SUBSTANCE EVALUATION CONCLUSION
as required by REACH Article 48
and
EVALUATION REPORT

for

Chromium (III) oxide
EC No 215-160-9
CAS RN 1308-38-9

Evaluating Member State(s): France

Dated: January 2022

Evaluating Member State Competent Authority

French Agency for Food, Environmental and Occupational Health Safety (ANSES) on behalf French Ministry of Environment
14 rue Pierre et Marie Curie
94701 MAISONS-ALFORT
France
Email: reach@anses.fr

Year of evaluation in CoRAP: 2019

Member State concluded the evaluation without any further need to ask more information from the registrants under Article 46(1) decision.

Further information on registered substances here:

http://echa.europa.eu/web/guest/information-on-chemicals/registered-substances

DISCLAIMER

This document has been prepared by the evaluating Member State as a part of the substance evaluation process under the REACH Regulation (EC) No 1907/2006. The information and views set out in this document are those of the author and do not necessarily reflect the position or opinion of the European Chemicals Agency or other Member States. The Agency does not guarantee the accuracy of the information included in the document. Neither the Agency nor the evaluating Member State nor any person acting on either of their behalves may be held liable for the use which may be made of the information contained therein. Statements made or information contained in the document are without prejudice to any further regulatory work that the Agency or Member States may initiate at a later stage.

Foreword

Substance evaluation is an evaluation process under REACH Regulation (EC) No. 1907/2006. Under this process the Member States perform the evaluation and ECHA secretariat coordinates the work. The Community rolling action plan (CoRAP) of substances subject to evaluation, is updated and published annually on the ECHA web site[1].

Substance evaluation is a concern driven process, which aims to clarify whether a substance constitutes a risk to human health or the environment. Member States evaluate assigned substances in the CoRAP with the objective to clarify the potential concern and, if necessary, to request further information from the registrant(s) concerning the substance. If the evaluating Member State concludes that no further information needs to be requested, the substance evaluation is completed. If additional information is required, this is sought by the evaluating Member State. The evaluating Member State then draws conclusions on how to use the existing and obtained information for the safe use of the substance.

This Conclusion document, as required by Article 48 of the REACH Regulation, provides the final outcome of the Substance Evaluation carried out by the evaluating Member State. The document consists of two parts i.e. A) the conclusion and B) the evaluation report. In the conclusion part A, the evaluating Member State considers how the information on the substance can be used for the purposes of regulatory risk management such as identification of substances of very high concern (SVHC), restriction and/or classification and labelling. In the evaluation report part B the document provides explanation how the evaluating Member State assessed and drew the conclusions from the information available.

With this Conclusion document the substance evaluation process is finished and the Commission, the Registrant(s) of the substance and the Competent Authorities of the other Member States are informed of the considerations of the evaluating Member State. In case the evaluating Member State proposes further regulatory risk management measures, this document shall not be considered initiating those other measures or processes. Further analyses may need to be performed which may change the proposed regulatory measures in this document. Since this document only reflects the views of the evaluating Member State, it does not preclude other Member States or the European Commission from initiating regulatory risk management measures which they deem appropriate.

[1] http://echa.europa.eu/regulations/reach/evaluation/substance-evaluation/community-rolling-action-plan

Contents

Part A. Conclusion

1. CONCERN(S) SUBJECT TO EVALUATION

The Substance, Chromium (III) oxide (EC No 215-160-9, CAS RN 1308-38-9) was originally selected for substance evaluation in order to clarify concerns about:

- Suspected reprotoxic;
- Suspected sensitiser;
- High (aggregated) tonnage

During the evaluation additional concerns were identified:

- genotoxicity of chromium(III) oxide nanoparticles
- repeated dose toxicity

During the evaluation, environment was not assessed.

2. OVERVIEW OF OTHER PROCESSES / EU LEGISLATION

Not applicable.

3. CONCLUSION OF SUBSTANCE EVALUATION

Table 1

CONCLUSION OF SUBSTANCE EVALUATION Conclusions	
Need for follow-up regulatory action at EU level	X
Harmonised Classification and Labelling	X
Identification as SVHC (authorisation)	
Restrictions	
Other EU-wide measures	X
No need for regulatory follow-up action at EU level	

Notably, a datagap has been identified for chromium(III) oxide for reproductive toxicity.

It was identified during substance evaluation that the reproductive toxicity datagap of chromium(III) oxide can be addressed in a grouping approach as the datagap was identified for the whole group. The eMSCA considers that the following data are to be requested by ECHA under CCH:

- Annex X, section 8.7.3: Extended One-Generation Reproductive Toxicity Study (OECD TG TG 443), with cohorts 1A, and 1B without extension to include a F2 generation, one species, route of administration representing the likely route of human exposure;
- Annex X, section 8.7.2: Developmental toxicity study (OECD TG 414), one species, most appropriate route of administration, having regards to the likely route of human exposure.

4. FOLLOW-UP AT EU LEVEL

4.1. Need for follow-up regulatory action at EU level

4.1.1. Harmonised Classification and Labelling

In parallel to substance evaluation, ECHA had identified several other registered chromium(III) substances, which contain data proposed to be used by the registrants for the assessment of chromium(III) oxide.

Following ECHA request, the evaluating MSCA (eMSCA) considered the group proposed by ECHA in order to get a more complete picture of chromium(III) toxicity.

A **group assessment** (i.e. not focusing on a conclusion for chromium(III) oxide only) is currently under developpement by the eMSCA for chromium(III) compounds.

The eMSCA identified a concern on skin sensitisation for chrome(III) oxide and for the group of chromium(III) compounds.

The eMSCA considered that chromium(III) compounds whole group should be classified for their skin sensitisation properties and that a CLH dossier on the group should be initiated.

Severe local pulmonary effects were observed following inhalation of chromium(III) oxide. A classification of chromium(III) oxide as STOT RE 2, H373 (lung) is also warranted.

In addition, as mentioned in the Justification Document of the Substance[2], the chromium (III) oxide can be classified as Aquatic Acute Cat. 1 H400 and Aquatic Chronic Cat 1 H410.

4.1.2. Identification as a substance of very high concern, SVHC (first step towards authorisation)

Not applicable.

4.1.3. Restriction

Not applicable.

4.1.4. Other EU-wide regulatory risk management measures

An **enforcement action** has been identified and is warranted to check the composition of the Substance from each registrant included in the joint submission of Chromium(III) oxide.

i. Chromium (VI) contents

The available information on the manufacture of the Substance suggest that the substance chromium(III) oxide may contain chromium(VI) compounds as impurities (e.g. chromium(VI) trioxide). There are several relevant existing entries in Annex VI to CLP which refer to chromium(VI): (i) in a group entry for "Chromium(VI) compounds, with the exception of barium chromate and of compounds specified elsewhere in this Annex", (ii) Chromium(VI) trioxide. In both cases a classification as a carcinogen (Cat 1B and 1A, respectively) applies, but chromium(VI) trioxide has additional hazard classifications associated (e.g. Muta 1B, Repr. 2 and Resp Sens 1).

