



Occupational exposure to hexavalent chromium. Part I. Hazard assessment of non-cancer health effects

Ellen V.S. Hessel, Yvonne C.M. Staal, Aldert H. Piersma, Shalenie P. den Braver-Sewradj, Janine Ezendam^{*}

National Institute for Public Health and the Environment (RIVM), P.O. Box 1, 3720 BA, Bilthoven, the Netherlands

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ABSTRACT

Hexavalent chromium (Cr(VI)) compounds have been studied extensively and several agencies have described their toxicological profile. In the past, personnel of the Dutch Ministry of Defence may have been exposed to Cr(VI) during maintenance activities. To investigate if this exposure may have caused irreversible adverse health effects, the Dutch National Institute for Public Health and the Environment (RIVM) summarized all available knowledge from previous evaluations. This information was complemented with a scoping review to retrieve new scientific literature. All scientific evidence was evaluated in workshops with external experts to come to an overview of irreversible adverse health effects that could be caused by occupational exposure to Cr(VI) compounds. This review focuses on non-cancer health effects.

It was concluded that occupational exposure to Cr(VI) can cause perforation of the nasal septum by chromium ulcers, chronic lung diseases, including asthma, rhinitis, pulmonary fibrosis and COPD, skin ulcers and allergic contact dermatitis in humans. It is currently insufficiently clear if Cr(VI) can cause irreversible diseases due to disturbances of the immune system (other than allergic contact eczema, allergic asthma and rhinitis and chronic lung diseases) or adverse effects on fertility or prenatal development in humans.

1. Introduction

Hexavalent chromium (Cr(VI)) compounds include a large group of chemicals with variable properties with regard to corrosion-resistance, durability, and hardness (NIOSH, 2014) and therefore are employed for a variety of different uses. Examples of Cr(VI) compounds are calcium chromate, potassium chromate, sodium chromate, zinc chromate, chromic acid, chromium trioxide and ammonium chromate.

Humans can be exposed to Cr(VI) at the workplace or via non-occupational sources. The routes of exposure can be oral, respiratory or dermal. Inhalation exposure can occur, for example, through industrial air emissions, cigarette smoke and workplace exposure. Dermal exposure can occur via leather consumer products or at the workplace, e.g. via cement. Oral exposure can occur via drinking water (ATSDR, 2012). The largest release of Cr(VI) compounds into the environment is due to industrial activities. Materials containing Cr(VI) include various paint and primer pigments, graphic art supplies, fungicides, corrosion inhibitors and wood preservatives. Industrial activities in which the largest numbers of workers may be exposed to the highest

concentrations of Cr(VI) compounds include electroplating, welding, and painting (NIOSH, 2013).

In the European Union, many Cr(VI) compounds are classified as reprotoxic substances according to EU Regulation (EC) No 1272/2008. The European harmonized classification of the Cr(VI) compounds was supported by the Dutch Expert Committee on Occupational Safety (DECOS) of the Health Council of the Netherlands (DECOS, 2001; 2016). In addition, some Cr(VI) compounds are classified as skin and respiratory sensitizers. These Cr(VI) compounds must be labeled with the risk phrases H317 ('can cause an allergic skin reaction') and H334 ('may cause allergy or asthma symptoms or breathing difficulties if inhaled').

Due to the known toxic and carcinogenic effects of Cr(VI) compounds several legislative measures were taken to reduce chromium exposure in workers and consumers. Many uses have been restricted or banned in the past decades in the European Union (EU). For example, the EU reduced the maximum allowed level in cement in order to reduce exposure in workers (EC, 2003). Chromates are also authorized in the EU, which further restricts their use in paints. Cr(VI) has been recently added to EU Carcinogens and Mutagens directive (CMD) with a binding occupational limit value. In US, ACGIH proposed in 2018 a TLV of 0.2

^{*} Corresponding author.

E-mail address: Janine.Ezendam@rivm.nl (J. Ezendam).

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Abbreviations

ATSDR	Agency for Toxic Substances and Disease Registry
BAL fluid	bronchial lavage fluid
ConA	concanavalin A
NCvB	Dutch Centre for Occupational Diseases
DECOS	Dutch Expert Committee on Occupational Safety
RIVM	Dutch National Institute for Public Health and the Environment
E2	estradiol
ECB	European Chemicals Bureau
EFSA	European Food Safety Authority
GD	gestational day
HSE	Health and Safety Executive
Cr(VI)	Hexavalent chromium

IARC	International Agency for Research on Cancer
LPS	lipopolysaccharide
MCH	Mean Corpuscular Hemoglobin
MCV	Mean Corpuscular Volume
NK cells	natural killer cells
NIOSH	National Institute of Occupational Safety and Health
NTP	National Toxicology Program
PHA	phytohaemagglutinin
PWM	pokeweed mitogen
SCOEL	Scientific Committee on Occupational Exposure Limits
SRBC	sheep red blood cell
POMS-locations	Prepositioned Organizational Material Storages
Cr(III)	trivalent chromium
US EPA	US Environmental Protection Agency

$\mu\text{g}/\text{m}^3$ which is based on non-cancer (lung) effects. Despite the knowledge on potential hazardous effects of Cr(VI) compounds, the awareness of these hazards may have been limited in certain occupational settings. Furthermore, potential release of Cr(VI) may have been underestimated at certain workplaces. This could have led to occupational exposures to Cr(VI) compounds to undesired levels for extended periods.

An example of an occupational setting where exposure to Cr(VI) compounds occurred in the past are the former Prepositioned Organizational Material Storages (POMS-locations) of NATO (North Atlantic Treaty Organisation), in the Netherlands. The Dutch army used materials containing Cr(VI) compounds, such as paints, primer pigments and corrosion inhibitors to protect military vehicles and material against corrosion. In the period from 1984 to 2006, Dutch Ministry of Defense employees worked with these materials. The Dutch National Institute for Public Health and the Environment (RIVM) coordinated a project to investigate if these workers were exposed to Cr(VI) compounds and whether this exposure may have caused irreversible adverse health effects. A series of 10 Dutch reports were published (RIVM, 2018), including an exposure assessment aiming at characterization and, if possible, (semi-)quantification of occupational Cr(VI) exposure at the POMS locations (Van der Meer et al., 2018). From the exposure assessment it was concluded that the primary exposure route at the POMS locations was inhalation. Dermal and oral exposure could not be ruled out as exposure routes as a result of the nature of the work and industrial hygiene practices (Van der Meer et al., 2018). In addition, an occupational hazard assessment was performed to identify irreversible adverse health effects that could be caused by Cr(VI) compounds (Hessel et al., 2018; Palmen et al., 2018). After this, a risk assessment was performed to assess if exposure to Cr(VI) compounds at the former POMS-locations could lead to these adverse irreversible health effects (Palmen et al., 2018).

Cr(VI) compounds have been studied extensively and several agencies have assessed the toxicological profile of these compounds. Therefore, RIVM used these evaluations as a starting point to summarize all available scientific knowledge on the occupational hazards of exposure to Cr(VI) compounds. This information was complemented with a scoping review to identify peer-reviewed scientific literature that was published after the latest reports from the agencies to evaluate if new scientific evidence emerged on adverse health effects that were not reported in those earlier reports. The outcomes of the occupational hazard assessment were published in 2018 (Hessel et al., 2018; Palmen et al., 2018). In 2020 an update of this hazard assessment that was based on novel scientific publications as well as a more comprehensive assessment of the literature on cancer, immunological diseases and dental effects in relation to occupational Cr(VI) exposure (Hessel et al., 2020) was published.

The hazard assessments performed by the RIVM were published in

Dutch reports. Since occupational hazard assessment is valuable for risk assessments that may be required for other locations where workers may have been exposed to Cr(VI) compounds, it was decided to translate these reports and merge them in two reviews. This review provides an overview of the existing scientific knowledge about irreversible non-cancer health effects that can be related to occupational exposure to Cr(VI) compounds, based on evidence from human and animal studies. In part II, the scientific knowledge on the relationship between occupational Cr(VI) compounds exposure and cancer is published (Den Braver et al., 2021, this issue).

2. Methods

The methodology that was used for RIVM reports 2018 and 2020 (Hessel et al., 2018, 2020) was quite similar, with some exceptions for specific adverse health effects for which questions were raised among former employees of the POMS locations or by the external advisory board of the project. The methodology section describes the procedures for both reports.

2.1. Methodology RIVM report 2018

2.1.1. Evaluations by international agencies

A search was conducted to retrieve evaluations performed by international agencies. These were used as a starting point to summarize all known occupational hazards of Cr(VI) compounds. Health effects that can be related to (occupational) Cr(VI) exposure and the underlying scientific evidence have been extensively reviewed by the Agency for Toxic Substances and Disease Registry (ATSDR) (ATSDR, 2012), the International Agency for Research on Cancer (IARC) (IARC, 2012), the National Institute of Occupational Safety and Health (NIOSH) (NIOSH, 2014), the European Food Safety Authority (EFSA) (Casalegno et al., 2015; EFSA, 2014), the Health Council of the Netherlands (Health Council, 2016a; b), and the Scientific Committee on Occupational Exposure Limits (SCOEL) (SCOEL, 2004; 2017).

2.1.2. Search strategy 1 (2012–2016)

A literature search was conducted to retrieve literature that was published after the publications from the official agencies and bodies. At the time, the most recent extensive review of the full toxicological profile of Cr(VI) compounds was performed by the ATSDR in 2012 (ATSDR, 2012). The search strategy aimed at retrieving literature on both non-cancer as well as cancer health effects (Den Braver et al., 2021, this issue).

The literature search covered the time period January 2012 to June 2016 and included the following databases: MEDLINE, EMBASE, Scopus and Toxicology Literature Online (TOXLINE). To find all human and

animal studies about occupational exposure to Cr(VI) compounds in relation to adverse health effects, the following search terms were used:

Chromium Compounds OR Chromium OR chrome* of chromi* or cr) adj ('6' or VI)) OR ((chrome* or chromi* or cr) adj hexaval*) OR (hexaval* adj (chrome* or chromi* or cr))) AND (Occupational Diseases OR Occupational Exposure OR Chronic Disease OR Environmental Health OR Environmental Exposure OR Health OR Maximum Allowable Concentration OR Risk OR Risk Assessment OR Accidents OR Hazardous Substances OR diseases).

This search resulted in 1858 articles.

2.1.3. Screening and selection

As a first step, the titles and abstracts of all articles were screened by one person for potential exclusion against the exclusion criteria listed in Table 1. If there were some uncertainties a second person or expert was consulted to discuss the inclusion and exclusion. If needed the full text article was read. This step resulted in 124 human studies and 145 animal studies.

A further selection was made based on the full texts of the selected articles. A summary of the additional inclusion and exclusion criteria for this selection step is listed in Table 1. This step resulted in 49 human studies and 24 studies in animals on all adverse health effects (cancer and non-cancer). The results of the selection can be found in Fig. 1.

2.1.4. Data extraction

Data extraction started with information from the evaluations of international agencies. The conclusions on irreversible adverse health effects from all these reports were merged together with a concise summary of the human and animal studies on which these conclusions are based. This resulted in an overview of all studies evaluated by the international agencies and their health effects were summarized. In this overview relevant studies per health effect can be easily selected. For the human studies retrieved in the literature search the following data were extracted and summarized by one person. If there were some uncertainties a second person and/or expert was consulted: 1) study

objectives (the health effects that are assessed); 2) study design (study population including the number of subjects, exposure assessment including the exposure route, exposure frequency, exposure concentration and type of Cr(VI) compound); 3) results (relative risk estimates and/or dose-response relationship, statistical methods applied).

For each animal study retrieved in the literature search, the following data was summarized by one person. If there were some uncertainties a second person and/or expert was consulted: 1) study objectives (effects that are examined); 2) animal characteristics (animal model, species, strain); 3) number of animals per dose group; 4) study design (administration route, dose groups, exposure conditions and type of Cr(VI) compound); 5) statistical methods applied; 6) results including the dose-response relationship (NOAEL/LOAEL).

This all together resulted in an overview of all the available studies per health effect.

2.1.5. Quality assessment

The quality assessment was based on predefined quality criteria which were compiled by consultation with epidemiological experts as well as toxicological experts with knowledge on toxicological studies in experimental animals. A minimum of 2 persons were involved in the quality assessment. If there were some uncertainties an (external) expert was consulted before the expert workshop.

For human studies, the quality criteria adopted were as follows:

1. An appropriate comparison group is selected;
2. Potential confounders are considered and corrected for;
3. The exposure is characterized and clearly defined;
4. A clear description is provided on the study design and the statistical methods used;
5. The quality of the study design.

For animal studies, the quality criteria were:

1. Multiple dosages are tested;
2. An appropriate control group is included;
3. The study design and setting is properly reported;
4. The quality of the study design
5. All results, including unadjusted and non-significant results, are reported.

2.1.6. Interpretation and evaluation of the scientific data and weight of evidence

For each hazard/health effect, all available data was collected from three lines of evidence:

- Evidence line 1. Conclusions from international agencies;
- Evidence line 2. Recent publications on human studies;
- Evidence line 3. Recent publications on animal studies.

2.1.7. Expert consultations and weight-of-evidence approach

The available data from the three lines of evidence and the subsequent weight of evidence for a causal relationship with the adverse health effects (cancer and non-cancer) and occupational exposure to Cr(VI) was drawn up by RIVM and evaluated with external experts during a one-day meeting. Experts in toxicology, (occupational) epidemiology, occupational medicine and occupational hygiene were consulted. The experts that participated in this workshop are mentioned in the Acknowledgements.

A weight-of-evidence approach was used, integrating all three lines of evidence. The conclusions from the international agencies were considered to be relevant and reliable and were taken over if they were conclusive. Human studies that were relevant, reliable and of sufficient quality based on the predefined criteria, were considered to provide convincing evidence that an adverse health effect was related to exposure to Cr(VI) compounds. Animal studies, if available, were included as supporting evidence, as long as the experimental setup was relevant for

Table 1
Summary of inclusion- and exclusion criteria, abstract and full-text based.

