 200 mg Cr/L as potassium dichromate in drinking water (0, 6, 12 or 25 mg Cr/kg bw/d)/oral	No maternal toxicity for main study described	Increased follicular atresia and decreased steroidogenesis in PND 25, 45 and 65, Vitamin C (partly) inhibited these effects; ic hydrogen peroxide and lipid hydroperoxide in plasma and ovary; decreased antioxidant enzymes (AOXs)GPx1, GR, SOD, and catalase; ic glutathione S-transferase in plasma and ovary LOAEL=6 mg Cr/kg bw/d

Banu et al. investigated lactational exposure to chromium VI on the puberty of female offspring from Wistar rats.[46] Litters from pregnant rats (n = 18) were culled to four female pups on the day of birth. Dams were given 0 or 200 mg/L potassium dichromate in the drinking water (equivalent to 25 mg Cr/kg bw/d) from the day of parturition to postnatal day 21. On postnatal day 21, 45 and 65 each, female pups from 6 dams/group (n = 24) were sacrificed and blood and ovaries collected. Chromium levels in plasma and ovarian tissue were higher in exposed female offspring than in controls and decreased with aging of the pups. Vaginal opening was delayed (55 days in treated rats compared to 33 days in control rats). While the dioestrous phase of the estrous cycle was extended, the length of the pro-oestrous, oestrous and metoestrous phases was not affected. On postnatal day 21 with chromium CI treatment decreased primordial, primary and secondary follicle numbers and resulted in no antral follicle development. On postnatal day 45 all follicle numbers were decreased. On postnatal day 65 only primordial and primary follicle numbers were decreased. Plasma levels of oestradiol, testosterone and progesterone were decreased in all three age groups

compared to controls. Chromium VI treatment did not change plasma luteinizing hormone, but increased follicle stimulating hormone on postnatal day 21 and 45. Both hormones were not affected on postnatal day 65. Growth hormone and prolactin plasma levels were decreased compared to controls in all three age groups. In the main study no maternal effects were described, but in the pilot study where dams were exposed to 50, 100, 200 or 400 mg/L potassium dichromate (6, 12, 25 or 50 mg Cr/kg bw/d) for postnatal day 1-20, severe toxic symptoms such as weight loss, weakness, and reduced intake of food and water, reduced uterus and ovary weights were observed at 50 mg Cr/kg bw, while these symptoms were low to moderate at 25 mg Cr/kg bw. Simultaneous administration of vitamin C (500 mg/L) protected against the CrVI developmental effects.

The same group hypothesized that lactational exposure to chromium VI would induce oxidative stress and disrupt uterine function in female offspring from Wistar rats.[47] Litters from pregnant rats (n = 12) were culled to four female pups on the day of birth. Dams were given 0, 50 or 200 mg/L potassium dichromate in the drinking water (equivalent to 6 and 25 mg Cr/kg bw/d) from the day of parturition to postnatal day 21. On postnatal day 42 and 65 each, female pups from 6 dams/group (n = 24) were sacrificed and blood and uterus collected. Chromium levels in plasma and uterine tissue were higher in exposed female offspring than in controls and decreased with aging of the pups. Pup weight was decreased in exposed groups in a dose-related manner at postnatal day 42 and 65. Uterus weight was decreased at 200 mg/L only at both times. As in the former study the dioestrous phase of the oestrous cycle was extended at both dose levels, while the metoestrous stage was extended at 200 mg/L. Uterine activities of superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and glutathione S-transferase were decreased at both dose levels with only partial recovery at postnatal day 65. Increased concentrations of lipid peroxidation and hydrogen peroxide were observed in chromium VI-treated pups with higher levels at postnatal day 65. The steroid hormones (testosterone, oestradiol and progesterone) showed a dose-related decrease in both age groups. Follicle stimulating hormone was elevated at both dose levels and age groups. Luteinizing hormone was only markedly increased at 200 mg/L at postnatal day 45.

In a subsequent study with a similar design, this research group tested the hypothesis that lactational exposure to potassium dichromate accelerates follicle atresia in F1 offspring by increasing reactive oxygen species and decreasing

cellular antioxidants.[52] The results showed that lactational exposure to potassium chromate increased follicular atresia and decreased steroidogenesis at postnatal day 25, 45, and 65 in a dose dependent manner. In addition, potassium chromate increased hydrogen peroxide and lipid hydroperoxide in plasma and ovary and decreased several antioxidant enzymes while glutathione S-transferase was increased.

In a study of Al-Hamood et al.[38] mice were exposed from day 12 of gestation till day 20 of lactation and in the study of Soudani et al.[33-35] rats were exposed from gestation day 14 to postnatal day 14. The effects observed are described under developmental toxicity. Since it is not clear whether effects observed are due to exposure in utero or via lactation, these studies were not considered for drawing conclusions on labelling for lactation.

Human studies

Chromium concentrations in human breast milk within Europe range from 0.09 to 19.8 µg/L as presented in the review of University of East Anglia.[48] Two studies from this review measured maternal chromium intake. In the first study, a mean maternal chromium intake of 41.08 ± 0.416 mg/day (n = 17) was found with a concomitant chromium level of 0.184 ± 0.021 µg/L in breast milk (number of samples not specified). In the second study, the mean maternal chromium intake was 256 ± 187 mg/day (n = 19) with a chromium level of 10.8 µg/L (range 3.1-19.4 µg/L; 536 samples) in breast milk. Both studies showed no direct correlation between chromium intake and breast milk concentration. This was confirmed by an isotope study in which mothers were given ^{53}Cr orally, which was, subsequently, not found in their breast milk. No reliable data on occupationally exposed women are available.

5.4 Summary and discussion of reproductive toxicity

Fertility and developmental effects of chromium VI have been studied in humans and animals.

Human studies investigated male and female fertility parameters in relation to stainless steel welding, which involves chromium VI exposure in the fumes.[12,27-31,49] Inconsistent results were described and in all cases co-exposure to other substances was present in the fumes.