Therefore, chromium(III) oxide containing chromium(VI) trioxide or chromium(VI) compounds as impurity at ≥ 0.1% shall be classified at least as carcinogen. In some of the registration dossiers, identification of impurities below 1% was not performed. Potential hazard arising from the presence of chromium(VI) compounds between 0.1% and 1 % was

[2] https://echa.europa.eu/fr/information-on-chemicals/evaluation/community-rolling-action-plan/corap-table/-/dislist/details/0b0236e1820e1d0f

thus not identified. In addition, some of the compositions containing Chrome(VI) impurity between 0.1% and 1% did not appropriately classify the substance as CMR due to the presence of the relevant impurity.

The compositions submitted by the registrants are considered to be mono-constituents according to the REACH guidance for the identification and naming of chrome(III) oxide. However, it is notified that the manufactoring process can used chromium (VI) compounds as starting materials. As all registrants did not provide details of the manufacturing process or the exact profile of the impurities, there is a need to clarify if the substance contains chromium (VI) with a content higher than 0.1%. Therfore, enforcement is needed to check that composition of registrants containing chrome(VI) impurity ≥ 0.1% are appropriately identified and classified for appropriate hazard communication.

ii. Nanomaterial

According to the recommendation set in the EU nanomaterial approved definition (2011/696/EU), a "Nanomaterial" means:
"*A natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50% or more of the particles in the number size distribution, one or more external dimensions is in the size range 1 nm-100 nm. In specific cases and where warranted by concerns for the environment, health, safety or competitiveness the number size distribution threshold of 50% may be replaced by a threshold between 1 and 50%.*"

According to the lead registrant's boundary composition of chromium (III) oxide, nanomaterials are present below the threshold of 50% and therefore does not fulfill the nanomaterial definition.

However, on the ECHA website and in the French R-Nano database, chromium(III) oxide is "known to be on the EEA market in nanomaterial form". In addition, chromium(III) oxide is listed in the nanopigments on the EU markets according to the European union observatory for nanomaterials (https://euon.echa.europa.eu/nano-pigments-inventory). The tonnage notified in the French database is 10-100 kg in 2017 but the tonnage at the EU level is not known.

According to the lead registrant of the chromium (III) oxide, nanomaterials are present below the threshold of 50%. Nevertheless, nanomaterial uses have been reported and chromium(III) oxide containing more than 50% nanomaterials should have been included in a separate registration submission.

The eMSCA would like to raise the potential enforcement issue on the lack of registration of nanoforms.

5. CURRENTLY NO FOLLOW-UP FORESEEN AT EU LEVEL

5.1. No need for regulatory follow-up at EU level

Not applicable.

5.2. Other actions

Not applicable.

6. TENTATIVE PLAN FOR FOLLOW-UP ACTIONS (IF NECESSARY)

Indication of a tentative plan is not a formal commitment by the evaluating Member State. A commitment to prepare a REACH Annex XV dossier (SVHC, restrictions) and/or CLP Annex VI dossier should be made via the Registry of Intentions.

Table 2

FOLLOW-UP		
Follow-up action	**Date for intention**	**Actor**
Preparatory group screening document in view for ECHA to consider group assessment regulatory needs (RMOA)	2023	ANSES/ECHA
Classification and labelling	To be initiated	ANSES

Part B. Substance evaluation

7. EVALUATION REPORT

7.1. Overview of the substance evaluation performed

The Substance Chromium (III) oxide (EC No 215-160-9, CAS RN 1308-38-9) was originally selected for substance evaluation in order to clarify concerns about:

- Suspected reprotoxic;
- Suspected sensitiser;
- High (aggregated) tonnage

During the evaluation an additional concern was identified:
- Genotoxicity of chromium(III) oxide nanoparticles
- Repeated dose toxicity

Table 3

EVALUATED ENDPOINTS	
Endpoint evaluated	**Outcome/conclusion**
Skin sensitisation	Concern confirmed. CLH to be initiated
Repeated dose toxicity	Concern confirmed. CLH to be initiated
Mutagenicity	Concern confirmed. Concern identified on chromium oxide containing particles in nanosize. No additional information required in the absence of registered nanomaterials
Reproductive toxicity	Concern unresolved. CCH to be initiated on EOGRTS and a developmental toxicity study (datagap identified for the whole group)

7.2. Procedure

The Substance chromium oxide was included in the Community rolling action plan (CoRAP) for substance evaluation to be evaluated in 2019 due to initial ground of concerns related to, sensitisation, reproductive toxicity, high(aggregated) tonnage.

On 19 March 2019, the eMSCA began the chromium (III) oxide evaluation, with a particular focus on toxicity to fertility and skin sensitisation.

On 5 September 2019, the eMSCA met the Lead registrant. Clarification on identity and toxicological data (eg skin sensitisation) were also provided.

During the evaluation, the eMSCA also identified genotoxicity of chromium(III) oxide nanoparticles as a potential new concern.

On 19 March 2020, the eMSCA sent a Draft Decision (DD) to ECHA on substance identity and on an in vivo comet assay to clarify potential genotoxicity concern on nanoparticles of chromium(III) oxide.

On 22 June 2020, the eMSCA received the registrant’s comment on the draft decision for chromium(III) oxide.

Following the assessment of registrant's comments, the eMSCA withdrew its draft decision as the concern was no longer substantiated. Indeed, the eMSCA acknowledge that major deficiencies were found in the published studies raising the concern. Indeed, the content of Cr(VI), that may have been present in the tested batch was not provided. In addition, chromium(III) oxide is not registered as a nanomaterial as such. Therefore, no request of a study on the nanomaterial form of the Substance could be requested. Further research work would be needed to confirm the concern and to identify the most relevant form to be tested.

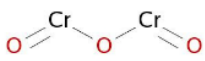
7.3. Identity of the substance

Table 4

SUBSTANCE IDENTITY	
Public name:	Chromium (III) oxide
EC number:	215-160-9
CAS number:	1308-38-9
Index number in Annex VI of the CLP Regulation:	none
Molecular formula:	Cr_2O_3
Molecular weight range:	151.99 g/mol
Synonyms:	• Chrome sesquioxide • Chromic oxide • Chromium (III) Oxide • Chromium (III) oxide dihydrate • Chromium (lll) oxide • Chromium III Oxide • CHROMIUM OXIDE • Chromium oxide (Cr2O3) • Chromium(III) oxide • Chromium(III) sesquioxide • Chromium(III)Oxide • Cr2O3 • dichromium trioxide • dichromium(3+) trioxidandiide • oxo(oxochromiooxy)chromium • oxo-(oxochromiooxy)chromium • oxo[(oxochromio)oxy]chromium • Tlenek chromu III • trioxochromium

Type of substance ✈ Mono-constituent ☐ Multi-constituent ☐ UVCB

Structural formula:



The compositions submitted by the registrants are considered as monoconstituent according to REACh guidance for identification and naming of substances.

Manufacturing process can be classified in two categories: by thermic process or by reduction of chromium (VI) with iron sulfate. Information on the manufacturing process is not provided by all registrants.

Regarding the impurities profile, there is some differences between the registrants.