Title- and abstract screening
<i>For inclusion</i>
The article is written in Dutch or English
Study on Cr(VI)
<i>For exclusion</i>
Articles that do not contain original data (e.g. summary articles or reviews, editorial letters)
Studies on Cr(VI) in medical devices
Studies in which exposure to Cr(VI) was not evaluated
Articles in languages other than English and Dutch
Studies on environmental hazard
Studies on treatment of wastewater
Studies on trivalent chromium compounds
Additional for full-text analysis, human studies
<i>For inclusion</i>
A causal or other relationship between the adverse health effects and exposure to Cr(VI) was examined in humans
<i>For exclusion</i>
Adverse health effects and diseases in humans from exposure to Cr(VI) were not examined
Studies on chromium (III), for example as a nutritional supplement
Additional for full-text analysis, animal studies
<i>For inclusion</i>
A causal or other relationship between the negative health effects and exposure to Cr(VI) was examined in animals
<i>For exclusion</i>
No causal or other relationship between the adverse health effects and/or diseases and exposure to Cr(VI) was examined in laboratory animals
Only mixtures of substances were tested and not only exposure to Cr(VI)
The exposure was intravenous (IV) or intraperitoneal (IP), as these exposure routes are not representative of occupational exposure

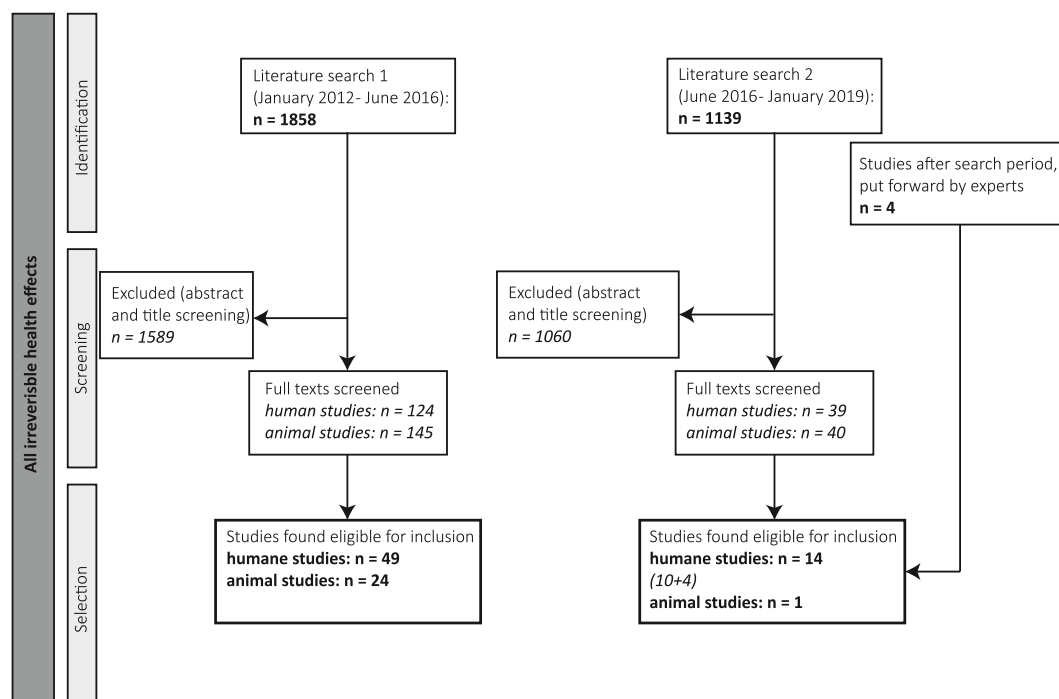


Fig. 1. Results of the screening and selection procedures in RIVM report 2018 (literature search 1) and RIVM report 2020 (literature search 2). These literature searches were aimed to retrieve literature on Cr(VI) and irreversible health effects, both non-cancer health effects and carcinogenic effects, published after the last extensive review of the full toxicological profile of Cr(VI) in 2012 (ATSDR, 2012). Screening and selection of abstract and full-text was done according to the inclusion- and exclusion criteria listed in Table 1. The studies selected by literature search 2 were further complemented with 4 studies that were published after the literature search but which were put forward by experts consulted in this project.

occupational exposure of humans (Table 1). For several adverse health effects evidence from human and/or animal studies were negative, insufficient, inconclusive or absent. For those adverse effects, all available evidence was weighted during the expert consultations to conclude if there was insufficient evidence or if it was unlikely that occupational Cr(VI) exposure caused the effect.

In the evaluation, the route of exposure was taken into consideration. Occupational exposure of Cr(VI) primarily occurs via the respiratory tract (SCOEL, 2004; 2017; Van der Meer et al., 2018). Studies in which the exposure route was via the airways (inhalation, intratracheal, intrabronchial, intrapleural) were therefore deemed most relevant. However, exposure via the dermal and oral routes cannot be excluded because of inadequate industrial hygiene practices, such as insufficient personal protective equipment or personal hygiene practices. Oral exposure can also result from secondary ingestion of inhaled particles cleared from the lungs (ATSDR, 2012; NIOSH, 2014; SCOEL, 2004; 2017; Van der Meer et al., 2018). Therefore, on the basis of the occupational exposure routes, a critical assessment was carried out with experts in dedicated expert sessions (see below) on the relevance of the findings in studies in which animals were exposed via the dermal or oral route. Toxicokinetic studies show that reduction of Cr(VI) to Cr(III) and therefore the local and systemic distribution of Cr(VI) depends on the exposure route (Cohen et al., 1993; den Braver-Sewradj, submitted; Heringa and Janssen, 2018; Petrilli et al., 1986; Suzuki et al., 1984; WHO, 2013). As a consequence, it may be expected that the adverse effects that can be related to Cr(VI) in portal of entry and systemic tissues will depend on the exposure route. Therefore, extrapolation between exposure routes is not performed in this hazard assessment. The US Environmental Protection Agency (EPA) reached a similar conclusion in the systematic review protocol for the Hexavalent Chromium IRIS assessment (US EPA, 2019). These discussions and the conclusions are captured in chapter 3.

The workshop resulted in an overall conclusion on a causal relation between occupational exposure to Cr(VI) compounds and an adverse

health effect, ranking from clear evidence to insufficient or no evidence.

- **Cr(VI) can cause the adverse health effect in humans.**

There is sufficient evidence from human studies and supporting evidence from animal studies that occupational exposure to Cr(VI) can cause the adverse health effect in humans.

- **Cr(VI) is suspected to cause the adverse health effect in humans.**

There is limited evidence from human studies, supported by limited evidence from animal studies that occupational exposure to Cr(VI) can cause the adverse health effect in humans.

- **It is currently insufficiently clear if Cr(VI) can cause the adverse health effect in humans**

There are some scientific indications from animal or human studies that exposure to Cr(VI) may cause the adverse health effect. Taking all scientific literature together, these indications were considered not to provide sufficient evidence to be able to state that occupational exposure to Cr(VI) can cause the adverse health effect in humans.

- **There are no or no convincing indications that Cr(VI) can cause the adverse health effect in humans**

There are no or no convincing scientific indications from animal or human studies that occupational exposure to Cr(VI) can cause the adverse health effect in humans.

2.2. Methodology RIVM report 2020

The second RIVM report was based on an update of the scientific literature. The literature search covered a period between June 2016 to

January 2019. The same procedures were followed as described in section 2.1.

Importantly, the second report specifically focused on three adverse health effects: carcinogenic effects (described in Den Braver et al., this issue), immunological conditions other than those that were already linked to Cr(VI) compounds and dental effects. This focus was chosen after concerns were raised by former employees and by the independent external advisory board of the project. For each of these three types adverse health effects, specific external expert consultations were held in 2019.

Within this period, SCOEL published a new recommendation on occupational exposure to Cr(VI) compounds (SCOEL, 2017). The conclusions on non-cancer adverse health effects remained unchanged compared to the previous report in 2004 (SCOEL, 2004), changes were only made to the cancer hazard assessment (Den Braver et al., this issue).

Literature search 2 resulted in 1139 articles. After title and abstract screening 39 human studies and 40 animal studies remained. This selection was complemented with four additional human studies that were put forward by experts consulted in this project. The analysis of the full texts based on the criteria listed in Table 1 yielded 10 human studies and 1 animal study. The results of the selection can be found in Fig. 1.

For the majority of non-cancer adverse health effects that were linked to Cr(VI) exposure no new literature was published. The exception was effects of Cr(VI) compounds on reproduction and prenatal development and systemic immunological effects. After applying the exclusion criteria, two new animal studies on reproduction toxicity (Banu et al., 2016, 2017) were included in the update. For systemic immunological effects or diseases, one animal study (Shipkowski et al., 2017) and one study in humans (Hu et al., 2017) remained after applying the selection criteria. One article that was published after the search period, and was brought forward by a colleague. This publication (Islam et al., 2019) was considered relevant and was therefore included.

For systemic immunological effects, the external advisory board recommended to evaluate all available literature published earlier than 2012, that met the predefined criteria, since these effects were not described in detail in earlier evaluations of other agencies. A total of 11 human studies and four animal studies were evaluated in the expert consultation.

For dental effects, it was also decided to evaluate all available literature on dental erosion and discoloration of the teeth. All studies were published before 2012 and met the predefined criteria. There were five studies in humans (Dulgergil et al., 2007; Gomes, 1972; Gupta, 1990; Suyama et al., 2010; Wiegand and Attin, 2007) and one animal study (De Lucca et al., 2009). During the discussions, the expert provided an additional human study (Edeer and Martin, 2010).

2.2.1. Expert consultation on systemic immunological diseases

The external advisory board requested a more in-depth evaluation of scientific literature that may support that exposure to Cr(VI) compounds may cause immunological diseases, other than allergic contact eczema, allergic asthma, allergic rhinitis and chronic lung diseases. In the first report, RIVM concluded that '*Chromium (VI) can have immunomodulatory effects, but there is currently no scientific evidence available that chromium (VI) can induce irreversible immune-mediated diseases other than those already described*'.

A dedicated workshop was organized with external experts on this topic to discuss all available scientific literature that assessed a relationship between Cr(VI) exposure and systemic immunological effects and/or conditions, were discussed in detail.

The following charge questions were addressed for each individual study:

- Are the control groups in the studies in humans appropriate, given the endpoints of those studies?
- Are the individual studies good enough in terms of quality to be included in the interpretation of the available scientific data?

- Are the changes to immunological parameters from the animal studies indicative of irreversible adverse effects and/or immunological conditions in humans?
- Are the changes to immunological parameters from the studies in humans indicative of irreversible adverse effects and/or immunological conditions?

The weight-of-evidence approach described in paragraph 2.1.7. was used to come to an overall conclusion on a causal relation between occupational exposure to Cr(VI) compounds and the risk for immunological diseases.

2.2.2. Expert consultation on dental effects

An expert was consulted who is specialized in cariology and endodontology (dentistry). The aim of the expert consultation was to discuss whether the available literature provides evidence that adverse dental effects can be caused by exposure to Cr(VI) compounds. The report from 2018 was sent to the expert beforehand, and the interpretation and conclusions about dental effects were presented during the discussion.

The following points were discussed:

- What kind of dental effects are there and what does 'crumbling teeth' mean?
- Can exposure to Cr(VI) compounds cause dental effects or make the teeth fragile/crumbling?
- The supporting evidence and conclusions from the RIVM report from 2018 were discussed and presented to the expert.

The weight-of-evidence approach described in paragraph 2.1.6. was used for categorization of dental effects.

3. Occupational health hazard assessment

The occupational health hazard assessment used evaluations of international agencies as a starting-point. For each health hazard a short summary of these evaluations is provided, supplemented with new scientific evidence, if available. Then, a summary of the expert consultation is provided together with a conclusion.

3.1. Respiratory tract

The respiratory tract is the most important route of occupational exposure to Cr(VI) compounds. Human and animal studies provide evidence that inhalation of Cr(VI) compounds lead to different reversible and irreversible adverse effects in both the upper as well as the lower respiratory tract (ATSDR, 2012).

3.1.1. Nasal septum perforation

3.1.1.1. International agencies. There is evidence from several studies in humans that intermediate-duration and chronic-duration exposure of workers to Cr(VI) compounds can lead to irritation and damage of the nasal mucosa. In older studies, it is shown that prolonged exposure to relatively high concentrations (above 0.01 mg Cr (VI)/m³) can lead to irreversible effects such as chromium ulcers and perforation of nasal septum (ATSDR, 2012; SCOEL, 2004). Several regulations and risk management measures are currently in place for Cr(VI) compounds, making it unlikely that nowadays exposure concentrations will be reached that lead to permanent damage of the nasal septum.

3.1.1.2. Interpretation of available scientific data in consultation with experts. There is sufficient evidence for these nasal septum effects from occupational studies in humans exposed to Cr(VI) compounds. It was therefore concluded that Cr(VI) compounds can cause perforation of the nasal septum by chromium ulcers.

3.1.2. Respiratory allergy and other chronic lung diseases

3.1.2.1. International agencies. Different respiratory effects were observed in workers that worked in chromium-related industries, including bronchitis, decreased pulmonary function, and pneumonia. Occupational asthma has been reported as well in several case reports. In addition, some case reports describe that very high exposure to Cr (VI) compounds can lead to acute respiratory effects such as dyspnoea, cough and wheezing. Experimental studies in rodents confirm that inhalation of Cr(VI) compounds can lead to respiratory effects, such as hyperplasia, macrophage infiltration, pulmonary inflammation and fibrosis (ATSDR, 2012).

Chromium (VI) trioxide, potassium dichromate, ammonium dichromate and sodium dichromate dehydrate are classified as category 1 respiratory sensitizers and need to be labeled as H334 (“May cause allergy or asthma symptoms or breathing difficulties if inhaled”) according to the EU CLP regulation (EC 1272/2008) (EC, 2016). This classification is based on evidence in humans that has shown that asthma and/or rhinitis primarily occur after occupational respiratory exposure.

SCOEL summarized respiratory effects seen in workers who had long-term exposure to Cr(VI) compounds. Effects in the lower respiratory tract include inflammation, various obstructive disorders and transient impairment of lung function (SCOEL, 2004; 2017).

The Health and Safety Executive (HSE) in the UK evaluated all available studies providing human evidence for asthma arising from exposure to Cr(VI) compounds (HSE, 1997). A bronchial provocation test is the gold standard that can be used for determining whether a substance causes the condition. In such tests, patients are exposed to the specific substance by inhalation. When the substance elicits allergic symptoms, e.g. dyspnoea or shortness of breath, there is sufficient evidence that there is a causal relationship between the compound and the respiratory allergy.