In multiple species, effects on the gonads were observed after exposure to chromium VI; the chromium VI was administered as sodium dichromate, potassium dichromate or chromium trioxide (one study only). In two studies in monkeys, and two studies in rabbits, various sperm parameters were affected (involving a decrease in testis weight, disruption of spermatogenesis, and a decrease in sperm number and motility).[10,11,23,26,27] Sperm quality and quantity were also affected in several studies with rats and mice.[12,15,20,22,24] In contrast, two NTP-studies in rats and three in mice did not show any adverse effects on sperm.[3,5,16-18,50] The Committee notes that in rats the NTP applied relatively low doses of chromium compared to other oral studies, and only mild signs of general toxicity were observed and only at the highest dose (i.e. effects on blood parameters, small decreases of body weight and absolute liver weight, cytoplasmic vacuolisation in hepatocytes). In mice, the oral results were inconsistent as one study[20] showed sperm effects at dose levels of 11 mg Cr/kg bw/day and above, which did not induce any effects in the NTP studies. However, effects on testes and fertility were observed in the only study with 148 mg Cr/kg bw/day and above.[22] In this study, a correlation was observed of decreased seminal vesicle and preputial gland weight with a decreased number of implantations and number of viable foetuses when non-exposed female mice were mated with exposed males. Overall, all four tested species show clear effects on testes, sperm counts and/or fertility. The absence of effects in some studies can at least partly be explained by the low dose levels tested.

Table 29 Summary of effects on fertility in male animals.

Males	Species	Substance	Exposure route	General toxicity	Effects on fertility
Yousef et al., 2006	Rabbit	Potassium dichromate	Oral (gavage)	NOAEL=4 mg/kg bw/d (no effects observed)	LOAEL=4 mg/kg bw/d (various sperm parameters)
Behari et al., 1978	Rabbit	Potassium dichromate	i.p.	NOAEL=0.7 mg/kg bw/d (no effects observed)	LOAEL=0.7 mg/kg bw/d (number of spermatocytes)
Subramanian et al., 2006; Aruldas et al. 2005	Monkey	Potasium dichromate	Oral (drinking water)	NOAEL=23 mg/kg bw/d (no effects observed)	NOAEL<6 mg/kg bw/d (several sperm parameters)
Glaser et al., 1984	Rat	Sodium dichromate	Inhalation	NOAEL=0.2 mg/kg bw/d (no effects observed)	NOAEL=0.2 mg/kg bw/d (no effects observed)
Li et al., 2001	Rat	Chromium trioxide	Oral (diet)	NOAEL=10 mg/kg bw/d (no effects observed)	LOAEL=5 mg/kg bw/d (several sperm parameters)
Chowdhur and Mitra, 1995	Rat	Sodium dichromate	Oral (gavage)	NOAEL=8 mg/kg bw/d (decreased body weight gain)	LOAEL=8 mg/kg bw/d (reduction testicular protein and serum, testosterone)
NTP, 1996; 2007	Rat	Potassium dichromate	Oral (diet)	NOAEL=2 mg/kg bw/d (haematotoxicity)	NOAEL=9 mg/kg bw/d (no effects observed)

Ernst, 1990	Rat	Sodium chromate	i.p.	LOAEL= 1 mg/kg bw/d (decreased body weight gain)	LOAEL=1 mg/kg bw/d (number of testicular parameters (cellular and morphology))
Ernst and Bond, 1992	Rat	Sodium chromate	i.p.	NOAEL= 0.5 mg/kg bw/d (no effects observed)	LOAEL=0.5 mg/kg bw/d (hormones; sperm motility)
Zahid et al., 1990	Mouse	Potassium dichromate	Oral (diet)	NOAEL=46 mg/kg bw/d (no effects observed)	LOAEL=11 mg/kg bw/d (several sperm parameters)
NTP, 1996	Mouse	Potassium dichromate	Oral (diet)	NOAEL=1 mg/kg bw/d (haematotoxicity)	NOAEL=41 mg/kg bw/d (no effects observed)
NTP, 1997	Mouse	Potassium dichromate	Oral (diet)	NOAEL F0=30 mg/kg bw/d (no effects observed) NOAEL F1=8 mg/kg bw/d (haematotoxicity)	NOAEL F0=30 mg/kg bw (no effects observed) NOAEL F1=37 mg/kg bw/d (no effects observed)
NTP, 2007	Mouse	Potassium dichromate	Oral (diet)	LOAEL=3 mg/kg bw/d (haematotoxicity)	NOAEL=9 mg/kg bw/d (no effects observed)
Elbetieha and Al-Hamood, 1997	Mouse	Potassium dichromate	Oral (drinking water)	NOAEL=74 mg/kg bw/d (reduced body weight)	NOAEL=74 mg/kg bw/d (testis weight; number of implantations)

For female reproduction toxicity, several studies with rats and mice are available with different results. Effects on the oestrous cycle were observed in rats and mice.[13,14,21] Further, a reduced mating index was observed in female rats.[13,14] In studies with female mice, also effects on ovary were observed, and a reduced number of implantations. In contrast, no histological lesions were observed in the ovaries of rats and mice in studies by the NTP.[5,16,17] Also no effect was observed on fertility of the F0- and F1-generation in an NTP continuous breeding study with mice and no effect on the oestrous cycle or gonadal organ weights were noted either.[18] However, the highest dose levels (30 and 41 mg Cr/kg bw/d) applied in the NTP studies were below the LOAEL (60 mg Cr/kg bw/d and above) in the oral studies that showed reductions in implantations and decreased number of foetuses in mice.

Table 30 Summary of effects on fertility in female animals.

Females	Species	Substance	Exposure route	General toxicity	Effects on fertility
Kanojia et al., 1996	Rat	Potassium dichromate	Oral (drinking water)	LOAEL=31 mg/kg bw/d (body weight)	LOAEL=31 mg/kg bw/d (reduced mating index)
Kanojia et al., 1998	Rat	Potassium dichromate	Oral (drinking water)	NOAEL=45 mg/kg bw/d (mortality)	LOAEL=45 mg/kg bw/d (persistent dioestrous phase)
NTP, 1996; 2007	Rat	Potassium dichromate	Oral (drinking water)	LOAEL=4 mg/kg bw/d (haematotoxicity)	NOAEL = 21 mg/kg bw/d (no abnormalities ovaries)