Based on the data provided by the registrants, 3 categories of dossiers can be defined:
- Dossiers in which all impurities are identified;
- Dossiers containing « unknown impurities » but impurities with an harmonized classification are quantified (including Chromium(VI)) and total unspecified impurities are less than 1% w/w.
- Dossiers with unknown impurities, without information on presence or absence of substances with harmonized classification.

The corresponding registrants used the similar argument for not providing data on impurities profile:
« As the substance is a part of the chemical matrix of an inorganic catalyst (i.e. regarded as a preparation according to Guidance on substance identification), impurities cannot be meaningfully assigned to the substance ".

Overall, despite uncertainties, the substance is considered by FR-MSCA as similar across registrations, except when its contains chromium(VI) with a level higher than 0.1%. Chromium(III) oxide may contain Chromium(VI) trioxide between 0.1 and 10%.

Concentration of Chromium(VI) is not always specified and it cannot be excluded that some registrations with unknown impurities do also include Chromium(VI).

Due to the hazardous properties of Chromium(VI) and in particular its carcinogenicity and enforcement actions should be taken to clarify Chromium(VI) content in the relevant composition.

There are three registrants that made a partial opt-out due to the presence of chromium(VI) impurity at ≥ 0.1%. The assessment of Cr III follows the assessment of the lead registrant and an additional safety report covers Cr VI safety assessment.

7.4. Physico-chemical properties

Table 5

Physico-chemical properties are available on the ECHA dessiminated website (consulted on May 2021)

OVERVIEW OF PHYSICOCHEMICAL PROPERTIES	
Property	**Value**
Physical state at 20°C and 101.3 kPa	solid, inorganic substance crystalline Colour: Light to dark green *Data from a peer review Handbook (Merck Index and CRC Lide).* *Data is available in literature (Dean) and gives same result.*
Melting point	Key value: 2435°C at 101325 Pa *Data from a peer review Handbook (Merck Index and CRC Lide).* *Data is available in literature (Dean) and gives consistent result.*
Boiling point	*In accordance with column 2 of REACH Annex VII, no test is required for a solid melt above 300°C* **Key value: 4000°C at 101.3 kPa**

	Data from a peer review Handbook (Merck Index and CRC Lide). *Data is available in literature (Dean) and gives 3000°C.*
Relative density	5.22 g/cm^3 at 20°C *Data from a peer review Handbook (Merck Index and CRC Lide).* *Data is available in literature (Dean) and gives similar result.*
Vapor pressure	In accordance with column 2 of REACH Annex VII, no vapour pressure's data (required in section 7.5.) is required for a high melting point solid (>300°C).
Water solubility	<1 mg/L *Data from a peer review Handbook (Merck Index and CRC Lide).* *Data is available in literature (Dean) and gives consistent result.*
Surface tension	In accordance with column 2 of REACH Annex VII, no Surface tension data's (required in section 7.6.) is required if the water solubility is below 1 mg/L at 20°C.
Flash point	In accordance with column 2 of REACH Annex VII, Flash point (required in section 7.9.) does not need to be conducted as the substance is inorganic.
Partition coefficient n-octanol/water (Log Kow)	In accordance with column 2 of REACH Annex VII, Partition coefficient n-octanol/water (required in section 7.8.) does not need to be conducted as the substance is inorganic.
Self ignition temperature	No exothermic effects were recorded at temperatures up to 400 °C. Chromium (III) oxide is classified as not undergoing spontaneous combustion
Flammability	Non flammable
Explosive properties	No data is required: Chromium oxide does not contain any chemical groups with explosive properties.
Oxidising properties	Chromium oxide has no oxidising properties.
Granulometry	100% of particles are of size <12.21 µm. The Median mass aerodynamic diameter (MMAD) is calculated to be 0.57 µm. No information on the ratio of nanoparticles for most of the registrants. The lead registrant declares having nanoparticles below the threshold of 50%.
Stability in organic solvents and identity of relevant degradation products	In accordance with column 2 of REACH Annex IX, no data is required for inorganic substances
Dissociation constant	Cr_2O_3 is an inorganic salt with a low water solubility and does not dissociate
Viscosity	In accordance with column 2 of REACH Annex IX, Viscosity (required in section 7.17.) does not need to be conducted as the substance is solid.

7.5. Manufacture and uses

7.5.1. Quantities

The Substance is manufactured and/or imported in the European Economic Area in 10 000 + tonnes per year.

Table 6

AGGREGATED TONNAGE (PER YEAR)				
☐ 1 – 10 t	☒ 10 – 100 t	☒ 100 – 1000 t	☒ 1000- 10,000 t	☒ ≥10,000t
☒ 50,000 – 100,000 t	☒ 100,000 – 500,000 t	☐ 500,000 – 1000,000 t	☒ > 1000,000 t	☐ Confidential

[3]

The following active registrants of Chromium (III) oxide (EC No 215-160-9 ; CAS No 1308-38-9) have been identified via the following link: https://echa.europa.eu/fi/registration-dossier/-/registered-dossier/15477/1/2

7.5.2. Overview of uses

The following uses of chromium (III) oxide (EC No 215-160-9 ; CAS RN 1308-38-9) have been identified on the ECHA website[4] :

Table 7

USES	
	Use(s)
Manufacture	ERC 1, 2, 7 PROC 0, 1, 2, 3, 4, 5, 8a, 8b, 9, 14, 15, 19, 21, 22, 24, 26, 28
Uses as intermediate	Catalyst Manufacture: ERC 6a; PROC: 1, 2, 3, 8b, 9, 15; PC 0, 9, 19; SU 8, 9, 0
Formulation	Catalyst manufacture, metal manufacture, production of chromium containing alloys, pigments Formulation or re-packing: ERC 2, 3, 10a, 11a PROC 1, 2, 3, 4, 5, 6, 8a, 8b, 9, 10, 13, 14, 15, 19, 21, 22, 23, 24, 26 PC 0, 7, 9a, 9b, 9c, 14, 15, 18, 20, 21, 32, 39
Uses at industrial sites	Industrial use of chromium III oxide, welding and soldering, coating, metal manufacture, pigment, catalyst Pigment manufacture: ERC 6a, 0; PROC 1,2,3,4,5,8a, 8b, 9, 21, 22, 23, 26; PC 9a, 9b, 9c, 39, 0 Coating: ERC 5, 7; PROC: 7, 8b, 23, 24; PC: 7, 14, 15, 38; SU: 14, 15, 17 Manufacture metal: ERC 6b, 7; PROC: 1,3,4, 8b, 9, 22, 24, 26; SU 14

[3] https://echa.europa.eu/fr/brief-profile/-/briefprofile/100.013.783, viewed on 17-06-21
[4] https://echa.europa.eu/fr/registration-dossier/-/registered-dossier/15477