Six electroplating workers exposed to Cr(VI) compounds developed asthma after latency periods ranging from 8 months to 6 years. Single-blinded bronchial challenge testing with potassium dichromate confirmed the diagnoses. HSE concluded that this was a well-conducted human study that provides strong evidence that Cr(VI) compounds can induce asthma. A case report was included on a welder who developed dyspnoea and urticarial rash after being exposed to chromium trioxide vapours for 10 years. The respiratory complaints and urticarial rash were confirmed in a single-blinded bronchial challenge test with nebulized sodium chromate (Cr VI). In another study, occupational asthma developed in four workers exposed to chromium (probably Cr(VI)) after latency periods of 3 months–9 years. Two workers also developed rhinitis and one developed skin rash (urticaria). Bronchial challenge tests with a nebulized Cr(VI) solution resulted in respiratory complaints in these four employees. They did not show a respiratory response in a control test using a saline solution (HSE, 1997).

The Health Council of the Netherlands evaluated all the available evidence from studies in humans for occupational respiratory allergies as a possible consequence of various chemicals, including Cr(VI) compounds. In accordance with the HSE’s assessment, the Health Council found evidence of a causal link between occupational exposure to Cr(VI) compounds by inhalation and respiratory allergies as asthma and rhinitis. The prevalence of respiratory tract allergies due to exposure to Cr(VI) compounds could not be determined from these studies because most of the human data was from case studies or small studies with just a few workers (Health Council, 2008).

The underlying mechanism by which Cr(VI) compounds cause asthma or rhinitis has not been fully elucidated yet. Both allergic as well as irritative mechanisms may play a role, as is often the case for occupational rhinitis and asthma. The lack of a predictive animal model for these respiratory adverse effects makes it difficult to support the human evidence with animal data and elucidate the mechanisms involved.

There are several inhalation studies performed with Cr(VI) compounds in rodents demonstrating that inhalation exposure leads to inflammatory responses and immune activation in the airways (Glaser et al., 1985, 1990; Nettesheim and Szakal, 1972). These mechanisms may be involved in the induction of respiratory sensitization and subsequent development of respiratory allergies, such as rhinitis or asthma.

3.1.2.2. Interpretation of available scientific data in consultation with experts. There is sufficient scientific evidence for a causal link between occupational inhalation exposure to Cr(VI) compounds and respiratory tract allergies, such as asthma and rhinitis. Asthma does not seem to occur frequently in workers exposed to Cr(VI) compounds, evidence comes mostly from case reports. The threshold for induction of respiratory sensitization by Cr(VI) compounds is unknown. The clinical experts present at the workshop presumed, based on their experience, that high exposure concentrations are often needed for the induction of respiratory allergy. The same holds true for other chronic lung diseases, such as lung fibrosis and chronic obstructive diseases. They have been linked to occupational Cr(VI) exposure as well, but only in some case reports. In a previous evaluations performed by the Dutch Centre for Occupational Diseases, it was assumed that long-term (>10 years) inhalation of Cr(VI) compounds could lead to lung fibrosis or chronic obstructive pulmonary disease (COPD), due to chronic inflammation of the airways, bronchitis, bronchopneumonia and emphysema (Lenderink and Laan, 2014).

Animal studies support the effects observed in workers. Inhalation of Cr (VI) compounds can lead to inflammation, fibrosis and macrophage activation in the airways. These respiratory effects observed in inhalation studies in rodents are time and concentration-dependent. It is difficult to translate these respiratory effects to a specific lung disease in humans.

The conclusion reached at the expert workshop was that prolonged occupational exposure to Cr(VI) compounds can cause chronic lung diseases including rhinitis, asthma, pulmonary fibrosis and COPD in humans.

3.2. Skin

Although the primary route of occupational exposure is inhalation, dermal exposure can occur as well if no risk management measures are taken. Adverse effects after dermal exposure have been reported in workers.

3.2.1. Skin irritation

3.2.1.1. International agencies. Some soluble Cr(VI) compounds, e.g. Cr (VI) trioxide, are corrosive and can cause burns after acute skin contact (ATSDR, 2012). According to SCOEL, highly soluble Cr(VI) compounds can have skin irritating effects, which can lead to “chrome ulcers”. Evidence for these adverse effects is mainly found in case reports, in particular in chrome plating workers or chromate production workers, showing that these effects occur after high exposure (SCOEL, 2004; 2017).

The European Chemicals Bureau (ECB), also report that Cr(VI) compounds can cause skin irritation in humans and that these effects were observed in animal studies as well. Cr(VI) trioxide is considered to be a corrosive substance, due to its low pH. Corrosive effects were observed in humans as well as in several animal studies (ECB, 2005).

3.2.1.2. Interpretation of available scientific data in consultation with experts. There is evidence that relatively high occupational dermal exposure to highly soluble Cr(VI) compounds can cause skin ulcers. Although these ulcers often, they can leave (permanent) scars. Other dermal effects, i.e. irritation are considered reversible and should therefore not be included in the risk assessment done for the POMS

location which focuses on irreversible health effects.

3.2.2. Allergic contact dermatitis (contact allergy)

3.2.2.1. International agencies. Cr(VI) compounds are well-known skin sensitizers and dermal exposure can cause allergic contact dermatitis, a delayed-type immune-mediated disease. Typical clinical symptoms include redness of the skin, rash, itching and edema. These symptoms will resolve once exposure is discontinued, but renewed exposure will lead to elicitation of contact allergy. Sensitization cannot be cured, but clinical symptoms can be prevented by avoiding exposure to Cr(VI) compounds.

Allergic contact dermatitis has been reported in numerous occupational studies, that employed diagnostic patch tests to demonstrate that sensitization to Cr(VI) was the cause of the disease (ATSDR, 2012; SCOEL, 2004; 2017).

Many chromium VI compounds are classified as skin sensitizers category 1 and need to be labeled with H317 (May cause an allergic skin reaction) according to the CLP regulation (EC 1272/2008).

ECB summarized all available animal data for Cr(VI) compounds and skin sensitization. Cr(VI) compounds (sodium/potassium dichromate) elicited skin allergic responses in guinea pig tests, indicative for the skin sensitizing potential of Cr(VI). This was also confirmed in a murine skin sensitization test methods, the mouse ear swelling test and the Local Lymph Node Assay (ECB, 2005).

3.2.2.2. Interpretation of available scientific data in consultation with experts. There is sufficient scientific evidence from human and animal studies that dermal exposure to Cr(VI) compounds can cause allergic contact dermatitis.

It was concluded that occupational exposure to Cr(VI) compounds can cause allergic contact dermatitis in humans.

3.3. Systemic effects on the immune system

3.3.1. International agencies

ATSDR summarized studies showing that exposure to Cr(VI) compounds in humans and in experimental animals has effects on several immunological parameters. Based on the data from these studies, ATSDR concluded that exposure to Cr(VI) can result in immunological effects. This may be indicative of changes in the functioning of the immune system, although these immunological effects were not clearly adverse. ATSDR concluded therefore that the toxicological significance of the immunological effects induced by Cr(VI) compounds is unclear (ATSDR, 2012).

The Dutch Centre for Occupational Diseases (NCvB) concluded in 2014 that the evidence that Cr(VI) compounds can lead to immunological diseases other than respiratory and contact allergies is limited (confidential report issued as part of the Compliance arrangements for former employees of the POMS locations; this report is not publicly accessible). This conclusion was based on a single scientific report in which various immunological parameters were measured for leather industry employees who had been exposed to Cr(VI) (Mignini et al., 2009). The results of this study are discussed later in this chapter. NCvB concluded that Cr(VI) compounds appear to play a role in an immunological stress reaction that was subclinical in the workers studied in the study of Mignini et al. (2009). According to the NCvB, the data from that study is as yet insufficient to be certain that there is a link between Cr(VI) exposure and immunological diseases, other than contact and respiratory allergies.

In none of the other evaluations done by official bodies, a causal link between occupational Cr(VI) exposure and systemic immune effects and diseases was reported. Since concerns on this issue were raised, a dedicated workshop was organized to evaluate all available studies in humans and experimental animals that studies the effects on Cr(VI) on

systemic immune effects and diseases.

3.3.2. Immunological parameters assessed in human studies

Before the individual studies were discussed in detail, there was a general discussion at the expert workshop about the immunological parameters that had been evaluated. The central question here was the extent to which differences between the groups in terms of these parameters were representative and predictive for a medical problem as a consequence of exposure to Cr(VI) compounds and/or whether these differences could also be caused by other factors (such as lifestyle).

Several studies measured concentrations of cytokines or immunoglobulins in serum. Cytokines are secreted by various types of cells and they are important in immunological processes. Some cytokines are only secreted by activated cells; others are produced continuously. The question was therefore whether changes in cytokine concentrations can be used as the basis for statements about possible harmful clinical effects. Cytokine levels fluctuate throughout the day or may be produced throughout the day. The half-life of cytokines is short. Additionally, cytokines work primarily at a local level, whereas the human studies could only take measurements in serum. The conclusion was that studies that only measure cytokines and no other functional immunological parameters cannot be used for determining whether there is a harmful immunological effect that could potentially have clinical consequences.

Some studies measured immunoglobulins in the serum. Immunoglobulins, also referred to as antibodies, are proteins that are produced by B cells, primarily as a response to exogenous substances (antigens). They have longer half-lives and are not so sensitive to fluctuations compared to cytokine levels. The distribution of antibodies between blood and tissues often varies over the course of the day. The participants of the workshop agreed that it is important to compare the immunoglobulin values that were measured against the normal value ranges in humans. These ranges are known and can be quite broad. If the values do not deviate from the normal values, the chance of biologically relevant effects occurring is limited.

Several human studies have performed experiments with lymphocytes that were isolated from peripheral blood. In the majority of studies, the lymphocytes were activated *ex vivo* by adding mitogens to the culture medium. Mitogens are substances that stimulate lymphocyte cell proliferation (mitosis). The mitogens concanavalin A (ConA) and phytohaemagglutinin (PHA) activate T lymphocytes; the mitogen lipopolysaccharide (LPS) activates B lymphocytes and pokeweed mitogen (PWM) activates both B and T cells. After the cells have been activated, proliferation and cytokine production can be measured. These studies are considered informative, since they inform on functioning of the lymphocytes. It was noted, though, that mitogenic stimulation is non-specific; using CD3 as the stimulus for T lymphocytes was considered to be better.

There was one study that measured the activity of natural killer (NK) cells. NK cells are lymphocytes that belong with the non-specific (innate) immune system, playing a key role in the response to viruses in particular. Complement factors were also measured in some studies. The complement system is also part of the innate immune system and, together with antibodies and phagocytes, it plays a key role in hosting resistance against microorganisms.

Finally, the participants agreed that evidence for an adverse effects on the immune system is stronger when functional cohesion is found between the different immunological parameters measured. During the workshop such cohesion was evaluated by the participants. Additionally, the evidentiary strength is greater when functional parameters are measured in the studies; those are parameters that give the best information for determining whether a substance can disrupt the way the immune system functions.

3.3.3. Studies in humans

Eleven human studies were evaluated during the workshop. First, the quality of each study was assessed. The discussion points that were

formulated beforehand were used for this (see paragraph 2.2.6) and used to assess study design and quality of the individual studies. Table 2 gives a brief summary of all the studies, including the quality assessment. Based on their evaluation, the experts decided to exclude three of the human studies (Hu et al., 2017; Islam et al., 2019; Taioli et al., 1995).

The population exposed to Cr(VI) compounds in most of the studies consisted of employees who were exposed during the course of their work. There was also one study in which the exposed group consisted of people who lived or worked in an area that was heavily contaminated with Cr(VI) compounds (Snyder et al., 1996). In this study, lymphocyte proliferation and IL-6 levels were measured using blood samples of 46 individuals who lived and/or worked in this contaminated area. A group of 47 people that did not live in the polluted area was included as a

control group in this study. The experts noticed an important shortcoming of this study, being that no Cr(VI) levels were measured in the group that supposedly was exposed to Cr(VI) compounds, nor in the control group. In this study, lymphocytes isolated from peripheral blood were stimulated with various mitogens. Lymphocyte proliferation and IL-6 production were then measured. Only the production of IL-6 was statistically significantly lower in the exposed group compared against the control group. There was no difference in lymphocyte proliferation between the exposed group and the control group.

During the workshop, the experts concluded that an isolated observation of a reduction in IL-6 production by activated lymphocytes without affecting other functional immunological parameters (such as a reduction in proliferation) cannot be used to determine whether a biologically relevant effect is involved. Furthermore, the absence of

Table 2
Overview of studies in humans.

Reference	Immunological parameters	Findings ¹	Quality assessment	Included in evaluation
Mignini (2009)	- Subpopulations of lymphocytes in the peripheral blood - Proliferation of lymphocytes isolated from blood after mitogenic stimulation - NK cell activity - Cytokines in plasma - Cytokines in serum - Immunoglobulins in the serum - Complement factors in the serum	- Increased lymphocyte proliferation in the highest-exposure group - Higher plasma concentrations of IL-6 and IL-2 - Lower plasma concentration of IL-12	The study has qualitative deficiencies. An important methodological shortcoming is that the exposed group was suddenly split into two subgroups during the study on the basis of the chromium concentration in the urine. This was however not statistically significantly different.	Yes
Qian (2013)	- Cytokines in plasma - Cytokines in serum - Immunoglobulins in the serum - Complement factors in the serum	- Cytokine concentrations in the serum were below the limit of detection for the test method - Immunoglobulins and complement factors were within the normal range of values in all groups	The percentage of smokers in group 1 (exposed employees) is higher than in the control group. This is a potential confounding factor for immunological parameters.	Yes
Taniwaga (1991)	- Absolute and relative numbers of lymphocytes in the peripheral blood - Number and activity of NK cells	- Lower absolute and relative numbers of lymphocytes - Higher NK cell activity (for the number of peripheral blood mononuclear cells)	Exposure to chromium was not determined. The employees who had been exposed were 10 years older than the control group.	Yes
Taniwaga (1995)	Absolute and relative numbers of lymphocytes, T lymphocytes and CD4⁺ and CD8⁺ T lymphocytes in the peripheral blood.	- Lower absolute and relative numbers of lymphocytes, T lymphocytes and CD4⁺ lymphocytes - Lower absolute number of CD8⁺ lymphocytes	Exposure to chromium was not determined.	Yes
Taioli (1995)	None	N/A	N/A – the study was excluded because no immunological parameters were measured.	No
Snyder (1996)	- Proliferation of lymphocytes isolated from blood after mitogenic stimulation - IL-6 production by lymphocytes after mitogenic stimulation	Lower IL-6 production	It is not known whether the exposed group was exposed to chromium.	Yes
Kuo & Wu (2002)	- Cytokines in the peripheral blood - Lymphocyte subsets in the peripheral blood	- Higher concentration of IL-8 in the blood - Lower percentage of B cells in the peripheral blood	Smoking is an important confounding factor that can affect the lymphocyte subsets. It is not known whether the percentages of smokers differ between the groups. It is not known whether a correction was made for confounding factors.	Yes
Katiyar (2008)	- Proliferation of lymphocytes isolated from blood after mitogenic stimulation - IL-2, IL-4, IL-10, TNF-α and IL-6 production after mitogenic stimulation	- Lower proliferation of T lymphocytes - Lower IL-6 production	Inclusion and exclusion criteria were not stated. The control group was not defined: there is only a statement that they were healthy volunteers.	Yes
Katiyar (2009)	IL-12 and IFN-γ production after mitogenic stimulation	Higher IL-12 production	Inclusion and exclusion criteria were not stated. The control group was not defined: there is only a statement that they were healthy volunteers.	Yes
Hu (2017)	Proteomics to identify biomarkers for chromium exposure	There was no difference between the biomarkers identified	Methodology is incorrect: pooled samples were used for identifying biomarkers. These were not demonstrated in the individual samples during the verification.	No
Islam (2019)	- Medical examination - Concentration of immunoglobulin and complement factors in the serum - Antibacterial activity of the serum	- The majority had a condition (contact allergy, other allergies) and suffered from infections more often - Concentrations of IgM, IgG, IgA and complement factors were within the normal range of values in all groups - Serum IgE is elevated - The antibacterial activity of the serum is reduced	The majority of the exposed group had one or more chronic conditions. Having a chronic disease was an exclusion criterion for the control group. This is a large difference between the groups. The control group consisted of volunteers who worked in shops and offices. The exposed group consisted of tannery workers. The control group came from a completely different socioeconomic environment than the tannery workers. In this study, there is a difference between the control group and the exposed group that could affect the immunological parameters. This study was therefore not included.	No

information on the actual exposure to Cr(VI) in the exposed versus the control group makes it impossible to link any effects to Cr(VI) exposure.