NTP, 1996; 2007	Mouse	Potassium dichromate	Oral (drinking water)	NOAEL=1 mg/kg bw/d (haematotoxicity)	NOAEL = 41 mg/kg bw/d (no abnormalities ovaries)
Trivedi et al., 1989	Mouse	Potassium dichromate	Oral (drinking water)	NOAEL=59 mg/kg bw/d (reduced body weight)	NOAEL=59 mg/kg bw/d (pre-implantation loss)
Murthy et al., 1996	Mouse	Potassium dichromate	Oral (drinking water)	Sub-acute: NOAEL=163 mg/kg bw/d (no effects observed) 90-d: NOAEL=1.1 mg/kg bw/d (no effects observed)	Sub-acute: LOAEL=54 mg/kg bw/d (decreased number follicles, ova, etc.) 90-d: NOAEL=0.1 mg/kg bw/d (abnormailities follicular and fecal cells)
Elbetieha and Al-Hamood, 1997	Mouse	Potassium dichromate	Oral (drinking water)	NOAEL=74 mg/kg bw/d (reduced body weight)	NOAEL=74 mg/kg bw/d (ovary weight; number of implantations)

In conclusion, animal studies show effects on structural and functional fertility parameters in males and females of several species after exposure to chromium VI compounds, although a relation of the effects on structural parameters with functional fertility is not clear. Therefore, classification of chromium VI compounds for effects on fertility is warranted.

With regard to developmental toxicity, one of the three human studies available indicates an increased risk after exposure to stainless steel welding.[31,43,44] However, no conclusions can be drawn as the stainless steel welding fumes have not been analysed for chromium VI and co-exposures to other toxic substances occurred.

Developmental toxicity studies with chromium VI in rats, mice and hamsters are available, exposed via various routes. Developmental toxicity studies with chromium VI in rats and mice have shown effects of exposure via drinking water pre-mating (mice) and during gestation (both species). Male or female mice exposed before mating resulted in an increased number of resorptions, reduced number of implantations and viable foetuses.[13,14,22] After oral gestational exposure in rats and mice, clear adverse effects on offspring were seen in absence of maternal toxicity (i.e. increased number of resorptions, pre- and post-implantation loss, reduced litter size, foetal weight and length, and retarded ossification).[19,32,37-39] Furthermore, an increased number of cleft palate, skeletal defects and hydrocephalus were noted after intravenous administration of chromium trioxide hamster in the absence and presence of maternal toxicity.[41,42]

Table 31 Summary of effects on development in animals.

Females	Species	Substance	Exposure route	General toxicity	Effects on development
Kanojia et al., 1996	Rat	Potassium dichromate	Oral (drinking water)	LOAEL = 31 mg/kg bw/d (decreased body weight gain)	LOAEL = 31 mg/kg bw/d (decreased number of live foetuses, increased number of resorptions, post-implantation loss)
Kanojia et al., 1998	Rat	Potassium dichromate	Oral (drinking water)	LOAEL = 45 mg/kg bw/d (decreased gestational body weight gain)	LOAEL = 45 mg/kg bw/d (decreased number of live foetuses/litter, foetal weight and crown-rump length, increased number of resorptions and implantation loss
Elsaieed and Nada, 2002	Rat	Potassium dichromate	Oral (drinking water)	LOAEL = 7 mg/kg bw/d (decreased gestational weight)	LOAEL = 7 mg/kg bw/d (increased post-implantation loss, resorptions, dead foetuses, visceral and skeletal anomalies, decrease foetal weight)
Soudani et al., 2011a,b,c	Rat	Potassium dichromate	Oral (drinking water)	LOAEL = 24 mg/kg bw/d (decreased body weight, changes in biochemical parameters and histopathology in dams)	LOAEL = 24 mg/kg bw/d (decreased body weight, changes in biochemical parameters and histopathology in pups)
Sivakum et al., 2014	Rat	Potassium dichromate	Oral (drinking water)	(not reported)	LOAEL = 1 mg/kg bw/d (decreased litter size, cellular abnormalities)
Glaser et al., 1984	Rat	Sodium dichromate	Inhalation	LOAEL= 0.2 mg/kg bw/d (local hyperplasia, increased relative organ weight)	NOAEL= 0.2 mg/kg bw/d (no effects observed)
Maroua et al., 2010	Rat	Potassium dichromate	ip	LOAEL = 1 mg/kg bw/d (decreased body weight gain and relative uterus weight)	LOAEL = 1 mg/kg bw/d (decreased foetal body weight gain, reduced number of foetuses/ mother, increased incidences of dead foetuses and resorption, morphological abnormalities and incomplete ossification)

Trivedi et al., 1989	Mouse	Potassium dichromate	Oral (drinking water)	NOAEL = 120 mg/kg bw/d (decreased body weight)	LOAEL = 60 mg/kg bw/d (increased number of resorptions, postimplantation loss, decreased litter size, retarded ossification)
Junaid et al., 1996	Mouse	Potassium dichromate	Oral (drinking water)	NOAEL = 125 mg/kg bw/d (decreased body weight gain)	LOAEL = 67 mg/kg bw/d (increased number of resorptions, post-implantation loss, decreased number of foetuses)
Elbetieha and Al-Hamood 1996	Mouse	Potassium dichromate	Oral (drinking water)	LOAEL = 148 mg/kg bw/d (decreased body weight)	LOAEL = 148 mg/kg/ bw/d (decreased number of viable foetuses, increase number resorptions)
NTP, 1997	Mouse	Potassium dichromate	Oral (diet)	NOAEL(F0) = 7 mg/kg bw/d (decreased body weight) LOAEL (F1) = 8 mg/kg bw/d (decreased MCH and haemoglobin)	NOAEL(F0) = 30 mg/kg bw/d (no effects observed) NOAEL (F1) = 37 mg/kg bw/d (no effects observed)
Al-Hamood et al., 1998	Mouse	Potassium dichromate	Oral (drinking water)	NOAEL = 71 mg/kg bw/d (no effects observed)	LOAEL = 71 mg/kg bw/d (increased number of resorptions, implantations and viable foetuses)
De Flora et al., 2006 (genotoxicity study)	Mouse	Sodium dichromate dihydrate and potassium dichromate	Oral (drinking water)	NOAEL = 2 mg/kg bw/d (no effects observed)	NOAEL 2 mg/kg bw/d (no effects observed)
Endo and Watana, 1988	Mouse	Chromium trioxide	ip	NOAEL = 8 mg/kg bw/d (no effects observed)	NOAEL = 8 mg/kg bw/d (no effects observed)
De Flora et al., 2006 (genotoxicity study)	Mouse	Potassium dichromate	ip	NOAEL = 50 mg/kg bw/d (no effects observed)	NOAEL 50 mg/kg bw/d (no effects observed)
Gale (1978)	Hamster	Chromium trioxide	iv	NOAEL= 2 mg/kg bw/d (decreased body weight, nephrotoxicity and hepatotoxicity)	LOAEL= 2 mg/kg bw/d (increased number of resorptions and malformations, retarded ossifcation)
Gale and Bunch (1979)	Hamster	Chromium trioxide	iv	LOAEL= 4 mg/kg bw/d (decreased body weight, nephrotoxicity and hepatotoxicity)	LOAEL= 4 mg/kg bw/d (increased number of resorptions and malformations, decrease crown-rump length)

In conclusion, despite the lack of clear evidence from human studies, animal studies with several species demonstrate chromium VI-induced developmental toxicity in the absence of maternal toxicity. Therefore, classification for developmental toxicity is warranted.