	Catalyst manufacture: ERC 6a, 6b; PROC 0, 1,2,3,4,8a, 8b, 9, 28; PC 0, 20; SU 0, 9, 10 Refractory material: ERC 6a, 10a, 10b, 11a; PROC 5; SU 0 PC 0; SU 19, 0, 3
Uses by professional workers	Pigment, cosmetics and artists colours/paints/coating, refractory and foundry material, products of pigments, small scale laboratory use, ERC 8a, 8b, 8c, 8d, 8e, 8f, 9a, 9b; PROC: 1 to 27; PC
Consumer Uses	Pigment, use of pigment formulations, cosmetics and artists colours/paints/coating ERC 8a, 8b, 8c, 8d, 8f, 9a, 9b, PROC 0, 1, 7, 9a, 9b, 9c, 14, 20, 23, 33, 39; PC 9a, 9b, 9c, 39, 0; SU 0, 13, 19
Article service life	AC 1, 2, 3, 4, 7, 10, 13, 0; ERC 2, 10a, 10b, 11a; 12a PROC 14, 21, 22, 24

Descriptors used in Table above:

Environmental release categories (ERC):

- ERC 1: Manufacture of the substance
- ERC 2: Formulation into mixture
- ERC3: Formulation into solid matrix
- ERC 5: Use at industrial site leading to inclusion into/onto article
- ERC 6a: Use of intermediate
- ERC 6b: Use of reactive processing aid at industrial site (no inclusion into or onto article)
- ERC 7: Use of functional fluid at industrial site
- ERC 8a: Widespread use of non-reactive processing aid (no inclusion into or onto article, indoor)
- ERC 8b: Widespread use of reactive processing aid (no inclusion into or onto article, indoor)
- ERC 8c: Widespread use leading to inclusion into/onto article (indoor)
- ERC 8d: Widespread use of non-reactive processing aid (no inclusion into or onto article, outdoor)
- ERC 8f: Widespread use leading to inclusion into/onto article (outdoor)
- ERC 9a: Widespread use of functional fluid (indoor)
- ERC 9b: Widespread use of functional fluid (outdoor)
- ERC 10a: Widespread use of articles with low release (outdoor)
- ERC 11a: Widespread use of articles with low release (indoor)
- ERC 12a: Processing of articles at industrial sites with low release

Process categories (PROC):

- PROC 1: Chemical production or refinery in closed process without likelihood of exposure or processes with equivalent containment conditionsPROC 2: Chemical production or refinery in closed continuous process with occasional controlled exposure or processes with equivalent containment conditions
- PROC 2: Chemical production or refinery in closed continuous process with occasional controlled exposure or processes with equivalent containment conditions
- PROC 3: Manufacture or formulation in the chemical industry in closed batch processes with occasional controlled exposure or processes with equivalent containment conditions
- PROC 4: Chemical production where opportunity for exposure arises

- PROC 5: Mixing or blending in batch processes
- PROC 6: Calendering operations
- PROC 7: Industrial spraying
- PROC 8a: Transfer of substance or mixture (charging and discharging) at non-dedicated facilities
- PROC 8b: Transfer of substance or mixture (charging and discharging) at dedicated facilities
- PROC 9: Transfer of substance or mixture into small containers (dedicated filling line, including weighing)
- PROC 10: Roller application or brushing
- PROC 11: Non industrial spraying
- PROC 13: Treatment of articles by dipping and pouring
- PROC 14: Tabletting, compression, extrusion, pelletisation, granulationPROC 13: Treatment of articles by dipping and pouring
- PROC 15: Use as laboratory reagent
- PROC 16: Use of fuels
- PROC 17: Lubrication at high energy conditions in metal working operations
- PROC 18: General greasing /lubrication at high kinetic energy conditions
- PROC 19: Manual activities involving hand contact
- PROC 20: Use of functional fluids in small devices
- PROC 21: Low energy manipulation and handling of substances bound in/on materials or articles
- PROC 22: Potentially closed processing operations with minerals/metals at elevated temperature. Industrial setting
- PROC 23: Open processing and transfer operations at substantially elevated temperature
- PROC 24: High (mechanical) energy work-up of substances bound in materials and/or articles
- PROC 26: Handling of solid inorganic substances at ambient temperature
- PROC 28: Manual maintenance (cleaning and repair) of machinery

Product categories (PC):

- PC 0: Other: Building/construction
- PC 7: Base metals and alloys
- PC 9a: Coatings and paints, thinners, paint removes
- PC 9b: Fillers, putties, plasters, modelling clay
- PC 9c: Finger paints
- PC 14: Metal surface treatment products
- PC 15: Non-metal-surface treatment products
- PC 18: Ink and toners
- PC 19: Removed from PC list and relocated in the technical function list (Table R.12-15).
- PC 20: Products such as ph-regulators, flocculants, precipitants, neutralisation agents
- PC 21: Laboratory chemicals
- PC 23: Leather treatment products
- PC 32: Polymer preparations and compounds
- PC 38: Welding and soldering products, flux products
- PC 39: Cosmetics, personal care products

Sector of end-uses (SU):

- SU 0: Other
- SU 8: Manufacture of bulk, large scale chemicals (including petroleum products)
- SU 9: Manufacture of fine chemicals
- SU 10: Formulation [mixing] of preparations and/or re-packaging (excluding alloys)
- SU 13: Manufacture of other non-metallic mineral products, e.g. plasters, cement
- SU 14: Manufacture of basic metals, including alloys
- SU 15: Manufacture of fabricated metal products, except machinery and equipment

- SU 17: General manufacturing, e.g. machinery, equipment, vehicles, other transport equipment
- SU 19: Building and construction work

Article category (AC):

- AC 0: Other: C19.1 - Constructional articles and building material for indoor use (no intended release)AC 0: Other: C19.2 - Constructional articles and building material for outdoor use (no intended release)
- AC 1: Vehicles
- AC 2: Machinery, mechanical appliances, electrical/electronic articles
- AC 3: Electrical batteries and accumulators
- AC 4: Stone, plaster, cement, glass and ceramic articles
- AC 7: Metal articles
- AC 8: Paper articles
- AC 13: Plastic articles

7.6. Classification and Labelling

7.6.1. Harmonised Classification (Annex VI of CLP)

No harmonized classification.

7.6.2. Self-classification

- The Substance is not classified in the lead registration dossier.

- In registration dossiers of the Substance containing chromium(VI) trioxide between 0.1 and 10%, chromium(III) oxide is classified as follows. CMR classification proposal may be due to the presence of chromium(VI) impurities above the threshold for classification.

  Carc. 1A ; H350
  Muta. 1B ; H340
  Repr. 2 ; H361f
  Acute tox. 4, H302
  Acute Tox 3, H312
  Acute Tox 3, H332
  Skin Corr. 1A, H314
  Eye dam. 1 , H318
  STOT RE 2 ; H373
  Resp. Sens. 1 ; H334
  Skin Sens. 1; H317
  STOT SE 3; H335
  Aquatic chronic 2, H411

- The following hazard classes are in addition notified among the aggregated self-classifications in the C&L Inventory*:*

  Aquatic Chronic 4 - H413

7.7. Environmental fate properties

Not addressed in this substance evaluation. As indicated in the Justification document in 2018, chromium (III) oxide could be classified as Aquatic Acute Cat. 1 H400 and Aquatic Chronic Cat 1 H410 based on the available data in 2018. Taking into account these information, it was not deemed necessary to reassess the environmental hazards.