In a cross-sectional occupational study from Italy, various immunological parameters were measured in employees who had worked in the shoemaking and leather industries. The control group consisted of office workers from the same companies. Cr (VI) was measured in the air as well as in the blood and urine of all the volunteers. The Cr(VI) concentrations in the urine were statistically significantly higher in the exposed group compared to the control group. The immunological parameters that were measured in this study were lymphocyte subpopulations in the peripheral blood, cytokine concentrations (IL-1 β , IL-6, TNF- α , IFN- γ , IL-4 and IL-2) and soluble adhesion molecules (ICAM-1, VCAM and E-selectin) in plasma, NK cytotoxic activity and lymphocyte proliferation from peripheral blood after mitogenic stimulation.

During the study, the exposed group was split into two groups based on chromium concentrations in the urine: a high-exposure group and a low-exposure group. The differences in chromium concentrations in the urine between the two exposed groups were however not statistically significant. The chromium concentration in the urine of the high-exposure group was statistically significantly higher in comparison with the control group. The low-exposure group did not have an elevated concentration of chromium in urine when compared to the control group.

In the group with the highest exposure level, the proliferation of lymphocytes after stimulation with the mitogens PHA or ConA and plasma concentrations of the cytokines IL-6 and IL-2 were statistically significantly higher than in the control group. The plasma concentration of IL-12 was statistically significantly lower in the highest-exposure group. For the other measured immunological parameters no differences between the groups were found (Mignini et al., 2009). The experts' conclusion was that this study had large methodological deficiencies in terms of its scientific quality. Splitting the exposed population using an arbitrarily chosen cut-off point created a number of effects that would otherwise not have been present. Furthermore, deficiencies in statistics were noted as well. Because of these methodological shortcomings, it is not possible to use the results of this study to determine whether occupational exposure to Cr(VI) has adverse effects on the immune system.

In a cross-sectional occupational study from China, various immunological parameters were measured in the male employees of a factory where potassium dichromate is used. The control group consisted of men who were not exposed. The exposed group consisted of two subgroups. In group 1, serum concentrations of cytokines IL-2, IL-4, IL-6, IL-10, TNF- α , IFN- γ and IL-17a were measured. In group 2, the concentrations of immunoglobulins IgA, IgM, IgG and IgE and complement factors (C3 and C4) in the serum were determined. The percentages of smokers and alcohol users was statistically significantly higher in group 1 than in the control group. No corrections were done for these differences. This was considered as a methodological limitation of this study by the experts.

Chromium was measured in the air as well as in the blood and urine of all the volunteers. The chromium concentrations in the air, blood and urine were statistically significantly elevated for the exposed groups when compared to the control group. For volunteers in the exposed as well as the control groups, cytokine concentrations were below the limit of detection and therefore not reliable. In group 2, the concentrations of IgG and IgA were statistically significantly lower in the exposed workers compared to the control group. Furthermore, the concentrations of complement factors were higher in the exposed group compared to the control group (Qian et al., 2013). A comparison was made with the values that are known for all of these parameters. It turned out that the concentrations of IgG, IgA and complement factors in the exposed workers were within the range of the normal values.

It was concluded that although statistically significant differences were demonstrated in this study for a number of immunological parameters, these effects were considered not to be biologically relevant, because they were within the range of normal values.

In two studies from Japan, several immunological parameters were measured in retired male workers who had been employed in a factory that produced chromate (Tanigawa et al., 1991, 1995). Chromium levels were not measured in serum or urine in these studies, so it is not known whether and to what extent the employees had been exposed to chromium. It was reported that nine of the exposed workers had nasal septum perforations, suggesting a relatively high level of exposure to chromium.

In the first study, 23 retired male chromate productions workers were included that worked seven to 35 years in the factory. The control group consisted of 12 healthy male taxi drivers. In this study, the total (absolute) and relative numbers of lymphocytes in the peripheral blood were determined. Additionally, the number and activity of NK cells was examined. The total and relative numbers of lymphocytes from the blood of exposed employees were statistically significantly lower than in the control group. There was no statistically significant difference between the groups in the total number of NK cells. The relative number of NK cells (with respect to the number of lymphocytes) was statistically significantly higher in the exposed employees. The relative NK activity (expressed per 10⁷ peripheral blood mononuclear cells, or PBMCs) was statistically significantly elevated in the exposed employees. There was no difference in the NK activity as expressed per 10⁵ NK cells. This indicates that the activity of the NK cells themselves was not increased. The increase in the relative NK activity (expressed for the number of lymphocytes) was explained by the authors by a decrease in the number of lymphocytes (Tanigawa et al., 1991).

In the second study, the exposed group consisted of 19 retired male employees who had worked 7–39 years in a factory that produced chromate. The control group consisted of 33 male volunteers who worked as security or office staff in other factories where they were not exposed to chromium. The total numbers of lymphocytes, T lymphocytes and the T lymphocyte subpopulations (CD4⁺ and CD8⁺) were determined in the peripheral blood. The total and relative numbers of lymphocytes, T-lymphocytes and CD4⁺ T-lymphocytes and the total number of CD8⁺ T lymphocytes were statistically significantly lower in exposed employees when compared against the controls (Tanigawa et al., 1995).

During the expert workshop, it was mentioned that both studies demonstrated a decrease in peripheral blood lymphocytes in the employees who had been exposed to Cr(VI) compounds. In the first study (Tanigawa et al., 1991), the number of NK cells and their activity was also examined. There were no differences between the groups in the absolute number of NK cells and the NK cell activity. The increase in relative NK activity was explained by the reduction in the number of lymphocytes.

These studies showed that the workers had lower absolute and relative numbers of lymphocytes. This is an observational immunological parameter that cannot be used in its own right to determine whether this decrease impairs the functioning of the immune system. It was therefore concluded that these studies cannot be used to draw conclusions as to whether exposure to Cr(VI) compounds is linked to clinically relevant adverse effects.

In a Taiwanese study, the employees of an electroplating workplace were examined. Chromium concentrations in the urine and air were measured. The type of work together with the urine concentrations were used to divide the cohorts into three groups: high urine concentration (electroplating workers; n = 10), moderate urine concentration (finishing workers – polishing, painting; n = 17) and the control group (administrative staff; n = 19). The following immunological parameters were measured in the peripheral blood: cytokines (IL-2, IL-4, IL-6, IL-8, IL-10, TNF- α and IFN- γ) and lymphocyte subpopulations (B lymphocytes, T lymphocytes, CD4⁺ T cells and CD8⁺ T cells) in the blood.

In the group with moderate urine chromium concentration, no statistically significant differences were found with the control group. In the high chromium urine concentration group, the concentration of IL-8 was statistically significantly higher when compared to the control group. There were no differences between the groups for the other

cytokines measured. The percentage of B cells was statistically significantly lower in the group with high chromium urine concentrations when compared to the control group. The absolute numbers of white blood cells, lymphocytes, monocytes and granulocytes did not vary between the groups (Kuo and Wu, 2002).

The parameters that differed statistically significantly in the group with the high chromium urine concentration were discussed at the workshop. In this group, the relative percentage of B lymphocytes was statistically significantly lower compared to the control group. It was noted that only looking at relative numbers of lymphocytes is not sufficient to draw conclusions. It is more informative to look at the total numbers of lymphocytes (both B and T) as well. However, total number of B lymphocytes was not mentioned in the publication, which was considered to be a methodological limitation. Remarkably, the total number of lymphocytes did not vary between groups. A statistically significantly higher concentration of IL-8 was observed in the high-exposure group as well. The conclusion of the experts was that this isolated observation without effects on related functional immunological parameters cannot be used to determine whether this increase is a biologically significant effect.

There are two publications from India in which various immunological parameters were measured in workers exposed to Cr(VI) compounds in different occupational settings (Katiyar et al., 2008, 2009).

In the first study, the employees who had been exposed to Cr(VI) compounds were divided into three cohorts: leather tanners ($n = 37$), industrial painters ($n = 30$) and chrome platers ($n = 33$). The control group consisted of 35 healthy, non-exposed volunteers. The concentration of chromium in the peripheral blood was measured for all those taking part. The tannery and chrome plating workers had significantly higher chromium concentrations in the blood when compared against the control group. The chromium concentrations in the blood of the industrial painters did not differ from the control group. The following immunological parameters were measured: proliferation and cytokine production (IL-2, IL-4, IL-10, TNF- α and IL-6) by lymphocytes from peripheral blood that had been activated using various mitogens. No statistically significant effects were found in the group containing industrial painters when compared against the control group. In both the leather tanning workers and the chrome platers, the proliferation of T lymphocytes after stimulation with the mitogen PHA was statistically significantly lower when compared against the control group. Of all the cytokines that were measured, only the PHA-induced production of IL-6 was statistically significantly lower in the tannery workers when compared against the control group (Katiyar et al., 2008).

In the second study, the cohort of exposed workers consisted only of the leather tanner workers and chromeplaters. A group of 30 workers was included and all of them had chromium blood levels above 100 $\mu\text{g/L}$. The control group consisted of 20 healthy volunteers. In this study, the production of the cytokines IL-12 and IFN- γ was measured after mitogenic stimulation of the lymphocytes. In the exposed workers, the concentrations of IL-12 and IFN- γ were statistically significantly higher than in the control group (Katiyar et al., 2009).

The results of both studies were discussed during the workshop. The first study showed that proliferation and IL-6 production by lymphocytes after mitogenic stimulation are lower in exposed workers. The lymphocyte response to mitogenic activation appears to be suppressed. In the second study, there was an increase in the production of the cytokines IL-12 and IFN- γ . Lymphocyte proliferation was not measured in this study, making it impossible to know if the suppression of lymphocyte proliferation occurred in this group of Cr(VI) exposed workers as well. The effects on cytokine production were contradicting in the two studies. It was concluded that interpretation of the immunological parameters is difficult, because the studies did not reveal a consistent pattern. It was therefore not possible to draw conclusions about the biological relevance of the effects measured in workers exposed to Cr(VI) compounds.

3.3.4. Summary of studies in humans

A total of eight studies were included in the evaluation during the expert workshop. The study by Qian et al. (2013) did not find any biologically relevant differences between the exposed group and the control group. There were serious methodological shortcomings in the study by Mignini et al. (2002) and so the results of that study could not be used for assessing whether Cr(VI) has adverse effects that could impair the immune system. In the studies by Snyder et al. (1996) and Kuo and Wu (2002), only a single immunological parameter (cytokine production) in the exposed group was statistically significantly different from the control group. During the workshop, the experts concluded that an isolated observation of an effect on cytokine production without affecting other functional immunological parameters cannot be used to determine whether a biologically relevant effect is involved. In the studies by Taniwaga et al. (1991, 1995) and Katiyar et al. (2008, 2009), inconsistent effects were seen in the immunological parameters that were statistically significantly different from the control group.

3.3.5. Studies in experimental animals

The effects of Cr(VI) compounds on systemic immunological parameters have been examined in four animal studies. Different routes of exposure were used in these studies: oral exposure (Shipkowski et al., 2017; Snyder et al., 1996), dermal exposure (Fang et al., 2013) or respiratory exposure (Glaser et al., 1985). During the workshop it was decided to exclude the study from Fang et al. (2013), because effects of Cr(VI) exposure was studied in a severely immunodeficient model. The outcomes of such a model were considered to be irrelevant for humans.

The effects of inhalation exposure to aerosols containing sodium dichromate (25, 50, 100 or 200 $\mu\text{g/m}^3$ $\text{Na}_2\text{Cr}_2\text{O}_7$) have been investigated in a subacute study (28 days) and a subchronic study (90 days) in rats (Glaser et al., 1985). In both studies, hematological parameters were measured in the blood, several parameters were measured in the bronchial lavage (BAL) fluid, histopathology was carried out on various organs and total concentration of immunoglobulins in the serum was measured. In both the subacute and subchronic studies, half of the rats in each group were immunized using the SRBC (sheep red blood cell) antigen in order to measure the T-cell-dependent primary IgM production. Spleen cells were also stimulated using the ConA mitogen to measure the proliferation of T cells. In this subchronic study, a subgroup that was exposed to 50 $\mu\text{g/m}^3$ was not sacrificed, but was instead exposed to clean air for two months to see if the immunological effects were reversible.

No effects on the haematology were observed in the subacute and subchronic studies; nor were there effects on the histology of the lungs, kidneys, liver, stomach and sexual organs. The statistically significant pulmonary and systemic effects in the subacute and subchronic studies are summarized in Table 3.