Two studies are available in which rats were exposed during lactation only. In the first study dams were exposed to 25 mg Cr/kg bw/d via drinking water from parturition until postnatal day 21 and puberty of female pups was studied. Various adverse effects were observed, including delayed vaginal opening, extended diestrus phase, decreased follicle numbers, and lack of antral follicle development. In an additional study from the same group in which dams were exposed to 6 or 25 mg Cr/kg bw/d, female pups showed an extended metestrus phase and increased level of luteinizing hormone at the high dose, and at both dose levels decreased pup weight, and increased oxidative stress. In both studies, chromium levels in plasma and uterine/ovarian tissue of female pups were higher than in those of controls and decreased with aging of the pups. Since no levels in breast milk were measured, toxicity cannot be directly attributed to the chromium VI level in breast milk.

5.5 Comparison with criteria

For effects on fertility and development, substances can be classified in category 1 (further divided in 1A and 1B) and category 2. The classification of substances in category 1A (known human reproductive toxicants) is largely based on evidence from humans, whereas the classification in category 1B (presumed human reproductive toxicant) is largely based on specific reproduction toxic effects observed in animals, which are considered relevant for humans. When evidence is found (either in humans or animals) which is not sufficient for classification in category 1, a substance is classified in category 2.

From human data, no clear association between chromium VI exposure and fertility parameters was observed.

The Committee notes that in animals, effects on fertility parameters have been observed in multiple species. These studies show consistent adverse effects on sperm parameters. In addition, reduced fertility (i.e. reduced number of implantations, reduced number of viable fetuses) has been observed after exposure of males and females. The Committee notes that it is not completely clear to what extent general toxicity was induced at the conditions under which these studies were performed. Several studies however, suggest that toxicity to fertility is induced in absence of general toxicity. Thus, the fertility effects should

be regarded as direct effects on sex organ function. Given the convincing findings in the studies showing adverse effects on fertility, which are considered relevant for humans, the Committee proposes to classify chromium VI compounds for effects on fertility in category 1B (presumed human reproductive toxicant) and to label with H360F (may damage fertility) according to Regulation (EC) 1272/2008.

From human data, no clear association between chromium VI exposure and developmental parameters was observed.

Various adverse effects on development have been observed in animals exposed to chromium VI. For the developmental toxicity studies, the Committee also notes that it is not completely clear to what extent general toxicity was induced at the conditions under which these studies were performed. Several studies however, suggest that severe developmental toxicity (i.e. prenatal deaths and malformations) is induced in absence of maternal toxicity. Thus, the developmental toxicity observed should be considered as a direct effect on the developing offspring and is unlikely to have been mediated by maternal toxicity. The Committee therefore considers this relevant for humans, and recommends to classify chromium VI compounds in category 1B (presumed human reproductive toxicant) and label with H360D (May damage the unborn child) according to Regulation (EC) 1272/2008.

Several studies have shown developmental effects and oxidative stress in pups after chromium VI exposure to lactating rats. The Committee further notes that most chromium VI compounds are classified for carcinogenic and genotoxic properties (see Section 2.4). No threshold can be established for (stochastic) genotoxic carcinogens and the presence of chromium VI in breast milk cannot be excluded. Therefore, the Committee proposes to label chromium VI compounds for effects on or via lactation.

Although the vast amount of data is derived from sodium dichromate and potassium dichromate, the Committee considers the conclusions applicable to all chromium VI compounds specified in this report (see Chapter 4 on read-across and toxicokinetics).

5.6 Conclusions on classification and labelling

The Committee recommends classification according to Regulation (EC) 1272/2008 of the European Union. For chromium trioxide, sodium chromate, sodium

dichromate, potassium dichromate, chromic acid, ammonium chromate, ammonium dichromate, calcium chromate, potassium chromate, dichromium tris(chromate), the Committee recommends:

- for effects on fertility, to classify these compounds in *category 1B (presumed human reproductive toxicant)*, and to label them with *H360F (may damage fertility)*
- for effects on development, to classify these compounds in *category 1B (presumed human reproductive toxicant)* and to label them with *H360D (may damage the unborn child)*
- for effects during lactation, to label these compounds with H362 *(may cause harm to breastfed babies)*.

Proposed classification for fertility

Category 1B, H360F.

Proposed classification for developmental toxicity

Category 1B, H360D.

Proposed labelling for effect during lactation

H362.

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A	The Committee
B	The submission letter (in English)
C	Comments on the public review draft
D	Regulation (EC) 1272/2008 of the European Community
E	Additional considerations to Regulation (EC) 1272/2008

Annexes

Annex **A**

The Committee

- D. Lindhout, *chairman*
  Professor of Medical Genetics, Paediatrician (not practising), clinical geneticist, University Medical Centre, Utrecht
- N. Roeleveld
  Reproductive Epidemiologist, Radboud university medical center, Nijmegen
- J.G. Theuns-van Vliet
  Reproductive Toxicologist, Triskelion BV, Zeist
- T.G.M. Vrijkotte *(since February 1, 2016)*
  Epidemiologist, Academic Medical Center, Amsterdam
- D.H. Waalkens-Berendsen
  Reproductive Toxicologist, Zeist
- P.J.J.M. Weterings
  Toxicologist, Weterings Consultancy BV, Rosmalen
- A.H. Piersma, *structurally consulted expert*
  Professor of Reproductive and Developmental Toxicology, National Institute of Public Health and the Environment, Bilthoven
- S.R. Vink, *scientific secretary*
  Health Council of the Netherlands, Den Haag

With respect to the data presentation and interpretation, the Committee consulted Ing. J.J.A. Muller and dr. G. Tiesjema as additional experts from Bureau REACH, National Institute of Public Health and the Environment, Bilthoven.