7.8. Environmental hazard assessment

Not addressed in this substance evaluation. As indicated in the Justification document in 2018, chromium (III) oxide could be classified as Aquatic Acute Cat. 1 H400 and Aquatic Chronic Cat 1 H410 based on the available data in 2018. Taking into account these information, it was not deemed necessary to reassess the environmental hazards.

7.9. Human Health hazard assessment

7.9.1. Read-across assessment

Although some data were performed with chromium(III) oxide, read-across with other chrome(III) compounds was proposed by Registrants for numbers of endpoints. The read-across rationale is assessed below.

Read-across rationale

The read-across is based on a category approach with the hypothesis that the target substance chromium(III) oxide and the source substances Chromium(III) compounds have similar toxicological properties because they release upon dissolution chromium(III) cations that are considered the relevant toxicological unit.

Identity of substances
The target and source substances are chromium(III) compounds either soluble or insoluble in water, simple or complex compounds.

Impurity profile may differ between the target and source substances as some of the chromium(III) compounds may contain Chromium(VI) as an impurity (e.g. Chromium(III) oxide, Chromium basic sulfate). Nevertheless, in case such an impurity is known to be present at ≥ 0.1%, the data is not be used or use with care due to the known carcinogenic properties of such impurity.

Table 8: Target substance

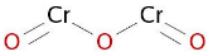
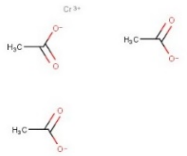
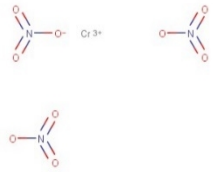
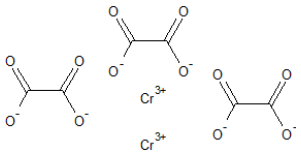
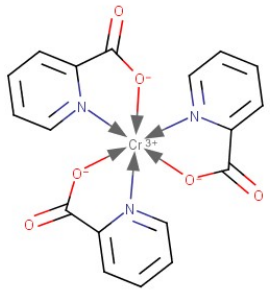
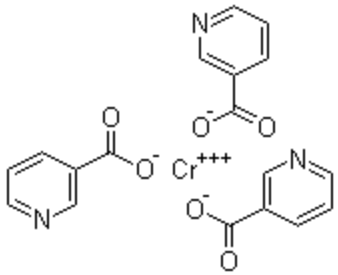
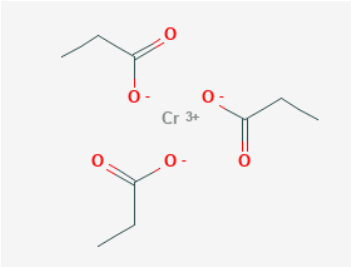
Constituents	CAS RN	Structure	Comments
Chromium(III) oxide	1308-38-9		Full (JS)

Table 9: Source substances used for read-across for hazard endpoints

Substance	CAS No	EC	Structure	Comments	Registration status/ongoing process
CHROMIUM(III) SALTS SOLUBLE IN WATER					
Chromium triacetate	1066-30-4	213-909-4		Mono-constituent	Full (JS)/ targeted CCH (closed)
Basic chromium sulfate (reaction mass of chromium(III) hydroxide sulfate and sodium sulfate)	-	914-129-3		Multi-constituent substance	Full (JS)
Chromium trichloride	10025-73-7	233-038-3	Cr^{3+} Cl- Cl- Cl-	Mono-constituent	Full (JS)
Chromium(III) nitrate	13548-38-4	236-921-1		Mono-constituent	Full (JS)
Dichromium trioxalate	30737-19-0	250-317-5		Mono-constituent	Full (JS)
CHROMIUM(III) COMPOUNDS INSOLUBLE IN WATER					
Chromium (III) hydroxide	215-158-8	1308-14-1	Cr3+ OH- OH- OH-	Mono constituent	Full (JS)/ /intermediate
CHROMIUM(III) COMPLEXES					

Substance	CAS No	EC	Structure	Comments	Registration status/ongoing process
Chromium picolinate	-	477-680-4		Mono-constituent	NONs (individual)
Chromium nicotinate	64452-96-6	456-568-6		Mono-constituent	Not registered
Chromium propionate complex	-	-		Mono-constituent	Not registered

Structural similarity

The source substances and target substance share structural similarity through the ion Cr^{+3}. They only differ by their counter anions.

Physico-chemical properties

Table 10

Constituents	CAS RN	Molecular weight	Solubility in water	Log Kow
Chromium(III) oxide	1308-38-9	151.99	Insoluble	technically not feasible
Chromium triacetate	1066-30-4	229.13	Soluble	0.2 (at 22°C)
Basic chromium sulfate	-	-	Soluble	Not feasible
Chromium trichloride	10025-73-7	158.36	Soluble (hydrated)	-3 (20°C) (QSAR)
Dichromium trioxalate	30737-19-0		soluble	-0.8 (other)
Chromium(III) nitrate	13548-38-4	238.03	Very soluble	-3 (20°C) (QSAR)
Chromium (III) hydroxide	215-158-8	165.06	Insoluble (soluble at low pH)	technically not feasible
Chromium picolinate	-	418.3	Soluble	Lipophilic
Chromium nicotinate	64452-96-6	418.3	-	Lipophilic
Chromium propionate complex	-	463.35	Soluble	Lipophilic

I: insoluble << 1g/l; S: soluble (between 100 and 1000 g/l), very soluble: > 1000 g/l

All the substances are in solid forms. Chromium(III) oxide is constituted of fine light to dark green crystals and contains < 50% nanoparticles (joint submission boundary composition). The nanosized particles may impact metal release/bioavailability of the substance and thus toxicological properties of the chromium(III) compounds (Singh *et al.,* 2016). Nevertheless, there are currently insufficient data to quantitatively assess potential differences toxicity.

Upon dissolution in aqueous media at physiologically relevant concentration and pH conditions, the only toxicologically relevant aqueous chromium species is the trivalent chromium cation. The trivalent chromium species is poorly soluble and is only stable under acidic condition. Thus, on this basis the read-across for systemic toxicity between chromium(III) compounds is justified. Nevertheless, there are differences in solubility between Chromium(III) compounds that may impact metal release/bioavailability of the substances.

Comparative toxicokinetics (modified from Riimaki and Marita, 2006 and EFSA, 2010 report)

The toxicokinetics of chromium(III) vary depending on the physico-chemical form of the compounds, solubility and the route of administration. The biochemistry is complex and there are still gaps in the understanding of the behavior of chromium in biological systems.

Lipid solubility or anionic charge seems to play key roles in increasing the passage of chromium complexes across membranes, in cellular uptake, and in excretion. Lipid soluble

species (e.g. chromium(III) picolinate), anionic complexes (e.g. chromium(III) acetate) may be absorbed more efficiently by oral route compare to other Cr(III) compounds. One comparative study revealed that there can be differences in the bioavailability and tissue levels of chromium resulting from intake of chromium(III) chloride, nicotinate or picolinate (Olin *et al.,*., 1994). Nevertheless, EFSA report (2010) concluded that the differences are small and the overall bioavailability of chromium from all these sources is low. Chromium(III) oxide is expected to be less bioavailable, read-across with other more soluble or lipophilic compounds is considered acceptable as a protective approach.