This inhalation study in rats showed that the pulmonary and systemic immunological effects of Cr(VI) are dose-dependent and time-dependent. In the subacute study, inhalation of Cr(VI) led to a moderate increase in lymphocytes, granulocytes and the number of multinucleated macrophages in the lungs, as well as an increase in phagocytic activity. The IgM response after immunisation with SRBC was also increased. In the subchronic study more pulmonary and systemic parameters were affected. Furthermore, the pulmonary effects were clearly dose-dependent. At lower doses, there was a mild increase in lymphocytes, granulocytes and multinucleated macrophages in the lungs, as well as an increase in phagocytic activity. In the highest dosage group, there was a decrease of phagocytic activity of the macrophages and also the pulmonary clearance of iron oxide was reduced. This suggests that at the highest exposure, Cr(VI) suppresses the pulmonary resistance. This study demonstrated that inhalation of Cr(VI) leads to changes in the systemic immunological parameters. Subacute and subchronic inhalation of Cr(VI) led to an increase in the IgM response against the SRBC antigen in all exposure groups. Additionally, the ConA-induced T cell proliferation increased in the highest dosage group in the subchronic

Table 3

Effects of Cr(VI) on immunological parameters after subacute and subchronic inhalation exposure*.

Subacute study (28 days)	Subchronic study (90 days)
Pulmonary effects	
increased weight of the lungs and spleen (all exposure groups)	increased weight of the lungs and spleen (all exposure groups)
	decrease in macrophages in pulmonary lavage (50 and 200 µg/m ³)
increase in the number of multinucleated macrophages (25 µg/m ³ group)	increase in the number of multinucleated macrophages (50 and 200 µg/m ³)
increase in the number of macrophages in telophase (25 and 50 µg/m ³)	increase in the number of macrophages in telophase (25, 50 and 100 µg/m ³)
increase in the number of lymphocytes in pulmonary lavage (25 and 50 µg/m ³)	increase in the number of lymphocytes in pulmonary lavage (25 and 50 µg/m ³)
increase in the number of granulocytes in pulmonary lavage (50 µg/m ³)	increase in the number of granulocytes in pulmonary lavage (50 µg/m ³)
increase in the phagocytic activity of alveolar macrophages (50 µg/m ³)	increase in the phagocytic activity of alveolar macrophages (25 and 50 µg/m ³)
	reduction in the phagocytic activity of alveolar macrophages (200 µg/m ³)
	reduced pulmonary clearance of iron oxide (200 µg/m ³)
Systemic effects	
dose-dependent higher primary IgM response to SRBC (all exposure groups)	dose-dependent higher primary IgM response to SRBC (all exposure groups)
	dose-dependent increase in the total concentration of immunoglobulins in the serum (50 and 100 µg/m ³)
	higher proliferation in ConA-stimulated T lymphocytes isolated from the peripheral blood (200 µg/m ³)

* Taken from Glaser et al., 1985. Abbreviations: SRBC: Sheep Red Blood Cells; ConA: Concanavalin A.

study and the total immunoglobulin concentration in the serum was increased in the rats that were exposed subchronically to 25 or 50 µg/m³ Cr(VI) (Glaser et al., 1985).

The expert workshop's conclusion was that inhalation of Cr(VI) compounds can cause immunotoxic effects, both after subacute as well as subchronic exposure. These effects occur both locally (in the lungs) and systemically. There are clear changes in functional immunological parameters, in both the subacute and the subchronic studies. These point towards an enhancement of the immune response after inhalation of Cr(VI).

Two oral studies were carried out studying the effects of Cr(VI) compounds on the immune system after intake through the drinking water. The more recent study by Shipkowski et al. (2017) is the more extensive. In the study, which was carried out as part of the National Toxicology Program (NTP) in the United States, two rat strains (Sprague Dawley and Fischer 344/N) and one mouse strain were used. The animals were exposed to various concentrations of sodium dichromate hydrate for 28 days. Several assays were used in this study to examine the effects of oral exposure to Cr(VI) on both the innate and the adaptive immune system (humoral and cell-mediated) (summarized in Table 4).

The effects observed for oral exposure to Cr(VI) depend on the animal species and strain, whereas most effects were observed in mice. The study in mice was repeated and a lot of effects could not be reproduced in the second study. A consistent change in all rodent study, is the increase of SRBC-specific IgM response in the spleen, that was observed in both rat strains as well as in mice. In Sprague Dawley rats, this increase was not statistically significant. In the F344/N rats and in the mouse study, the effects were only statistically significant for one of the exposure dose, no dose-response effects were observed. The increase in the spleen was not accompanied by an increase of SRBC-specific IgM in serum. In the cohorts immunized with KLH, no differences were found

Table 4

Statistically significant effects of Cr(VI) on immunological parameters in the oral rodent studies*.

Animal model	Immunological parameters
Fischer 344/N rats (n = 8)	Increase in the absolute number of NK cells and macrophages in the spleen after exposure to 14.3 and 172 ppm.
	Increased SRBC-specific IgM production in the spleen after immunisation with SRBC after exposure to 57.3 ppm.
Sprague Dawley rats (n = 10)	Increase in the percentage of macrophages in the spleen after exposure to 15.6, 62.5, 125 and 250 ppm.
B ₆ C ₃ F ₁ mice (n = 8)	Decrease in the relative weight of the spleen (16%) after exposure to 31.3 ppm. This effect was not reproduced in a second study.
	Increase in the relative number of B cells in the spleen after exposure to 250 ppm.
	Increase in the relative number of Th cells in the spleen after exposure to 15.6, 62.5, 125 and 250 ppm.
	Increase in the relative number of T _{CTL} cells in the spleen after exposure to 31.3 and 125 ppm.
	Decrease in the absolute and relative numbers of macrophages in the spleen after exposure to 31.3 and 125 ppm.
	Increased SRBC-specific IgM production in the spleen after immunisation with SRBC after exposure to 31.3 and 62.5 ppm; these effects could not however be reproduced in a second study in mice.

* Taken from Shipkowski et al., 2017. Abbreviations: SRBC: Sheep red blood cells; ppm: parts per million.

for KLH-specific IgM between the experimental and the control groups. There was also no effect on the anti-CD3 T lymphocyte proliferation between controls and animals exposed to Cr(VI).

The authors concluded that Cr(VI) had no effect or inconsistent effects on the immunological parameters being evaluated. The NTP concluded that Cr(VI) intake via the drinking water for 28 days produces minimal toxicological and immunotoxicological effects in mice and rats (Shipkowski et al., 2017).

All the findings were discussed at length during the expert workshop. In summary, the NTP studies were well-conducted, with clear descriptions of the methodology and statistical analysis done. The different studies were quite extensive, measuring a lot of parameters, including functional read-outs. Cr(VI) caused an increase in SRBC-specific IgM in the spleen in the first mouse study and in Fischer 344/N rats. It is remarkably that the SRBC-specific IgM response in the serum was not elevated. Additionally, there are other functional parameters (such as the IgM response against KLH immunization and anti-CD3-induced proliferation of T lymphocytes) that were not changed in any of the animal studies. This led to the conclusion that there were no clear and consistent patterns within any of the rodent studies or when the different studies were compared. As regards the studies in mice, it is noteworthy that the statistically significant decrease in the relative weight of the spleen and the statistically significantly higher SRBC-specific IgM response in the spleen could not be reproduced in a second study in mice.

In the other oral animal study, Fischer 344 (F344) rats were exposed orally for 21 days or 10 weeks to Cr(VI) via the drinking water (as K₂CrO₄). In the 21-day study, rats were exposed to 100 and 200 mg/ml; in the 10-week study, only to 100 mg/ml. In the 21-day study, there was a statistically significant increase in the proliferation of T and B lymphocytes after mitogenic activation of spleen cells in rats that had been exposed to 100 mg/ml. In the 200 mg/ml group, there was a statistically significant decrease in the proliferation of T lymphocytes after mitogenic stimulation. There was no difference in the proliferation of B lymphocytes in this exposure group. The authors state that the decrease in T cell proliferation in the highest dose group was probably caused by systemic toxicity. In the 10-week study, higher proliferation was measured in the spleen cells in the mixed lymphocyte test as compared to the control group. In this test, it was shown that adding Cr(VI) to the

cultured spleen cells from rats that were exposed to Cr(VI) resulted in an increased proliferation than the spleen cells from the control group. According to the authors, this indicates sensitization of the immune system by Cr(VI) (Snyder and Valle, 1991).

The experts pointed out that not many immunological parameters were measured in this study compared to the NTP study. The 21-day study shows that Cr(VI) at a lower dose leads to activation whereas at a higher dose it leads to suppression. This is in line with the inhalation study by Glaser et al. (1985). The results therefore do not add much to the animal studies discussed earlier, which were much more extensive.

3.3.6. Interpretation of available scientific data in consultation with experts

The literature search did not find studies in humans that could provide evidence that occupational exposure to Cr(VI) compounds leads to immunological diseases, other than respiratory and contact allergy. There were several publications found in which immunological parameters were measured in workers exposed to Cr(VI) compounds. Also, immunotoxic effects of Cr(VI) has been studied in some animal studies. In the absence of human studies that found an association between occupational Cr(VI) exposure and immunological diseases, such as autoimmune diseases, the central question during the workshop was whether the changes in immunological parameters from the human studies were indicative for permanent adverse effects ('adversity') and/or immunological conditions. The conclusion was that the evidence from these studies in humans cannot be translated to biologically or clinically relevant permanent effects or diseases. In the animal studies, oral or inhalation exposure does cause changes in functional immunological parameters but the effects were not clearly dose-related, are strain and species-dependent and cannot always be reproduced. Therefore, the clinical relevance and significance of these changes remains unclear.

The experts concluded that exposure to Cr(VI) compounds can lead to changes in immunological parameters both in humans and experimental animals. The clinical relevance of these changes remains unclear. The final conclusion of the workshop was that it is currently insufficiently clear if Cr(VI) can cause irreversible diseases due to disturbances of the immune system (other than allergic contact eczema, allergic asthma, allergic rhinitis and chronic lung diseases) in humans.

3.4. Effects on fertility and developmental toxicity

3.4.1. International agencies

The Health Council of the Netherlands has classified Cr(VI) compounds as toxic to fertility and as toxic to prenatal development (Health Council, 2016a). Classification was performed according to the criteria described in European Union Regulation (EC) 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures (EC, 2016). After its first report in 2001 (Health Council, 2001), the Health Council of the Netherlands updated its assessment in 2016 (Health Council, 2016a). In this assessment, Cr(VI) compounds included chromium trioxide, sodium chromate, sodium dichromate, potassium dichromate, chromic acid, ammonium chromate, ammonium dichromate, calcium chromate, potassium chromate, and dichromium tris(chromate). For effects on fertility, these compounds were classified in category 1B (presumed human reproductive toxicant), and labeled with hazard phrase H360F (may damage fertility). For effects on prenatal development, these compounds were classified in category 1B (presumed human reproductive toxicant) and labeled with hazard phrase H360D (may damage the unborn child). Classification of a substance in category 1B is largely based on data from animal studies. It is important to realize that the CLP regulation is intended for labeling purposes only, and is based on hazard identification (i.e. the intrinsic properties of a chemical), which does not in itself take exposure into consideration and does not inform about risk. In the following, a summary of the reproductive toxicity assessment of Cr(VI) compounds is given, largely based on the recent report of the Health Council of the Netherlands on the subject.

3.4.2. Effects on fertility

Human studies investigated male and female fertility parameters in relation to stainless steel welding, which involves exposure to Cr(VI) compounds in the fumes, but also possible other reprotoxic compounds such as manganese and nickel. Exposure levels were not determined. Rachootin et al. (1983) and Jernes and Knudsen (1988) found no association between welding and male fertility parameters (Bonde and Ernst, 1992; Jernes and Knudsen, 1988; Rachootin and Olsen, 1983). Bonde and Ernst (1992) observed that welders were more likely to have higher Cr(VI) blood levels, but these were not associated with changes in sperm parameters (Bonde and Ernst, 1992). Li et al. (2001) found lower sperm counts in a group of chemical workers as compared to controls, but this was not associated with a difference in blood Cr(VI) concentration between the groups (Li et al., 2001). Mortensen (1998) reported reduced sperm numbers and motility in a group of steel welders (Mortensen, 1988). This study has no data on chromium exposure. Kumar et al. (2005) reported increased numbers of abnormal sperm in chromium sulfate workers and they had higher chromium blood levels (Kumar et al., 2005). There were significant lifestyle differences between exposed and control groups. In a large cohort of metalworkers (n = 3507), Bonde (1993) found no association between fertility and welding exposure (Bonde, 1993). Overall, these data identified no clear association between Cr(VI) compounds exposure and fertility, and in all cases co-exposure to other substances in the fumes was present. The available human data do not provide sufficient evidence to conclude that Cr(VI) compounds affect human fertility under real life exposure conditions.

Animal studies on fertility effects of Cr(VI) compounds are relatively abundant, with 20 publications referred to by the Health Council of the Netherlands report (Health Council, 2016a). Different exposure scenarios, but mainly the oral route in monkeys, rabbits, rats and mice caused testicular atrophy, reductions in sperm counts and sperm parameters, estrus cycle effects, reduced fertility, and increased preimplantation loss. Seven studies reported LOAELs for testicular effects between 0.5 and 10 mg Cr(VI)/kg bw/day (Aruldas et al., 2005; Behari et al., 1978; Bonde and Ernst, 1992; Chowdhury, 1995; Ernst, 1990; Ernst and Bonde, 1992; Li et al., 2001; Yousef et al., 2006).

Remarkably, in a series of four oral exposure studies in rats and mice from the US National Toxicology Program, in the presence of mild general toxicity, no fertility effects were observed up to the highest dose levels tested, which varied from 9 to 41 mg Cr(VI)/kg bw/day. Only one inhalation study in animals is available. Within this study 0 or 200 µg Cr/m³ as sodium dichromate (0 or 0.2 mg Cr/kg bw/d (assuming a breathing volume of 200 ml/min))/inhalation was tested in Wistar rats. These rats were exposed for 130 days per generation for 3 generations. No effect on reproduction was identified in these animals (Glaser et al., 1984). The weight of evidence led the Health Council of the Netherlands to conclude on a Classification for fertility in Category 1B (Health Council, 2001, 2016a). This is in line with the ECHA/RAC evaluation on Cr(VI) compounds (ECHA, 2013).

3.4.3. Developmental effects

Three human studies on Cr(VI) exposure and developmental toxicity were identified by the Health Council of the Netherlands (Aschengrau et al., 1993; Bonde, 1993; Hjollund et al., 2000). One of them indicates an association of pregnancy loss with parental exposure to stainless steel welding (Hjollund et al., 2000). However, no conclusions on causality can be drawn as the stainless steel welding fumes have not been analyzed for Cr(VI) compounds and co-exposures to other toxic substances occurred.