The Health Council and interests

Members of Health Council Committees are appointed in a personal capacity because of their special expertise in the matters to be addressed. Nonetheless, it is precisely because of this expertise that they may also have interests. This in itself does not necessarily present an obstacle for membership of a Health Council Committee. Transparency regarding possible conflicts of interest is nonetheless important, both for the chairperson and members of a Committee and for the President of the Health Council. On being invited to join a Committee, persons are asked to submit a form detailing the functions they hold and any other material and immaterial interests which could be relevant for the Committee's work. It is the responsibility of the Health Council to assess whether or not someone can become a member. An expert who has no financial but another clearly definable interest, can become a member under the restriction that he will not be involved in the debate on the subject to which his interest relates. If a person's interest is not clearly definable, he can sometimes be consulted as an expert. Experts working for a ministry or governmental organisation can be structurally consulted. During the inaugural meeting the declarations issued are discussed, so that all members of the Committee are aware of each other's possible interests.

Annex **B**

The submission letter (in English)

Subject : Submission of the advisory report *Chromium VI compounds*
Your reference : DGV/BMO/U-932542
Our reference : U-963412/SV/jh/543-G16
Enclosure(s) : 1
Date : May 18, 2016

Dear Minister,

I hereby submit the advisory report on the effects of a group of chromium VI compounds on fertility and on the development of the progeny; it also concerns effects on lactation and on the progeny via lactation.

This advisory report is part of an extensive series in which reproduction toxic substances are classified in accordance with European guidelines. It concerns substances to which people may be exposed occupationally. This advisory report is an update of an advisory report that was published by the Health Council in 2001. This update was requested since the previously recommended classification deviates from the current classification in the EU.

The advisory report was prepared by a permanent Committee of the Health Council of the Netherlands, the Subcommittee on the Classification of Reproduction Toxic Substances. The advisory report was consequently reviewed by the Health Council's Standing Committee on Public Health.

Today I sent copies of this advisory report to the Minister of Health, Welfare and Sport and to the State Secretary of Infrastructure and the Environment, for their information.

Yours sincerely,
(signed)
Prof. dr. J.L. Severens,
Vice President

Annex C

Comments on the public draft

A draft of the present report was released in 2015 for public review. The following organisations and persons have commented on the draft document:

- T.J. Lentz, L. Greenawald, S.S. Leonard, National Institute for Occupational Safety and Health (NIOSH), Cincinnati, OH, USA
- J. Arts, AkzoNobel NV.

The comments received, and the reply by the Committee can be found on the website of the Health Council.

Annex **D**

Regulation (EC) 1272/2008 of the European Community

3.7 Reproductive toxicity

3.7.1 Definitions and general considerations

3.7.1.1 Reproductive toxicity includes adverse effects on sexual function and fertility in adult males and females, as well as developmental toxicity in the offspring. The definitions presented below are adapted from those agreed as working definitions in IPCS/EHC Document No 225, Principles for Evaluating Health Risks to Reproduction Associated with Exposure to Chemicals. For classification purposes, the known induction of genetically based heritable effects in the offspring is addressed in Germ Cell Mutagenicity (section 3.5), since in the present classification system it is considered more appropriate to address such effects under the separate hazard class of germ cell mutagenicity.

In this classification system, reproductive toxicity is subdivided under two main headings:

(a) adverse effects on sexual function and fertility;

(b) adverse effects on development of the offspring.

Some reproductive toxic effects cannot be clearly assigned to either impairment of sexual function and fertility or to developmental toxicity. Nonetheless, substances with these effects, or mixtures containing them, shall be classified as reproductive toxicants.

3.7.1.2 For the purpose of classification the hazard class Reproductive Toxicity is differentiated into:

- adverse effects
  - on sexual function and fertility, or
  - on development;
- effects on or via lactation.

3.7.1.3 Adverse effects on sexual function and fertility

Any effect of substances that has the potential to interfere with sexual function and fertility. This includes, but is not limited to, alterations to the female and male reproductive system, adverse effects on onset of puberty, gamete production and transport, reproductive cycle normality, sexual behaviour, fertility, parturition, pregnancy outcomes, premature reproductive senescence, or modifications in other functions that are dependent on the integrity of the reproductive systems.

3.7.1.4 Adverse effects on development of the offspring

Developmental toxicity includes, in its widest sense, any effect which interferes with normal development of the conceptus, either before or after birth, and resulting from exposure of either parent prior to conception, or exposure of the developing offspring during prenatal development, or postnatally, to the time of sexual maturation. However, it is considered that classification under the heading of developmental toxicity is primarily intended to provide a hazard warning for pregnant women, and for men and women of reproductive capacity. Therefore, for pragmatic purposes of classification, developmental toxicity essentially means adverse effects induced during pregnancy, or as a result of parental exposure. These effects can be manifested at any point in the life span of the organism. The major manifestations of developmental toxicity include (1) death of the developing organism, (2) structural abnormality, (3) altered growth, and (4) functional deficiency.

3.7.1.5 Adverse effects on or via lactation are also included in reproductive toxicity, but for classification purposes, such effects are treated separately (see Table 3.7.1 (b)). This is because it is desirable to be able to classify substances specifically for an adverse effect on lactation so that a specific hazard warning about this effect can be provided for lactating mothers.

3.7.2 Classification criteria for substances

3.7.2.1 Hazard categories

3.7.2.1.1 For the purpose of classification for reproductive toxicity, substances are allocated to one of two categories. Within each category, effects on sexual function and fertility, and on development, are considered separately. In addition, effects on lactation are allocated to a separate hazard category.

Table 3.7.1(a) Hazard categories for reproductive toxicants.

Categories		Criteria
CATEGORY 1		Known or presumed human reproductive toxicant Substances are classified in Category 1 for reproductive toxicity when they are known to have produced an adverse effect on sexual function and fertility, or on development in humans or when there is evidence from animal studies, possibly supplemented with other information, to provide a strong presumption that the substance has the capacity to interfere with reproduction in humans. The classification of a substance is further distinguished on the basis of whether the evidence for classification is primarily from human data (Category 1A) or from animal data (Category 1B).
	Category 1A	Known human reproductive toxicant The classification of a substance in Category 1A is largely based on evidence from humans.
	Category 1B	Presumed human reproductive toxicant The classification of a substance in Category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate.
CATEGORY 2		Suspected human reproductive toxicant Substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification. Such effects shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects.