Absorption from the respiratory system varies depending on the chemical, solubility, and particle size characteristics of the chromium compound involved. About 5- 10% of water soluble chromium(III) compounds, when inhaled as aerosols or intratracheally administered, may be taken up by the circulating blood within few hours to one day followed by a further slow release over weeks and months. Regarding insoluble particles, after removal from the respiratory tract by mucociliary clearance, uptake is a very slow process presumably mediated by gradual dissolution in phagocytic cells of the lungs and removal via the lymphatic system.

Chromium(III) salts (as well as chromium(III) complexes) are able to cross the skin penetration barrier and enter the epidermis. However, chromium(III) insoluble compounds were not shown to be absorbed across the skin into the systemic circulation.

Chromium compounds are expected to have similar biological target and tissue distribution. Nevertheless, lipophilic chromium species were shown to be taken up by the tissues more efficiently than cationic chromium(III).

In conclusion, the systemic toxicity of chromium(III) oxide and other chromium compounds following absorption are expected to be determined by the chromium(III) ions toxicity. Solubility in water and lipophilicity may influence the bioavailability of the target substance and toxicity. All the source substances are expected to have similar or higher bioavailability than the target substance chromium(III) oxide based on these properties. Nevertheless, the size of the particle may also greatly influence the TK of the substances. The presence of a fraction of nanosize particles of chromium(III) oxide may influence the bioavailability of the chrome(III) oxide (Singh *et al.,*., 2016) but there are no TK comparative data available.

Comparison of data from human health endpoints

Toxicity data of the target and source substances (information as available in ECHA disseminated website or in the registration dossier of chromium(III) oxide):

Table 11

	Chromium(III) oxide	Chromium triacetate	Chromium hydroxide	Basic chromium sulfate	Chromium chloride
CAS RN	1308-38-9	1066-30-4	1308-14-1	No CAS (EC no. 914-129-3)	10025-73-7
Acute toxicity, oral	Similar to OECD TG 401 Oral, gavage Rat, m/f $LD50_{oral}$ > 5000 mg/kg bw	OECD TG 423 Female Rat, oral, gavage $LD50_{oral}$ > 5000 mg/kg bw	Read-across	Similar to OECD TG 401 Male rats Oral, gavage LD50 = 3530 (3190-3790) mg/kg	Read-across
Acute toxicity, inhalation	OECD TG 403 Aerosol, nose-only LC50(rat, 4h)> 5.41 mg/l	Not required	Not required	OECD TG 403 LC50 rat > 4.58 mg/l	Not required
Acute toxicity, dermal	Not required	OECD TG 402 Rat, LD50 > 2000 mg/kg	Not required	Not required	Read-across
Eye irritation	OECD TG 404 Rabbit Not irritating	In vitro HET-CAM test : not corrosive Read-across with chromium	OECD TG 437 BCOP Not irritating	Similar to OECD TG 405 Rabbit Not irritating	OECD TG 405 Rabbit Not irritating
Skin irritation	OECD TG 405 Rabbit Not irritating	Read-across	OECD TG 439 In vitro human skin Not irritating	No,guideline study Rabbit Not irritating	OECD TG 404 Rabbit Not irritating
Skin sensitisation (animal data)	Read-across	OECD TG 429 Vehicle: DMF Skin Sens 1B	RA with chromium iron oxide not accepted (Negative LLNA)	OECD TG 406 (Buehler 3 applications) Not a skin sensitiser	Non-guideline studies Guinea-pigs Skin sensitiser
Short-term repeated dose toxicity study	Similar to OECD TG 413 Rat, inhaltation, dust (nose-only) LOAEC local = 4.4 mg/m^3 (lung) NOEC systemic = 4.4 mg/m^3 (thyroid)	Read-across	Read-across	Similar to OECD TG 413 Rat inhalation (nose-only) LOAEC local= 17 mg/m^3 (lung) NOEC systemic= 17 mg/m^3 (thyroid, brain, kidney, thyroid, liver, spleen,	Similar to OECD TG 408 20-week oral study NOAEL = 35.9 mg/kg (top dose used in the study)

				testes)	
***In vitro* gene mutation study (bacteria or mammalian cells)**	HPRT assays (non GLP): 1 positive + 1 negative	Ames (OECD TG 471) Negative	Ames (OECD TG 471) Negative	Read-across	Ames: Negative MLA TK: equivocal
***In vitro* micronucleus study or cytogenicity study in mammalian cells**	Read-across	Read-across	Read-across	Read-across	OECD TG 473 Negative
***In vivo* mutagenicity**	Micronucleus study (GLP) Mice, single ip Negative MN, CA, comet (unknown CrVI content, non -GLP) Rat, 28-day Positive (micro and nanoparticles)	Read-across	Read-across	Read-across	MN: negative in mice 2-d, ip
Carcinogenicity	Read-across	Read-across	Read-across	Read-across	Read-across
Reproductive toxicity	Read-across	Read-across	Read-across	Read-across	Read-across

Table 12

	Dichromium trioxalate	Chromium nitrate	Chromium nicotinate	Chromium picolinate	Chromium propionate complexes
CAS RN	64452-96-6	13548-38-4	456-96-6	14639-25-9	85561439
Acute toxicity, oral	Read-across	OECD TG 401 LD_{50} oral rat =1540/1410 (M/F) Acute tox 4 H302 LD_{50} nonahydrate > 3500 mg/kg	OECD TG 425 LD_{50} oral > 5000 mg/kg	No data available	LD_{50} oral, rat > 2000 mg/kg
Acute toxicity, dermal	Read-across	Not required	OECD TG 402 LD_{50}> 2000 mg/kg	No data available	No data available
Acute Toxicity, inhalation	Not required	Read-across	No data available	No data available	No data available
Eye irritation		Read-across	No data available	Non irritant	No data available
Skin irritation	OECD TG 409 Non irritant	Read-across	No data available	Non irritant	No data available
Skin sensitisation (animal data)	Read-across	Read-across	No data available	No data available	No data available
Short-term repeated dose toxicity study	Read-across	Read-across	90-day oral study, rat NOAEL > 62.25 mg/kg (top dose) 52-week study, rat, diet NOAEL ≥ 5.7 mg/kg (top dose)	90-day oral (diet) NOAEL > 4240 mg/kg in rats and > 9140 mg/kg in mice (top dose)	No data available
***In vitro* gene mutation study in bacteria or in mammalian cells**	Ames (OECD TG 471) negative	Ames: Negative	Ames: negative Mammalian cells: negative	Ames: negative Mammalian cells : positive at high concentration	No data available

***In vitro* micronucleus study or cytogenicity study in mammalian cells**	Read-across	Read-across	negative	WOE: conflicting results	No data available
***In vivo* mutagenicity**	Not required	MN assay: Single appl. Up to 500 mg/kg chromium trinitrate nonahydrate: negative	No data available	MN mice : equivocal Females, negative Males (14-w oral, feed) MN rat (3 oral exposure, gavage): negative	No data available
Carcinogenicity	Read-across	Read-across	No data available	OECD TG 451 oral NOAEL > 2400 mg/kg in rat and 2100 mg/kg in mice	No data available
Reproductive toxicity study	Read-across	Read-across	2-generation Rat, oral NOAEL > 8 mg/kg (max dose tested) Insufficient dose levels	No multigeneration study (non guideline screening study in male mice, negative up to 25 mg/kg Chromium (III) Picolinate + effects on sperm parameters and estous cycle in the 90-day study) Insufficient dose levels	No data available
Prenatal developmental toxicity	Read-across	Read-across	No data available-	No reliable data identified (Bailey *et al.,* 2006 rated K3)	Prenatal developmental (non guideline) Rat, oral NOAEL > 7.2 mg/kg

Concerning systemic toxicity, available data on repeated-dose toxicity, carcinogenicity and reproductive toxicity do not provide evidence of differences of toxicity between the chromium compounds. Nevertheless, few studies compare the toxicity between the different chromium compounds.