Animal studies on developmental toxicity of Cr(VI) compounds have been performed in rats, mice and hamsters. Sixteen reports are referenced in the report of the Health Council of the Netherlands, in most of which exposure was done via the drinking water. Common findings among multiple studies are pre- and postimplantation loss, retarded fetal growth, reduced numbers of live pups, low pup weight, fetal hemorrhages, reduced ossification, and increased numbers of malformations.

The only inhalation study available did not show effects at the highest dose tested, 200 $\mu\text{g}/\text{m}^3$ (when converted to compare it with oral doses 0.2 mg Cr(VI)/kg bw/day)) (Glaser et al., 1984). Among the studies using the oral exposure route, Elsaieed and Nada (2002) observed the lowest LOAEL at 7 mg Cr(VI)/kg bw/day (Elsaieed and Nada, 2002). Interestingly, in a study from the US National Toxicology Program no pup effects were observed up to oral exposure (diet) levels of 30 mg Cr(VI)/kg bw/day, which did induce maternal weight loss (NTP, 1997). The Health Council of the Netherlands concluded on a category 1B classification for prenatal development based on the weight of evidence (Health Council, 2016a).

Two new developmental studies in rats were published after the report of the Health Council of the Netherlands.

Lactating rats were orally exposed to Cr(VI) (50 ppm potassium dichromate) from postpartum days 1–21 through drinking water. On postnatal day (PND) 25, blood and the ovary, kidney and liver were collected from the F1 females for analyses. After oral exposure to Cr(VI), increased atresia of follicles, decreased estradiol (E2) biosynthesis and enhanced metabolic clearance of E2 was observed. Furthermore, after developmental exposure to Cr(VI), an increase in oxidative stress parameters accompanied by a decrease in endogenous antioxidants was found (Banu et al., 2016). This was a single dose level study in which no body weights nor drinking water consumption was given. No NOAEL could be established. The LOAEL is 50 ppm based on ovarian follicle atresia.

In the second study, pregnant dams (rats) were orally exposed to Cr(VI) (50 ppm $\text{K}_2\text{Cr}_2\text{O}_7$) through drinking water from gestational day (GD) 9.5–14.5, and placentas were analyzed on GD 18.5. Cr(VI) increased apoptosis of trophoblasts, vascular endothelium of the metrial glands and yolk sac epithelium in the placenta. This is a single dose level study neither body weights nor drinking water consumption is given so dose in mg/kg bw can only be estimated. No NOAEL could be established. The LOAEL is 50 ppm based on ovarian apoptosis in the placenta (Banu et al., 2017).

In summary, the two new developmental studies from Banu et al. (2016, 2017) show clear adverse health effects after oral exposure to Cr(VI), i. e. follicular atresia, apoptosis of trophoblasts, vascular endothelium of the metrial glands and yolk sac epithelium in the placenta. The LOAEL in these studies was 50 ppm in drinking water. Using a conversion factor of 0.121 for drinking water exposure to daily dose in female rats (EFSA, 2011), this amounts to 6 mg/kg per day. This exposure level is several orders of magnitude above the dose metric of 0.003 mg/kg per day used as the point of departure for reproductive and developmental toxicity risk assessment (Palmen et al., 2018). In addition, both are single dose studies, which does not allow analysis of dose-response. Taken together, the recent literature reviewed above does not provide novel insights that affect the existing hazard assessment of Cr(VI) exposure for reproductive and developmental toxicity (Health Council, 2016a).

3.4.4. Effects on lactation

No human studies on effects on or via lactation are available. One review reported on chromium levels in human breast milk (Mullee A et al., 2012). No studies on occupationally exposed in lactating woman are available.

Two studies in rats showed developmental effects including delayed vaginal opening, extended diestrus phase, decreased follicle numbers, lack of antral follicle development, decreased pup weight and oxidative stress in pups after Cr(VI) compound exposure to lactating rats (Samuel et al., 2011; Stanley et al., 2013). The LOAEL was established at 6 mg Cr(VI)/kg bw/day. Since no levels in breast milk were measured, toxicity cannot be directly attributed to the chromium level in breast milk.

The presence of chromium VI in breast milk cannot be excluded, and, since most Cr(VI) compounds are classified as genotoxic carcinogens and theoretical no threshold can be established for such compounds, therefore the Health Council of the Netherlands proposed to label Cr(VI)

compounds for effects on or via lactation.

3.4.5. Interpretation of available scientific data in consultation with experts

There is limited scientific evidence that occupational exposure to Cr(VI) compounds, especially in welders, can lead to adverse effects on fertility and prenatal development in humans. Data from animal studies using oral exposure are inconsistent. Several animal studies show that oral exposure to Cr(VI) compounds leads to adverse effects on sperm, fertility and prenatal development after chronic oral exposure. In other oral studies no effects were seen. No effects on lactation, fertility and development were found in inhalation studies in rats. The effects observed in orally exposed rodent studies occurred at exposures far exceeding those likely to occur in humans and the effects are therefore considered not to be relevant for occupational human exposure conditions.

It was therefore concluded that it is currently insufficiently clear if Cr(VI) can cause adverse effect on fertility or prenatal development in humans.

3.5. Other systemic effects

3.5.1. Gastrointestinal effects

The primary route of exposure for gastrointestinal effects caused by Cr(VI) compounds is the oral route. In occupational settings this is not the primary route, but oral exposure is possible via mucociliary movement of particles from the respiratory tract to the oral cavity, by transfer via hand-to-mouth activity and due to mouth breathing of a Cr(VI) aerosols.

3.5.1.1. International agencies. ATSDR summarizes different studies in humans reporting adverse gastrointestinal effects. Very high exposure to lethal or near-lethal doses of Cr(VI) compounds can lead to adverse effects, such as abdominal pain, vomiting, gastrointestinal ulceration, hemorrhage, necrosis and diarrhea. In the 1960s, humans were exposed to Cr(VI) compounds in China via heavily polluted drinking water that contained 20 mg Cr(VI) per liter water. Adverse effects such as oral ulcer, diarrhea, abdominal pain, ingestion and vomiting were reported. In occupational studies in chrome plating and chromate production workers, gastrointestinal complaints, such as epigastric pain, irritation and ulceration, have been reported. These workers were exposed to Cr(VI) via inhalation and presumably via the oral route as well (mucociliary movement from the lungs or indirect via hand-mouth activity). It was noted that the outcomes may be influenced by other factors, such as stress or diet (ATSDR, 2012).

Studies in animals show that the gastrointestinal tract is the primary target of intermediate and chronic duration oral exposure to Cr(VI) compounds. Different animal studies show that after oral exposure epithelial hyperplasia is present in the stomach and intestines. Higher concentrations result also in ulceration. Inhalation studies with Cr(VI) compounds in rodents resulted in mixed results. In rats, no gastrointestinal effects were found after inhalation exposure (0.2 mg/m^3 for 90 days). A chronic inhalation study in mice exposed to 4.3 mg/m^3 Cr(VI) resulted in occasional small ulcerations in the stomach and intestinal mucosa. In this study, oral exposure due to grooming behavior could not be excluded (ATSDR, 2012).

The National Toxicology Program (NTP) in the USA conducted chronic oral exposure studies in rats and mice. Cr(VI) compounds were administered via drinking water. After the 2-year exposure period, rats displayed histiocytic infiltration (0.77 and 2.4 mg Cr(VI)/kg bw/day as sodium dichromate dihydrate). In mice duodenal epithelial hyperplasia was found at 0.38 mg Cr(VI)/kg bw/day and histiocytic cellular infiltration of the duodenum at 2.4 mg Cr(VI)/kg bw/day (NTP, 2008).

In line with the NTP study, a more recent oral study in rats showed that effects are clearly time and dose-dependent. Histopathological changes in the gastrointestinal tract include villous atrophy and crypt

cell hyperplasia in the duodenum and jejunum of rats exposed for 8 days at 10.5 mg Cr(VI)/kg bw/day as sodium dichromate dihydrate. Longer exposure duration (3 months) of 2.9 mg Cr(VI)/kg bw/day resulted in duodenal histiocytic infiltration in rats (Thompson et al., 2012).

3.5.1.2. Interpretation of available scientific data in consultation with experts. Several gastrointestinal effects in humans were found after intentional or accidental ingestion of chromate solutions. In oral exposure studies in rodents these effects were only observed at exposures exceeding those likely to occur in humans in occupational settings. Although, oral exposure at work cannot be excluded, this is expected to be relatively low compared to the human and animal studies in which gastrointestinal complaints were reported. Adverse effects of Cr(VI) compounds on the gastrointestinal tract in occupational settings is not consistently reported. The limited number of inhalation studies in animals support this and show no gastrointestinal effects.

The conclusion of the experts was that there are no convincing indications in the current scientific human and animal studies that occupational Cr(VI) exposure can cause gastrointestinal effects in humans.

3.5.2. Hematological effects

3.5.2.1. International agencies. Cr(VI) compounds are distributed to and accumulate in erythrocytes. Once inside the cell, Cr(VI) rapidly reduces to Cr(III) and binds to hemoglobin and other ligands. This complex with hemoglobin is relatively stable and remains sequestered within the cell over the life-span of the erythrocyte. Occupational studies and other studies in humans have not consistently reported hematological effects (ATSDR, 2012).

Oral exposure in animal studies, shows that the hematological system is a sensitive target for Cr(VI) compounds. Short term, up to 5 days, exposure of rats to Cr(VI) compounds in drinking water resulted in decreased Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Volume (MCV) at doses of 2.7 and 7.4 mg Cr(VI)/kg bw/day as sodium dichromate dihydrate (NTP, 2008). More severe microcytic hypochromic anemia (decreased hematocrit, hemoglobin, MCV and MCH) was seen in rats and mice exposed for 22 or 23 days to 0.77 mg Cr(VI)/kg bw/day as sodium dichromate dihydrate via drinking water (Bucher, 2007; NTP, 2008). Also longer exposure results in similar effects on hematological parameters. No inhalation studies in rodents that studied hematological effects were found.

3.5.2.2. Interpretation of available scientific data in consultation with experts. There is no evidence that occupational exposure to Cr(VI) compounds can cause hematological effects in humans. Oral exposure studies in rodents show that Cr(VI) compounds can lead to reversible hematological effects. No inhalation studies were found, so it is unclear if such effects are induced after inhalation exposure.

The conclusion of the experts was that there are no convincing indications in the current scientific human and animal studies that occupational Cr(VI) exposure can cause irreversible hematological effects in humans.

3.5.3. Hepatic effects

3.5.3.1. International agencies. ATSDR summarized human and animal evidence on hepatic effects of Cr(VI) compounds. In an old study (1952), Cr(VI) compounds were reported to cause severe liver effects, including derangements of cells in the liver, necrosis, inflammation and increase in Kupffer cells, in four workers exposed in chrome plating industry. No information on the extent of exposure was provided. An increased incidence death due to liver cirrhosis was found in a French cohort of 4227 stainless steel workers compared to national rates. No measurements of exposure were reported and based on the information provided ATSDR concluded that exposure to Cr(VI) compounds was not

associated with these liver effects. A Japanese study, showed that liver function values were in the normal range for workers after a 3-year period of working with Cr(VI) compounds. In an Italian study, a survey was conducted in a chromate producing factory. Workers reporting hepatobiliary diseases (15 of the 65) were further investigated with liver function tests. A few slight impairments were found, but it could not be excluded that these were caused by other factors, such as heavy alcohol use (ATSDR, 2012).

Inhalation exposure to Cr(VI) compounds in animal studies resulted in no or minimal hepatic effects and were considered not to be adverse. The NTP chronic study showed that oral exposure to Cr(VI) resulted in effects on the liver of rats and mice. Exposure of 5 days to 4 mg Cr(VI)/kg bw/day as sodium dichromate dihydrate resulted in increased serum alanine aminotransferase activity. Exposure of 14 days resulted in histiocytic cellular infiltration in the livers of female rats (Bucher, 2007). Longer exposure durations resulted in hepatic effects indicated by biochemical alterations and histopathological abnormalities (vacuolization, increased sinusoidal space, necrosis, accumulation of lipids, chronic inflammation) (Bucher, 2007; NTP, 2008).

3.5.3.2. Interpretation of available scientific data in consultation with experts. There is inconclusive evidence from occupational studies that Cr(VI) compounds may cause irreversible adverse effects in the liver. Several studies show no effects on hepatic parameters, whereas others do show an effect, but in these studies it cannot be excluded that these effects were caused by other factors. Animal studies show that oral exposure to Cr(VI) compounds can lead to hepatic effects. Such effects were not observed in animal studies in which exposure to Cr(VI) compounds were via inhalation.

Taken all data together, it was concluded that there are no convincing indications in the current scientific human and animal studies that occupational Cr(VI) exposure can cause irreversible hepatic effects in humans.

3.5.4. Renal effects

3.5.4.1. International agencies. ATSDR summarized all human studies in which renal effects associated with occupational Cr(VI) exposure were studied. In a cohort of 3408 workers from facilities producing chromium compounds no increases in urinary diseases were found. Renal function was studied in different occupational settings, including chromate and dichromate production, in chrome platers, stainless steel welders, workers employed in ferrochromium production, in boilermakers and workers in an alloy steel plant. Only in workers exposed to Cr(VI) compounds in the chromate production plant, higher levels of a brush border protein antigen and retinol binding protein in urine were found compared to controls. Other studies among Cr(VI) exposed workers found increases in urinary N-acetyl- β -D-glucosaminidase activity and microalbumin and β 2-microglobulin levels. In contrast, several other studies focusing on renal function in chromate production workers found negative or equivocal results (ATSDR, 2012).

ATSDR summarizes several inhalation studies in rodents. In none of these studies, inhalation of Cr(VI) compounds induced abnormal renal function or histopathological abnormalities in the kidneys. Oral exposure of rats did, however, result in renal effects. Histopathological abnormalities, such as accumulation of lipids, triglycerides and phospholipids were seen in rats exposed for 20 days to 13.5 mg Cr(VI)/kg bw/day (ATSDR, 2012). A lower dose of 3.4 mg Cr(VI)/kg bw/day as potassium dichromate resulted in decreased creatinine clearance and renal hemorrhage, necrosis, and enlarged glomeruli space in rats (Soudani et al., 2010). Other observed renal effects were vacuolization in glomeruli, degeneration of basement membrane of Bowman's capsule and renal tubular epithelial degeneration in rats exposed for 22 weeks to 1.3 mg Cr(VI)/kg bw/day (potassium dichromate) (Acharya et al., 2001).