Table 3.7.1(b) Hazard category for lactation effects.

EFFECTS ON OR VIA LACTATION
Effects on or via lactation are allocated to a separate single category. It is recognised that for many substances there is no information on the potential to cause adverse effects on the offspring via lactation. However, substances which are absorbed by women and have been shown to interfere with lactation, or which may be present (including metabolites) in breast milk in amounts sufficient to cause concern for the health of a breastfed child, shall be classified and labelled to indicate this property hazardous to breastfed babies. This classification can be assigned on the: (a) human evidence indicating a hazard to babies during the lactation period; and/or (b) results of one or two generation studies in animals which provide clear evidence of adverse effect in the offspring due to transfer in the milk or adverse effect on the quality of the milk; and/or (c) absorption, metabolism, distribution and excretion studies that indicate the likelihood that the substance is present in potentially toxic levels in breast milk.

3.7.2.2 Basis of classification

3.7.2.2.1 Classification is made on the basis of the appropriate criteria, outlined above, and an assessment of the total weight of evidence (see 1.1.1). Classification as a reproductive toxicant is intended to be used for substances which have an intrinsic, specific property to produce an adverse effect on reproduction and substances shall not be so classified if such an effect is produced solely as a non-specific secondary consequence of other toxic effects.

The classification of a substance is derived from the hazard categories in the following order of precedence: Category 1A, Category 1B, Category 2 and the additional Category for effects on or via lactation. If a substance meets the criteria for classification into both of the main categories (for example Category 1B for effects on sexual function and fertility and also Category 2 for development) then both hazard differentiations shall be communicated by the respective hazard statements. Classification in the additional category for effects on or via lactation will be considered irrespective of a classification into Category 1A, Category 1B or Category 2.

3.7.2.2.2 In the evaluation of toxic effects on the developing offspring, it is important to consider the possible influence of maternal toxicity (see section 3.7.2.4).

3.7.2.2.3 For human evidence to provide the primary basis for a Category 1A classification there must be reliable evidence of an adverse effect on reproduction in humans. Evidence used for classification shall ideally be from well conducted epidemiological studies which include the use of appropriate controls, balanced assessment, and due consideration of bias or confounding factors. Less rigorous data from studies in humans shall be supplemented with adequate data from studies in experimental animals and classification in Category 1B shall be considered.

3.7.2.3 Weight of evidence

3.7.2.3.1 Classification as a reproductive toxicant is made on the basis of an assessment of the total weight of evidence, see section 1.1.1. This means that all available information that bears on the determination of reproductive toxicity is considered together, such as epidemiological studies and case reports in humans and specific reproduction studies along with sub-chronic, chronic and special study results in animals that provide relevant information regarding toxicity to reproductive and related endocrine organs. Evaluation of substances chemically related to the substance under study may also be included, particularly when information on the substance is scarce. The weight given to the available evidence will be influenced by factors such as the quality of the studies, consistency of results, nature and severity of effects, the presence of maternal toxicity in experimental animal studies, level of statistical significance for inter-group differences, number of endpoints affected, relevance of route of administration to humans and freedom from bias. Both positive and negative results are assembled together into a weight of evidence determination. A single, positive study performed according to good scientific principles and with statistically or biologically significant positive results may justify classification (see also 3.7.2.2.3).

3.7.2.3.2 Toxicokinetic studies in animals and humans, site of action and mechanism or mode of action study results may provide relevant information which reduces or increases concerns about the hazard to human health. If it is conclusively demonstrated that the clearly identified mechanism or mode of action has no relevance for humans or when the toxicokinetic differences are so marked that it is certain that the hazardous property will not be expressed in humans then a substance which produces an adverse effect on reproduction in experimental animals should not be classified.

3.7.2.3.3 If, in some reproductive toxicity studies in experimental animals the only effects recorded are considered to be of low or minimal toxicological significance, classification may not necessarily be the outcome. These effects include small changes in semen parameters or in the incidence of spontaneous defects in the foetus, small changes in the proportions of common foetal variants such as are observed in skeletal examinations, or in foetal weights, or small differences in postnatal developmental assessments.

3.7.2.3.4 Data from animal studies ideally shall provide clear evidence of specific reproductive toxicity in the absence of other systemic toxic effects. However, if developmental toxicity occurs together with other toxic effects in the dam, the potential influence of the generalised adverse effects shall be assessed to the extent possible. The preferred approach is to consider adverse effects in the embryo/foetus first, and then evaluate maternal toxicity, along with any other factors which are likely to have influenced these effects, as part of the weight of evidence. In general, developmental effects that are observed at maternally toxic doses shall not be automatically discounted. Discounting devel-

opmental effects that are observed at maternally toxic doses can only be done on a case-by-case basis when a causal relationship is established or refuted.

3.7.2.3.5 If appropriate information is available it is important to try to determine whether developmental toxicity is due to a specific maternally mediated mechanism or to a non-specific secondary mechanism, like maternal stress and the disruption of homeostasis. Generally, the presence of maternal toxicity shall not be used to negate findings of embryo/foetal effects, unless it can be clearly demonstrated that the effects are secondary non-specific effects. This is especially the case when the effects in the offspring are significant, e.g. irreversible effects such as structural malformations. In some situations it can be assumed that reproductive toxicity is due to a secondary consequence of maternal toxicity and discount the effects, if the substance is so toxic that dams fail to thrive and there is severe inanition, they are incapable of nursing pups; or they are prostrate or dying.

3.7.2.4 Maternal toxicity

3.7.2.4.1 Development of the offspring throughout gestation and during the early postnatal stages can be influenced by toxic effects in the mother either through non-specific mechanisms related to stress and the disruption of maternal homeostasis, or by specific maternally-mediated mechanisms. In the interpretation of the developmental outcome to decide classification for developmental effects it is important to consider the possible influence of maternal toxicity. This is a complex issue because of uncertainties surrounding the relationship between maternal toxicity and developmental outcome. Expert judgement and a weight of evidence approach, using all available studies, shall be used to determine the degree of influence that shall be attributed to maternal toxicity when interpreting the criteria for classification for developmental effects. The adverse effects in the embryo/foetus shall be first considered, and then maternal toxicity, along with any other factors which are likely to have influenced these effects, as weight of evidence, to help reach a conclusion about classification.