Concerning local toxicity, the read-across approach is excluded. Although toxic effects are estimated to be related to the soluble metal chromium(III) ion, local effects of the particle itself cannot be excluded.

Conclusion on the read-across

Systemic effects of the target substance can be addressed with information from source substances for the following endpoints: repeated-dose toxicity, genotoxicity, reproductive toxicity. Nevertheless, several recent papers have raised a concern on potential genotoxicity of chromium(III) oxide as nanoparticles. This is further discussed in the genotoxicity section below.

Local effects of the target substance cannot be addressed with the information from source substances. The assessment of local effects should therefore be excluded from the read-across approach. The corresponding endpoints are skin sensitisation and local site-of contact effects following acute or short or long-term exposure (e.g. lung toxicity following inhalation exposure).

7.9.2. Toxicokinetics

The main toxicokinetics data were performed by oral route of chromium administration whereas there are limited studies concerning dermal and inhalation routes of exposure more relevant for occupational exposure.

***In vitro* data**

The release of chromium(III) oxide has been investigated *in vitro* by simulating dissolution under physiological conditions considered to mimic artificial lysosomal fluid (ALF) and artificial sweat (ASW) (Unpublished report, 2010a/b).
ALF simulates intracellular conditions in lung cells occurring in conjunction with phagocytosis and represents relatively harsh conditions, whereas ASW simulates the hypoosmolar fluid, linked to hyponatraemia (loss of Na^+ from blood), which is excreted from the body upon sweating. The chromium release rate in ALF and ASW was determined with 0.0000024 µg/cm^2/h (corresponding to free chromium concentration of 2.06 µg/L after 168h, pH 4.5) and 0.00009 µg/cm^2/h (corresponding to < 2 µg/L after 168h, pH 6.5), respectively. A **transformation to chromium (VI) was not observed** under any test.

Although the *in vitro* studies do provide useful information, FR-MSCA noted that comparative data with other chromium(III) compounds are not available. Moreover, the complexity of chromium(III) oxide absorption is not fully taken into account (complexation with other biomolecules that could enhance absorption are for example not taken into account in this type of studies). Indeed, one of the important aspect is that transition elements (Cr, Cu, Zn) and amino acid (e.g. containing sulphur and oxygen) are known to increase intestinal absorption of the elements. Other biomolecules such as carbohydrates and carboxylic acids (citric, acetic and oxalic acid can also form complexes with chromium, impacting intestinal absorption. Therefore, although the *in vitro* studies provided by the registrant may give useful information, all the complexity of chromium(III) absorption was not taken into account.

Animal and human *in vivo* data (selected part of Riimaki and Marita, 2006 report)

Oral absorption

Following oral administration, trivalent chromium was reported to be very poorly absorbed *via* the gastrointestinal tract (0.4 to 2.8%) in both rats and humans.

Dermal absorption

No guideline dermal absorption studies are available with chromium(III) oxide or other chromium(III) compounds.

Chromium(III) insoluble compounds in water were not shown to be absorbed across the skin into the systemic circulation. Chromium(III) salts (as well chromium(III) complexes) are however able to cross the skin penetration barrier and enter the epidermis in the following decreasing order of efficiency: chromium chloride>(basic) chromium sulfate>chromium nitrate.

Absorption by inhalation

Absorption from the respiratory system varies depending on the chemical reactivity, solubility, and particle size characteristics of the chromium compound involved. About 5-10% of water soluble chromium(III) compounds, when inhaled as aerosols or intratracheally administered, may be taken up by the circulating blood within few hours to one day followed by a further slow release over weeks and months. Regarding insoluble particles, after removal from the respiratory tract by mucociliary clearance, uptake is a very slow process presumably mediated by gradual dissolution in phagocytic cells of the lungs and removal via the lymphatic system.

Distribution/metabolism/excretion

The following summary is available in the Finnish Institute of Occupational Health report (Riimaki and Marita, 2006): "In blood plasma, 95% of chromium(III) is bound to large molecular weight proteins, notably transferrin. Chromium also associates with the low molecular weight oligopeptide LMWCr. The prominent tissues of chromium distribution are the liver, kidneys and spleen as well as bone and the remaining carcass (muscle, skin and hair). Growing bone takes up more chromium. […]. Animal models suggest that the main storage functions reside in the bone and soft tissues where some 90% of the whole-body chromium was located after repeated oral dosing with chromium(VI) for 42 days. Some chromium may reach the interstitium of the testis, and significant amounts of chromium accumulate in the placenta, however, low amounts pass the placenta.

Chromium(III) is mainly excreted in the urine and to a lesser extent into the faeces. Small amounts are excreted in the bile, sweat, hair and presumably with desquamating cells. At physiological levels chromium is partly conserved in the kidneys. However, when plasma chromium concentrations increase significantly (about 10-fold), clearance increases abruptly. This may indicate a physiological regulation of the chromium body burden in which the oligopeptide low molecular weight Cr may be involved."

7.9.3. Acute toxicity and Corrosion/Irritation

The registrants concluded the substance is not acutely toxic and not irritant to the eyes and skin. Based on the available information, the FR-MSCA can support this conclusion.

7.9.4. Sensitisation

7.9.3.1 Skin sensitisation

The data comes from the chromium(III) oxide registration dossier (aggregated dataset), registration dossiers on other chromium compounds available in ECHA disseminated website and from a literature review. In the registration dossier**,** the only study described was the Buehler patch test (Unpublished report, 2006). The registrants also referred to conclusions of international reports on chromium: Health and safety executive (HSE),

1989; Agency for Toxic substances and Disease Registry (ATSDR), 2000; Riihimäki and Luotamo; 2006 (rapporteur FIOH).

A literature search was performed by FR-MSCA with the search terms "trivalent chromium" or "chromium(III)" AND "contact dermatitis" in the title, abstract and keywords until July 2018 in Pubmed database. The aim of the search was to retrieve experimental animal data or human patch test studies with chromium(III) oxide or other Chromium(III) salts.
Based on title and abstract, studies in Pubmed were further selected and full-text were retrieved.
Inclusion criteria (full-text) were the followings:

- The full study is published in English;
- It is not secondary literature, such as review, editorials, posters, oral abstracts, books;
- Full-text available to FR-MSCA or summary available in published reviews;
- Additionally, studies quoted by WHO, 2009 were also taken for analysis.