3.5.4.2. Interpretation of available scientific data in consultation with experts. Occupational studies on renal effects associated with Cr(VI) compounds show either no or equivocal effects. There are also studies that demonstrate elevated levels of urinary biomarkers. In these studies no impairment of renal function was shown. The biomarkers that were affected were considered reversible by the experts and in the absence of impaired renal function, not considered indicative of renal damage. Effects on the kidneys were seen in animal studies after oral, but not inhalation exposure. The effects in the kidney occur at relatively high oral exposure levels.

Taken all data together, it was concluded that there are no convincing indications in the current scientific human and animal studies that occupational Cr(VI) exposure can cause irreversible renal effects in humans.

3.5.5. Neurological effects

3.5.5.1. International agencies. Workers employed at chromium plating factories in South Korea showed decreased performance in olfactory perceptions tests, indicating that olfactory recognition thresholds were significantly higher in exposed workers compared to controls (mean employment duration of 7.9 years). Although the cause of this change was not determined, the authors suggest that inhalation of Cr(VI) may affect the olfactory nerve. In another study, a cohort of 3408 workers from four former facilities in New Jersey were studied. Workers were exposed to Cr(VI) by inhalation of chromite ore. No increases in vascular lesions in the central nervous system were found (ATSDR, 2012).

According to ATSDR, no information was found regarding neurological effects in humans or animals after inhalation exposure. Histopathological neurological effects were assessed in animals exposed by inhalation of Cr(VI) compounds for 3 months or 2 years. No histopathological lesions were found in the brain of male Sprague-Dawley rats exposed to 1.15 mg Cr(VI)/m³ as chromium trioxide for 3 months or in CDF rats exposed to 30 mg Cr(III)/m³ as chromic oxide or basic chromium sulfate for 3 months. No neurological or behavioral tests were conducted in these studies.

A decrease in motor activity and balance was reported in rats given 98 mg Cr(VI)/kg bw/day as sodium chromate in drinking water for 28 days, however it is unclear whether this is caused by general toxicity (Diaz-Mayans et al., 1986). No histopathological abnormalities were observed in the brain and nervous system of the rats orally exposed to Cr(VI) (sodium dichromate dihydrate) for 3 months to 2 years. Different species and strains were tested. The highest doses tested for intermediate and chronic exposure in these rodents were 27.9 and 8.7 Cr(VI)/kg bw/day in drinking water (Bucher, 2007; NTP, 2008). In none of these studies more sensitive neurological, neurochemical, or neurobehavioral tests were conducted.

3.5.5.2. Interpretation of available scientific data in consultation with experts. There is no evidence from occupational studies that neurological or cognitive effects occur after exposure to Cr(VI) compounds. Neurological effects after Cr(VI) compounds exposure are not sufficiently investigated in animal studies. There is evidence from one oral study in rats, that Cr(VI) is able to migrate to the brain (Behari and Tandon, 1980). Histopathological parameters and limited behavioral data do not show adverse effects in this study.

Taken all data together, it was concluded that there are no convincing indications in the current scientific human and animal studies that occupational Cr(VI) exposure can cause irreversible neurological effects in humans.

3.5.6. Cardiovascular effects

3.5.6.1. International agencies. Information regarding cardiovascular effects in humans after exposure to Cr(VI) compounds is limited. Case

reports of humans who died after accidental acute oral exposure to high levels of Cr(VI) compounds have described cardiovascular effects as part of the events leading to death. Cardiovascular effects have not been reported at nonlethal doses (ATSDR, 2012).

No histopathological changes to the heart were observed in male Sprague-Dawley rats exposed via inhalation to 1.15 mg Cr(VI)/m³ as chromium trioxide. After 3 weeks of oral exposure to Cr(VI) compounds (26 mg/kg/day), fibrosis, necrosis, vacuolization, and hemorrhage in the heart was found in a study rats. In other oral studies, histopathological examination of the heart of rats and mice exposed to oral Cr(VI) compounds for durations of 3 months to 2 years, no cardiovascular abnormalities were observed. None of these animal studies assessed other cardiovascular endpoints such as blood pressure or electrocardiograms (ATSDR, 2012).

3.5.6.2. Interpretation of available scientific data in consultation with experts. Cardiovascular effects in humans were only reported after accidental exposure to lethal doses. Animal studies show that after inhalation, no cardiovascular effects were found. Oral studies give inconsistent results and only assess histopathological parameters and no other relevant cardiovascular endpoints.

Taken all data together, it was concluded that there are no convincing indications in the current scientific human and animal studies that occupational Cr(VI) exposure can cause irreversible cardiovascular effects in humans.

3.6. Dental effects

3.6.1. International agencies

There is only limited data available about the dental effects of Cr(VI) exposure. The available scientific studies and their results are summarized briefly below.

The ATSDR report in 2008 on the toxicity of chromium described dental erosion and discoloration (yellowing) of the teeth as a possible effect of exposure to Cr(VI). The causal relationship between chromium and dental erosion or tooth discoloration was not detailed further or substantiated in that document (ATSDR, 2008).

3.6.2. Studies in humans

A questionnaire was given to 303 electroplating workers from São Paulo in Brazil. In that study, the electroplating workers were subdivided into three groups depending on the level of exposure to chromium trioxide vapour. The exposure levels were between 0.11 and 1 mg chromic acid per m³. 50% of the electroplating workers surveyed had developed nasal septum perforations, showing that the exposure to Cr(VI) must have been high. Additionally, 50% of the electroplating workers had dental problems, including discoloration of the teeth and dental wear (Gomes, 1972). The study concluded that dental effects occur due to exposure to chromic acid, with the acidity (low pH) explaining effects on the teeth.

A case report described a rare form of occupational dental erosion (Dulgergil et al., 2007). A 52-year-old man had worked in the alloy industry in Turkey for 20 years and had been exposed to chromic acid by inhalation on a daily basis for at least 8 h. The erosion was expressed as sensitivity of the teeth with excessive erosion of the neck of the tooth, even including exposing the dental pulp in certain teeth because of exposure to chromic acid. The man was referred to a dental expert because of the excessive pain. This form of dental erosion was probably caused by exposure to chromic acid and can therefore also be explained by the exposure to acidity (low pH) rather than by Cr(VI) itself. It should be noted that earlier studies have shown that exposure to acids, often combined with dust particles, causes dental erosion (Wiegand and Attin, 2007). The authors conclude that these types of dental effects occur very rarely nowadays thanks to the well-controlled working conditions, but occupational dental problems due to particles combined with acids were

observed more often in the past in chemical factory workers. A more recent report about occupational tooth erosion discussed similar causes of dental erosion. This report was put forward by the external expert. Here also, acids combined with dust particles play a key role in the occurrence of dental erosion (Edeer and Martin, 2010).

Qayyum et al. (2012) carried out an epidemiological study in India are looking at nickel and chrome-plating workers. The workers were split into three groups depending on the duration of exposure, i.e. a control group and two exposure groups with 1–9 years and 10–25 years respectively of exposure to nickel and chromium. A questionnaire was used to survey the health of 150 workers. Tooth discoloration and erosion occurred at frequencies of 8% and 3% in the long and short exposure groups respectively, as compared to 0% in the control group. No analysis was performed to work out the statistical significance and it was unclear whether the possible effect on dental erosion was caused by exposure to Cr(VI), nickel or other substances (Qayyum S. et al., 2012).

3.6.3. Interpretation of available scientific data in consultation with an expert

Based on the available data it was discussed if there was any scientific evidence that Cr(VI) exposure can cause dental problems. The examples in the literature were primarily about chromic acid: the acidity (low pH) alone can affect the tooth enamel (and the dentine in later stages). Exposure to acids causes the surface of the enamel to partially dissolve, making it softer and more vulnerable. If exposure to acids is associated with exposure to dust particles (during the work), the softer ceramic material can easily be abraded by the dust particles. People who grind their teeth when they work (when exerting force) can also be affected more badly by exposure to acids. There is no convincing scientific substantiation for a relationship between occupational exposure to Cr(VI) compounds and dental problems.

The mechanism of dental effects of Cr(VI) is still being investigated. Due to the direct conversion from Cr(VI) to Cr(III), it is expected that local effects can be caused by Cr(VI), depending on the level of exposure, at the place where it enters the body. This could potentially cause inflammation of the gums because Cr(VI) may cause an inflammatory response. This is however a reversible effect; the inflammation recedes when the exposure stops. Minor gum problems are reversible. Severe gum problems could hypothetically cause more severe dental effects (periodontitis, in which the bone of the tooth is affected as well), but it is not known whether this can be caused by exposure to Cr(VI). Cr(VI) exposure can cause chromium ulcers on the skin. This could possibly also occur in the mouth in case of high exposure and this could then affect the teeth. There are however no scientific studies available that would support this theory.

Only a limited number of studies of workers are available that investigated effects of Cr(VI) compounds on dental erosion. However, it was difficult to assess the quality of these studies. Exposure to acids, potentially combined with dust particles, was probably the cause of the dental effects identified in the studies. The available data was too limited to establish a causal link between Cr(VI) exposure and dental erosion.

The expert concluded that there are no convincing indications in the current scientific human and animal studies that occupational Cr(VI) exposure can cause adverse dental effects in humans.

4. Conclusion

This review provides a hazard assessment of occupational exposure to Cr(VI) compounds. The hazard assessment was done by integrating three lines of evidence: evidence line 1, conclusions from international agencies; evidence line 2, (recent) human studies; and evidence line 3, animal studies.

The following conclusions were drawn on adverse effects that can be related to occupational exposure to Cr(VI) compounds:

- Cr(VI) can cause perforation of the nasal septum by chromium ulcers, chronic lung diseases, including asthma, rhinitis, pulmonary fibrosis and COPD, skin ulcers and allergic contact dermatitis in humans.
- It is currently insufficiently clear if Cr(VI) can cause irreversible diseases due to disturbances of the immune system (other than allergic contact eczema, allergic asthma and rhinitis and chronic lung diseases) or adverse effects on fertility or prenatal development in humans.
- There are no convincing indications in the current scientific human and animal studies that occupational exposure to Cr(VI) can cause irreversible gastrointestinal, hematological, hepatic, renal, neurological cardiovascular effects nor adverse dental effects.

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CRedit authorship contribution statement

Ellen V.S. Hessel: Investigation, Writing – review & editing, Methodology, Project administration. **Yvonne C.M. Staal:** Investigation, Writing – review & editing. **Aldert H. Piersma:** Investigation. **Shalenie P. den Braver-Sewradj:** Writing – original draft, Methodology. **Janine Ezendam:** Investigation, Writing – original draft, Methodology.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: This project was funded by the Dutch Ministry of Defense. The authors declare that the research was carried out independently, which was ensured by the structure of the project. The research was led and prioritized by a paritary committee, that consists of four representatives of the employees and four representatives of the employers and an independent chairman. RIVM organized consultations with external experts to discuss the conclusions made based on the available scientific evidence and included their input in the reports. The scientific results were also evaluated by an independent advisory board, that consisted of scientific and legal experts. RIVM was not involved in this selection procedure.

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Expert consultation systemic immunological diseases (2019): Dr. J. Damoiseaux (Maastricht University Medical Centre+), Prof. dr. G. Houben (TNO, University Medical Center Utrecht), Dr. F. Kuper (consultant, toxicologic pathologist), Dr. R. Pieters (IRAS, Utrecht University, HU University of Applied Sciences Utrecht).

Expert consultation dental effects (2019): Prof. dr. M.C. Huysmans (Radboud University Nijmegen).