3.7.2.4.2 Based on pragmatic observation, maternal toxicity may, depending on severity, influence development via non-specific secondary mechanisms, producing effects such as depressed foetal weight, retarded ossification, and possibly resorptions and certain malformations in some strains of certain species. However, the limited number of studies which have investigated the relationship between developmental effects and general maternal toxicity have failed to demonstrate a consistent, reproducible relationship across species. Developmental effects which occur even in the presence of maternal toxicity are considered to be evidence of developmental toxicity, unless it can be unequivocally demonstrated on a case-by-case basis that the developmental effects are secondary to maternal toxicity. Moreover, classification shall be considered where there is a significant toxic effect in the offspring, e.g. irreversible effects such as structural malformations, embryo/foetal lethality, significant post-natal functional deficiencies.

3.7.2.4.3 Classification shall not automatically be discounted for substances that produce developmental toxicity only in association with maternal toxicity, even if a specific maternally-mediated mechanism has been demonstrated. In such a case, classification in Category 2 may be considered more appropriate than Category 1. However, when a substance is so toxic that maternal death or severe inanition results, or the dams are prostrate and incapable of nursing the pups, it is reasonable to assume that developmental toxicity is produced solely as a secondary consequence of maternal toxicity and discount the developmental effects. Classification is not necessarily the outcome in the case of minor developmental changes, when there is only a small reduction in foetal/pup body weight or retardation of ossification when seen in association with maternal toxicity.

3.7.2.4.4 Some of the end points used to assess maternal effects are provided below. Data on these end points, if available, need to be evaluated in light of their statistical or biological significance and dose response relationship.

Maternal mortality:

an increased incidence of mortality among the treated dams over the controls shall be considered evidence of maternal toxicity if the increase occurs in a dose-related manner and can be attributed to the systemic toxicity of the test material. Maternal mortality greater than 10 % is considered excessive and the data for that dose level shall not normally be considered for further evaluation.

Mating index

(no. animals with seminal plugs or sperm/no. mated × 100) (*)

Fertility index

(no. animals with implants/no. of matings × 100)

Gestation length

(if allowed to deliver)

Body weight and body weight change:

Consideration of the maternal body weight change and/or adjusted (corrected) maternal body weight shall be included in the evaluation of maternal toxicity whenever such data are available. The calcula-

* () It is recognised that the Mating index and the Fertility index can also be affected by the male.

tion of an adjusted (corrected) mean maternal body weight change, which is the difference between the initial and terminal body weight minus the gravid uterine weight (or alternatively, the sum of the weights of the foetuses), may indicate whether the effect is maternal or intrauterine. In rabbits, the body weight gain may not be useful indicators of maternal toxicity because of normal fluctuations in body weight during pregnancy.

Food and water consumption (if relevant):

The observation of a significant decrease in the average food or water consumption in treated dams compared to the control group is useful in evaluating maternal toxicity, particularly when the test material is administered in the diet or drinking water. Changes in food or water consumption need to be evaluated in conjunction with maternal body weights when determining if the effects noted are reflective of maternal toxicity or more simply, unpalatability of the test material in feed or water.

Clinical evaluations (including clinical signs, markers, haematology and clinical chemistry studies):

The observation of increased incidence of significant clinical signs of toxicity in treated dams relative to the control group is useful in evaluating maternal toxicity. If this is to be used as the basis for the assessment of maternal toxicity, the types, incidence, degree and duration of clinical signs shall be reported in the study. Clinical signs of maternal intoxication include: coma, prostration, hyperactivity, loss of righting reflex, ataxia, or laboured breathing.

Post-mortem data:

Increased incidence and/or severity of post-mortem findings may be indicative of maternal toxicity. This can include gross or microscopic pathological findings or organ weight data, including absolute organ weight, organ-to-body weight ratio, or organ-to-brain weight ratio. When supported by findings of adverse histopathological effects in the affected organ(s), the observation of a significant change in the average weight of suspected target organ(s) of treated dams, compared to those in the control group, may be considered evidence of maternal toxicity.

3.7.2.5 Animal and experimental data

3.7.2.5.1 A number of internationally accepted test methods are available; these include methods for developmental toxicity testing (e.g. OECD Test Guideline 414), and methods for one or two-generation toxicity testing (e.g. OECD Test Guidelines 415, 416).

3.7.2.5.2 Results obtained from Screening Tests (e.g. OECD Guidelines 421 — Reproduction/ Developmental Toxicity Screening Test, and 422 — Combined Repeated Dose Toxicity Study with

Reproduction/Development Toxicity Screening Test) can also be used to justify classification, although it is recognised that the quality of this evidence is less reliable than that obtained through full studies.

3.7.2.5.3 Adverse effects or changes, seen in short- or long-term repeated dose toxicity studies, which are judged likely to impair reproductive function and which occur in the absence of significant generalised toxicity, may be used as a basis for classification, e.g. histopathological changes in the gonads.

3.7.2.5.4 Evidence from in vitro assays, or non-mammalian tests, and from analogous substances using structure-activity relationship (SAR), can contribute to the procedure for classification. In all cases of this nature, expert judgement must be used to assess the adequacy of the data. Inadequate data shall not be used as a primary support for classification.

3.7.2.5.5 It is preferable that animal studies are conducted using appropriate routes of administration which relate to the potential route of human exposure. However, in practice, reproductive toxicity studies are commonly conducted using the oral route, and such studies will normally be suitable for evaluating the hazardous properties of the substance with respect to reproductive toxicity. However, if it can be conclusively demonstrated that the clearly identified mechanism or mode of action has no relevance for humans or when the toxicokinetic differences are so marked that it is certain that the hazardous property will not be expressed in humans then a substance which produces an adverse effect on reproduction in experimental animals shall not be classified.

3.7.2.5.6 Studies involving routes of administration such as intravenous or intraperitoneal injection, which result in exposure of the reproductive organs to unrealistically high levels of the test substance, or elicit local damage to the reproductive organs, including irritation, must be interpreted with extreme caution and on their own are not normally the basis for classification.