References

- Acharya, S., et al., 2001. A subtoxic interactive toxicity study of ethanol and chromium in male Wistar rats. *Alcohol* 23, 99–108.
- Aruldas, M.M., et al., 2005. Chronic chromium exposure-induced changes in testicular histoarchitecture are associated with oxidative stress: study in a non-human primate (*Macaca radiata* Geoffroy). *Hum. Reprod.* 20, 2801–2813.
- Aschengrau, A., et al., 1993. Quality of community drinking water and the occurrence of late adverse pregnancy outcomes. *Arch. Environ. Health* 48, 105–113.
- ATSDR, 2008. Case studies in environmental medicine (CSEM), chromium toxicity. WB 1466, 2008, 18 12.
- ATSDR, 2012. Toxicological Profile for Chromium. Agency for Toxic Substances and Disease Registry, US Department of Health and Human Services.
- Banu, S.K., et al., 2016. Resveratrol protects the ovary against chromium-toxicity by enhancing endogenous antioxidant enzymes and inhibiting metabolic clearance of estradiol. *Toxicol. Appl. Pharmacol.* 303, 65–78.
- Banu, S.K., et al., 2017. Chromium VI - induced developmental toxicity of placenta is mediated through spatiotemporal dysregulation of cell survival and apoptotic proteins. *Reprod. Toxicol.* 68, 171–190.
- Behari, J., et al., 1978. Comparative toxicity of trivalent and hexavalent chromium to rabbits. III. Biochemical and histological changes in testicular tissue. *Acta Biol. Med. Ger.* 37, 463–468.
- Behari, J.R., Tandon, S.K., 1980. Chelation in metal intoxication. VIII. Removal of chromium from organs of potassium chromate administered rats. *Clin. Toxicol.* 16, 33–40.
- Bonde, J.P., 1993. The risk of male subfecundity attributable to welding of metals. Studies of semen quality, infertility, fertility, adverse pregnancy outcome and childhood malignancy. *Int. J. Androl.* 16 (Suppl. 1), 1–29.
- Bonde, J.P., Ernst, E., 1992. Sex hormones and semen quality in welders exposed to hexavalent chromium. *Hum. Exp. Toxicol.* 11, 259–263.
- Bucher, J.R., 2007. NTP toxicity studies of sodium dichromate dihydrate (CAS No. 7789-12-0) administered in drinking water to male and female F344/N rats and B6C3F1 mice and male BALB/c and am3-C57BL/6 mice. *Toxic Rep.* 1-g4.
- Casalegno, C., et al., 2015. Collate Literature Data on Toxicity of Chromium (Cr) and Nickel (Ni) in Experimental Animals and Humans. EFSA Supporting Publications.
- Chowdhury, A.R., 1995. Spermatogenic and steroidogenic impairment after chromium treatment in rats. *Indian J. Exp. Biol.* 33, 480–484.
- Cohen, M.D., et al., 1993. Mechanisms of chromium carcinogenicity and toxicity. *Crit. Rev. Toxicol.* 23, 255–281.
- De Lucca, R.C., et al., 2009. Effect of different doses of hexavalent chromium on mandibular growth and tooth eruption in juvenile Wistar rats. *Exp. Toxicol. Pathol.* 61, 347–352.
- DECOS, 2001. Chromium VI and its Compounds; Evaluation of the Effects on Reproduction, Recommendation for Classification. DECOS. Health Council of the Netherlands. publication no. 2001/01OSH.
- DECOS, 2016. Chromium VI compounds. Evaluation of the effects on reproduction, recommendation for classification. In: Dutch Expert Committee on Occupational Safety (DECOS). Health Council of the Netherlands publication no. 2016/04.
- den Braver-Sewradj, S. P., van Benthem J., Staai Y.C.M., Ezendam J., Piersma A.H., Hessel E.V.S., submitted. Occupational Exposure to Hexavalent Chromium. Part II. Hazard Assessment of Carcinogenic Effects.
- Diaz-Mayans, J., et al., 1986. Hexavalent chromium effects on motor activity and some metabolic aspects of Wistar albino rats. *Comp. Biochem. Physiol.* C 83, 191–195.
- Dulgergil, C.T., et al., 2007. An industrial dental-erosion by chromic Acid: a case report. *Eur. J. Dermatol.* 1, 119–122.
- EC, 2003. European Commission. Directive 2003/53/EC of the European Parliament and of the Council of 18 June 2003 amending for the 26th time Council Directive 76/769/EEC relating to restrictions on the marketing and use of certain dangerous substances and preparations (nonylphenol, nonylphenol ethoxylate and cement). Off. J. European Union, 178/24–178/28.
- EC, 2016. European Commission. Regulation No 1272/2008 of the European Parliament and of the council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006. Off. J. European Union L, 353/1. 2008.
- ECB, 2005. European Union Risk Assessment Report. Chromium Trioxide, Sodium Chromate, Sodium Dichromate, Ammonium Dichromate, Potassium Dichromate.
- ECHA, 2013. Amendment of the RAC Note “Application for Authorisation: Establishing a Reference Dose-Response Relationship for Carcinogenicity of Hexavalent Chromium” to Include the Intrinsic Property “Toxic to Reproduction” of the Cr(VI) Compounds. European Chemicals Agency, Helsinki: 2013. RAC/27/2013/06 Rev.1.
- Edeer, D., Martin, C., 2010. Occupational dental erosion. Richmond, BC: WorksafeBC evidence-based practice group. http://worksafebc.com/health_care_providers/Assets/PDF/occupational_dental_erosion.pdf.
- EFSA, 2014. Scientific Opinion on the risks to public health related to the presence of chromium in food and drinking water. EFSA Journal 12.
- Elsaied, E.M., Nada, S.A., 2002. Teratogenicity of hexavalent chromium in rats and the beneficial role of ginseng. *Bull. Environ. Contam. Toxicol.* 68, 361–368.
- Ernst, E., 1990. Testicular toxicity following short-term exposure to tri- and hexavalent chromium: an experimental study in the rat. *Toxicol. Lett.* 51, 269–275.
- Ernst, E., Bonde, J.P., 1992. Sex hormones and epididymal sperm parameters in rats following sub-chronic treatment with hexavalent chromium. *Hum. Exp. Toxicol.* 11, 255–258.
- Fang, J.Y., et al., 2013. Proteomics reveals plasma profiles for monitoring the toxicity caused by chromium compounds. *Clin. Chim. Acta* 423, 23–31.
- Glaser, U., et al., 1984. Inhalation Studies with Wistar Rats and Pathophysiological Effects of Chromium. Berlin. D-1 UFOPLAN F+E 10606007/2.
- Glaser, U., et al., 1985. Low level chromium (VI) inhalation effects on alveolar macrophages and immune functions in Wistar rats. *Arch. Toxicol.* 57, 250–256.
- Glaser, U., et al., 1990. Investigation of irritating properties of inhaled CrVI with possible influence on its carcinogenic action. In: Seemayer, N.H., Hadnagy, W. (Eds.), *Environmental Hygiene II*. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 239–245.
- Gomes, E.R., 1972. Incidence of chromium-induced lesions among electroplating workers in Brazil. *IMS Ind Med Surg* 41, 21–25.
- Gupta, B.N., 1990. Occupational diseases of teeth. *J. Soc. Occup. Med.* 40, 149–152.
- Health Council, 2001. Chromium VI and its compounds; Evaluation of the effects on reproduction, recommendation for classification. Health Council of the Netherlands, The Hague publication no. 2001/01OSH.
- Health Council, 2008. Prevention of Work-Related Airway Allergies. Recommended Occupational Exposure Limits and Periodic Screening. Health Council of the Netherlands, The Hague publication no. 2008/03E, 2008.
- Health Council, 2016a. Chromium VI Compounds. Evaluation of the Effects on Reproduction, Recommendation for Classification. Health Council of the Netherlands, The Hague publication no. 2016/04.
- Health Council, 2016b. Hexavalent Chromium Compounds. Healthbased Calculated Cancer Risk Values. Health Council of the Netherlands, The Hague publication no. 2016/13E.
- Heringa, M., Janssen, P., 2018. Achtergrondinformatie over chroom-6: gebruik, voorkomen in het leefmilieu en gedrag in het lichaam. RIVM Rapport 2018-0051.
- Hessel, E., et al., 2018. Nadelige gezondheidseffecten en ziekten veroorzaakt door chroom-6. Indeling in categorieën van causaliteit in relatie tot chroom-6-blootstelling. RIVM rapport 2018-0166.
- Hessel, E.V.S., et al., 2020. Nadelige gezondheidseffecten en ziekten veroorzaakt door chroom-6. Actualisatie van de wetenschappelijke literatuur en de risicobeoordeling voor strottenhoofdtkanker bij de POMs-locaties. RIVM-rapport 2020-0019. RIVM.
- Hjollund, N.H., et al., 2000. Male-mediated spontaneous abortion among spouses of stainless steel welders. *Scand. J. Work. Environ. Health* 26, 187–192.
- HSE, 1997. Asthmagen? Critical Assessments of the Evidence for Agents Implicated in Occupational Asthma. Health and Safety Executive.
- Hu, G., et al., 2017. Serum protein expression profiling and bioinformatics analysis in workers occupationally exposed to chromium (VI). *Toxicol. Lett.* 277, 76–83.
- IARC, 2012. IARC Monographs on the Evaluation of the Carcinogenic Risks to Humans: a Review of Human Carcinogens: Arsenic, Metals, Fibres, and Dusts, 100C. World Health Organization, International Agency for Research on Cancer, Lyon, France, pp. 147–168.
- Islam, L.N., et al., 2019. Serum immunoglobulin levels and complement function of tannery workers in Bangladesh. *J. Health Pollut* 9, 190308.
- Jelnes, J.E., Knudsen, L.E., 1988. Stainless steel welding and semen quality. *Reprod. Toxicol.* 2, 213–215.
- Katiyar, S., et al., 2008. Suppression of IL-6 level in human peripheral blood mononuclear cells stimulated with PHA/LPS after occupational exposure to chromium. *Sci. Total Environ.* 390, 355–361.
- Katiyar, S., et al., 2009. Effect of chromium on the level of IL-12 and IFN-gamma in occupationally exposed workers. *Sci. Total Environ.* 407, 1868–1874.
- Kumar, S., et al., 2005. Semen quality of industrial workers occupationally exposed to chromium. *J. Occup. Health* 47, 424–430.
- Kuo, H.W., Wu, M.L., 2002. Effects of chromic acid exposure on immunological parameters among electroplating workers. *Int. Arch. Occup. Environ. Health* 75, 186–190.
- Lenderink, A., Laan, v. d.G., 2014. Gezondheidsschade door blootstelling aan zwaarbaar chroom in de werksituatie.
- Li, H., et al., 2001. Effect of Cr(VI) exposure on sperm quality: human and animal studies. *Ann. Occup. Hyg.* 45, 505–511.
- Mignini, F., et al., 2009. Immunological pattern alteration in shoe, hide, and leather industry workers exposed to hexavalent chromium. *Environ. Toxicol.* 24, 594–602.
- Mortensen, J.T., 1988. Risk for reduced sperm quality among metal workers, with special reference to welders. *Scand. J. Work. Environ. Health* 14, 27–30.
- Mullea, A., et al., 2012. Literature search and review related to specific preparatory work in the establishment of Dietary Reference Values; preparation of an evidence report identifying health outcomes upon which Dietary Reference Values could potentially be based for chromium, manganese and molybdenum. Scientific report submitted to EFSA.
- Nettesheim, P., Szakal, A.K., 1972. Morphogenesis of alveolar bronchiolization. *Lab. Invest.* 26, 210–219.
- NIOSH, 2013. Criteria for a Recommended Standard Occupational Exposure to Hexavalent Chromium. U.S. Department of Health and Human Services, Center for Disease Control and Prevention, National Institute for Occupational Safety and Health, DHHS (NIOSH) Publication No, Cincinnati, OH, 2013–128.
- NIOSH, 2014. Criteria for a Recommended Standard Occupational Exposure to Hexavalent Chromium. U.S. Department of Health and Human Services, Center for Disease Control and Prevention, National Institute for Occupational Safety and Health, DHHS (NIOSH) Publication No, Cincinnati, OH, 2013–128.
- NTP, 1997. Final Report on the Reproductive Toxicity of Potassium Dichromate (CAS No. 7778-50-9) Administered in Diet to BALB/c Mice. US Department of Health and Human Services; 1997, RACB94014.
- NTP, 2008. Toxicology and carcinogenesis studies of sodium dichromate dihydrate (Cas No. 7789-12-0) in F344/N rats and B6C3F1 mice (drinking water studies). *Natl. Toxicol. Progr. Tech. Rep.* 1–192.

- Palmen, N.G.M., et al., 2018. Gezondheidseffecten en risicobeoordeling van blootstelling aan chroom-6 op de POMS-locaties van Defensie. Vol. RIVM Rapport 2018-0053. RIVM Bilthoven.
- Petrilli, F.L., et al., 1986. Metabolic reduction of chromium by alveolar macrophages and its relationships to cigarette smoke. *J. Clin. Invest.* 77, 1917–1924.
- Qayyum, S., et al., 2012. An epidemiological study of electroplaters occupationally exposed to Nickel and Chromium. *Biomed Res-India* 2012 23, 609–613.
- Qian, Q., et al., 2013. Alteration of Th1/Th2/Th17 cytokine profile and humoral immune responses associated with chromate exposure. *Occup. Environ. Med.* 70, 697–702.
- Rachootin, P., Olsen, J., 1983. The risk of infertility and delayed conception associated with exposures in the Danish workplace. *J. Occup. Med.* 25, 394–402.
- RIVM, 2018. Chroom-6 bij het re-integratieproject tROM. Gezondheidsrisico's en verantwoordelijkheden: Bevindingen uit het onderzoek op hoofdlijnen. Vol. RIVM report 2018-0164. RIVM.
- Samuel, J.B., et al., 2011. Lactational hexavalent chromium exposure-induced oxidative stress in rat uterus is associated with delayed puberty and impaired gonadotropin levels. *Hum. Exp. Toxicol.* 30, 91–101.
- SCOEL, 2004. Hexavalent Chromium. (Scientific Committee on Occupational Exposure Limits EC). SCOEL/SUM/86.
- SCOEL, 2017. Chromium VI Compounds. Scientific Committee on Occupational Exposure Limits EC). SCOEL/REC/386.
- Shipkowski, K.A., et al., 2017. Assessment of immunotoxicity in female Fischer 344/N and Sprague Dawley rats and female B6C3F1 mice exposed to hexavalent chromium via the drinking water. *J. Immunot.* 14, 215–227.
- Snyder, C.A., et al., 1996. Reduced IL-6 levels among individuals in Hudson County, New Jersey, an area contaminated with chromium. *Arch. Environ. Health* 51, 26–28.
- Snyder, C.A., Valle, C.D., 1991. Immune function assays as indicators of chromate exposure. *Environ. Health Perspect.* 92, 83–86.
- Soudani, N., et al., 2010. Protective effects of Selenium (Se) on Chromium (VI) induced nephrotoxicity in adult rats. *Ecotoxicol. Environ. Saf.* 73, 671–678.
- Stanley, J.A., et al., 2013. Postnatal exposure to chromium through mother's milk accelerates follicular atresia in F1 offspring through increased oxidative stress and depletion of antioxidant enzymes. *Free Radic. Biol. Med.* 61, 179–196.
- Suyama, Y., et al., 2010. Dental erosion in workers exposed to sulfuric acid in lead storage battery manufacturing facility. *Bull. Tokyo Dent. Coll.* 51, 77–83.
- Suzuki, Y., et al., 1984. Distribution of chromium in rats exposed to hexavalent chromium and trivalent chromium aerosols. *Ind. Health* 22, 261–277.
- Taioli, E., et al., 1995. Increased DNA-protein crosslinks in lymphocytes of residents living in chromium-contaminated areas. *Biol. Trace Elem. Res.* 50, 175–180.
- Tanigawa, T., et al., 1991. A decrease in Leu-11a negative lymphocytes in relation to natural killer cell activity in chromate workers. *Br. J. Ind. Med.* 48, 211–213.
- Tanigawa, T., et al., 1995. Decreases of CD4- and CD8-positive T lymphocytes in retired chromate workers. *Am. J. Ind. Med.* 27, 877–882.
- Thompson, C.M., et al., 2012. Comparison of the effects of hexavalent chromium in the alimentary canal of F344 rats and B6C3F1 mice following exposure in drinking water: implications for carcinogenic modes of action. *Toxicol. Sci.* 125, 79–90.
- US EPA, 2019. Systematic Review Protocol for the Hexavalent Chromium IRIS Assessment (Preliminary Assessment Materials) [CASRN 18540-29-9]. EPA/635/R-18/155.
- Van der Meer, N., et al., 2018. Eindrapport WP4 Blootstelling. Blootstelling aan chroom-6 op de NL-POMS-sites 1984-2006. RIVM Rapport 2018-0061.
- WHO, 2013. Inorganic Chromium (VI) Compounds. ISBN 978 92 4 153078 1.
- Wiegand, A., Attin, T., 2007. Occupational dental erosion from exposure to acids: a review. *Occup. Med. (Lond.)* 57, 169–176.
- Yousef, M.I., et al., 2006. Ameliorating effect of folic acid on chromium(VI)-induced changes in reproductive performance and seminal plasma biochemistry in male rabbits. *Reprod. Toxicol.* 21, 322–328.