3.7.2.5.7 There is general agreement about the concept of a limit dose, above which the production of an adverse effect is considered to be outside the criteria which lead to classification, but not regarding the inclusion within the criteria of a specific dose as a limit dose. However, some guidelines for test methods, specify a limit dose, others qualify the limit dose with a statement that higher doses may be necessary if anticipated human exposure is sufficiently high that an adequate margin of exposure is not achieved. Also, due to species differences in toxicokinetics, establishing a specific limit dose may not be adequate for situations where humans are more sensitive than the animal model.

3.7.2.5.8 In principle, adverse effects on reproduction seen only at very high dose levels in animal studies (for example doses that induce prostration, severe inappetence, excessive mortality) would

not normally lead to classification, unless other information is available, e.g. toxicokinetics information indicating that humans may be more susceptible than animals, to suggest that classification is appropriate. Please also refer to the section on maternal toxicity (3.7.2.4) for further guidance in this area.

3.7.2.5.9 However, specification of the actual 'limit dose' will depend upon the test method that has been employed to provide the test results, e.g. in the OECD Test Guideline for repeated dose toxicity studies by the oral route, an upper dose of 1 000 mg/kg has been recommended as a limit dose, unless expected human response indicates the need for a higher dose level.

3.7.3 Classification criteria for mixtures

3.7.3.1 Classification of mixtures when data are available for all ingredients or only for some ingredients of the mixture

3.7.3.1.1 The mixture shall be classified as a reproductive toxicant when at least one ingredient has been classified as a Category 1A, Category 1B or Category 2 reproductive toxicant and is present at or above the appropriate generic concentration limit as shown in Table 3.7.2 for Category 1A, Category 1B and Category 2 respectively.

3.7.3.1.2 The mixture shall be classified for effects on or via lactation when at least one ingredient has been classified for effects on or via lactation and is present at or above the appropriate generic concentration limit as shown in Table 3.7.2 for the additional category for effects on or via lactation.

Table 3.7.2 Generic concentration limits of ingredients of a mixture classified as reproduction toxicants or foreffects on or via lactation that trigger classification of the mixture.

Ingredient classified as:	Generic concentration limits triggering classification of a mixture as:			
	Category 1A reproductive toxicant	Category 1B reproductive toxicant	Category 2 reproductive toxicant	Additional category for effects on or via l actation
Category 1A reproductive toxicant	≥ 0,3 % [Note 1]			
Category 1B reproductive toxicant		≥ 0,3 % [Note 1]		
Category 2 reproductive toxicant			≥ 3,0 % [Note 1]	
Additional category for effects on or via lactation				≥ 0,3 % [Note 1]

Note The concentration limits in the table above apply to solids and liquids (w/w units) as well as gases (v/v units).
Note 1 If a Category 1 or Category 2 reproductive toxicant or a substance classified for effects on or via lactation is present in the mixture as an ingredient at a concentration above 0,1 %, a SDS shall be available for the mixture upon request.

3.7.3.2 Classification of mixtures when data are available for the complete mixture

3.7.3.2.1 Classification of mixtures will be based on the available test data for the individual ingredients of the mixture using concentration limits for the ingredients of the mixture. On a case-by-case basis, test data on mixtures may be used for classification when demonstrating effects that have not been established from the evaluation based on the individual components. In such cases, the test results for the mixture as a whole must be shown to be conclusive taking into account dose and other factors such as duration, observations, sensitivity and statistical analysis of reproduction test systems. Adequate documentation supporting the classification shall be retained and made available for review upon request.

3.7.3.3 Classification of mixtures when data are not available for the complete mixture: bridging principles

3.7.3.3.1 Subject to paragraph 3.7.3.2.1, where the mixture itself has not been tested to determine its reproductive toxicity, but there are sufficient data on the individual ingredients and similar tested mixtures to adequately characterise the hazards of the mixture, these data shall be used in accordance with the applicable bridging rules set out in section 1.1.3.

3.7.4 Hazard Communication

3.7.4.1 Label elements shall be used for substances or mixtures meeting the criteria for classification in this hazard class in accordance with Table 3.7.3

Table 3.7.3 Label elements for reproductive toxicity.

Classification	Category 1A or Category 1B	Category 2	Additional category for effects on or via lactation
GHS Pictograms			No pictogram
Signal Word	Danger	Warning	No signal word
Hazard Statement	H360: May damage fertility or the unborn child (state specific effect if known)(state route of exposure if it is conclusively proven that no other routes of exposure cause the hazard)	H361: Suspected of damaging fertility or the unborn child (state specific effect if known) (state route of exposure if it is conclusively proven that no other routes of exposure cause the hazard)	H362: May cause harm to breast-fed children.
Precautionary Statement Prevention	P201 P202 P281	P201 P202 P281	P201 P260 P263 P264 P270
Precautionary Statement Response	P308 + P313	P308 + P313	P308 + P313
Precautionary Statement Storage	P405	P405	
Precautionary Statement Disposal	P501	P501	

Annex **E**

Additional considerations to Regulation (EC) 1272/2008

The classification and labelling of substances is performed according to the guidelines of the European Union (Regulation (EC)1272/2008) presented in Annex D. The classification of compounds is ultimately dependent on an integrated assessment of the nature of all parental and developmental effects observed, their specificity and adversity, and the dosages at which the various effects occur. The guideline necessarily leaves room for interpretation, dependent on the specific data set under consideration. In the process of using the regulation, the committee has agreed upon a number of additional considerations:

- If there is sufficient evidence to establish a causal relationship between human exposure to the substance and impaired fertility or subsequent developmental toxic effects in the offspring, the compound will be classified in category 1A, irrespective of the general toxic effects (see Annex D, 3.7.2.2.1.).
- Adverse effects in a reproductive study, occurring without reporting the parental or maternal toxicity, may lead to a classification other than category 1B, when the effects occur at dose levels which cause severe toxicity in *general* toxicity studies.
- Clear adverse reproductive effects will not be disregarded on the basis of reversibility per se.

- The committee does not only use guideline studies (studies performed according to OECD* standard protocols) for the classification of compounds, but non-guideline studies are taken into consideration as well.

* Organisation for Economic Cooperation and Development.

Health Council of the Netherlands

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Prevention
Which forms of prevention can help realise significant health benefits?

Healthy nutrition
Which foods promote good health and which carry certain health risks?

Environmental health
Which environmental influences could have a positive or negative effect on health?

Healthy working conditions
How can employees be protected against working conditions that could harm their health?

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