In addition, data as provided in the ECHA disseminated website for other chromium(III) compounds were checked.

Table 13: Animal data

<table>
<tr><th>Method</th><th>Type of effect</th><th>Remarks</th><th>Reference</th></tr>
<tr><td>OECD TG Guideline 429 (Skin Sensitisation: Local Lymph Node Assay)
GLP compliant
CBA/CaOlaHsd female mice
0, 10, 25, 50%
4 animals/dose
Positive control: Hexyl cinnamic aldehyde
Limitation:
- Only the summary of the study is available to FR-MSCA
- Relative humidity in the animal room was between appr. 30 - 100% for few hours
- lower concentration should have been tested to assess potency and to calculate EC3 value with sufficient reliability</td><td>Positive study

Stimulation index:
7.42 at 10%
7.25 at 25%
13.60 at 50%</td><td>2 (reliable with limitations)

Test material: chromium(III) acetate

Vehicle: DMF
Purity: no data</td><td>Unpublished report, 2008 (quoted in ECHA disseminated website for EC 241-562-9)</td></tr>
<tr><td>OECD TG Guideline 406 (Buehler Patch Test, 3 applications)

Hartley Guinea-pigs
20 controls + 10 treated females

Induction : 3 epicutaneous, semi-occlusive at 80%
Challenge: epicutaneous, semi-oclusive at 80%

Positive control: alpha hecyl cinnamic aldehyde

Limitations:
- semi-occusive dressing is used instead of occlusive dressing recommended in Buehler patch test</td><td>Range findings study: no irritation up to 80%

Main test: negative
Induction: one animal with grade I erythema following 2nd and third applications
Challenge: no reactions in controls and treated animals at 24 or 48h following challenge</td><td>2 (reliable with limitations)

Test material: basic chromium(III) sulfate (mixture of chromium sulfate (60%) and sodium sulfate

Purity: confidential

Vehicle: physiological saline</td><td>Unpublished report, 2006</td></tr>
<tr><td>Non guideline boosted guinea-pig skin sensitisation assay
In vivo test
Induction : 5 injections of 0.2ml of potassium dichromate in FCA or chromium(III) chloride in FCA

Induction: weekly intradermal injection of potassium dichromate or chromium(III) chloride in saline and simultaneous weekly epicutaneous application of test materials in Triton X-100 until reaction occured (max. 6 weeks)</td><td><table><tr><th>Sensitisation and restimulation</th><th>Challenge</th><th>Positive/total</th></tr><tr><td rowspan="2">$K_2Cr_2O_7$</td><td>$K_2Cr_2O_7$</td><td>11/11</td></tr><tr><td>$CrCl_3$</td><td>7/11</td></tr><tr><td rowspan="2">$CrCl_3$</td><td>$CrCl_3$</td><td>7/10</td></tr><tr><td>$K_2Cr_2O_7$</td><td>3/10*</td></tr></table>*No differences in the intensity of skin reaction
Additional experiment:</td><td>3 (unreliable)

Test material: Chromium(III) chloride and potassium dichromate</td><td>Siegenthaler et al., 1983</td></tr>
</table>

Challenge: epicutaneous application with both substances simultaneously and the reactions were evaluated 24 hr later Reading: 24h after challenge Additional experiment : *In vitro* and *in vivo* selection of chromium specific lymphocytes Limitations: - No GLP status - no information on purity of test material - unknown number of animals used at the start of the study - reading only at 24h following challenge - concentration used in the study not clearly reported - no negative or positive controls used in the study - boosting protocol not adequate to conclude on skin sensitisation potential of Chromium(III) chloride	Based on the in *vitro/in vivo* selection of lymphocytes, the authors hypothetised that there is a common determinant which is chromium(III). Several common allergenic complexes are formed, as well as some additional allergenic complexes specific for the particular valence state.		
Non guideline guinea-pig skin sensitisation study 21 Guinea-pigs Chromium chloride in FCA Induction: intramuscular injection + intradermal injection + epicutaneous application Challenge: weekly epicutaneous and every 2 weeks intradermal challenges	Epicutaenous test: positive response with chromium(III) chloride in 38% of the animals (*vs* 95% with potassium dichromate) Intradermal test: positive response with chromium(III) chloride in 74% of the animals (*vs* 100% with potassium dichromate)	4 (secondary litterature) Test material: chromium chloride or potassium dichromate	Polak *et al.,* 1973 (cited by WHO, 2009)
Non guideline guinea-pigs skin sensitisation assay Guinea-pigs Main test: Induction: 3 subcutaneous injection one week appart: FCA + chromium(III) chloride or potassium dichromate Challenge: intradermal injection Reading: 48h Cross-reactivity experiments in animals with established delayed hypersensitivity Reactions to other trivalent salts Limitation	Potassium dichromate: positive in 26/27 animals Chromium(III) chloride: positive in 10/13 animals Cross-reactions: all sensitised animals to chromium(VI) reacted to Chromium(III). In the 10 animals sensitised to Chromium(III), 8 also reacted to Chromium(VI) Reaction to other trivalent salts: more reactions with more highly dissociated salts (e.g. chromium chloride or nitrate) than weakly dissociated salts (e.g. chromic oxalate)	4 (secondary litterature) Test chromium(III) chloride and potassium dichromate Vehicle: physiological saline	Gross *et al.,* 1968 (cited by WHO, 2009)

- Strain and number of the animals at the start of the study not specified - No GLP status - No information of purity - Environmental conditions not specified - No positive controls - Unknown number of negative controls - Only three tested animals in the experiment with other trivalent salts			
Non guideline guinea-pig skin sensitisation study 40 female guinea-pigs 8 animals/groups Induction: 2 subcutaneous injections of 0.1ml, one week interval (chromium sulfate 0.03% or potassium dichromate + FCA)	Positive reactions with both chromium sulfate or potassium dichromate	4 (secondary literature) Test material: hydrated chromium sulfate or potassium dichromate	Jansen and Berrens, 1968 (cited by WHO, 2009)

Table 14: Human experimental skin sensitisation studies

Method	Type of effect	Remarks	Reference
Human Maximisation test Induction test (repeated 5-times): Pretreatement : SLS 5%, 24-h occlusive patch Subsequent 48-h occlusive patch with: - 3 % chromium trioxide, - 25% chromium sulfate, or - 2% potassium dichromate Challenge test: 48h epicutaneous pretreatment with 10% SLS, followed by 48h epicutaneous exposure: - 0.5% chromium trioxide, - 2% chromium sulfate, or - 0.25% potassium dichromate, respectively A control patch with petrolatum was used for the same time intervals. Limitations: - non acceptable unethical experiment on prisonners - no information on clinical history of volunteers - no GLP status - no information on purity of the test materials	Sensitisation rate: Chromium trioxide: 13/23, Grade 3 (moderate) Chromium sulfate: 11/23, Grade 3 (moderate) Potassium dichromate: 23/23, grade 5	3 (non reliable) Test material: chromium trioxide, potassium dichromate, chromium sulfate	Kligman *et al.,* 1989

Table 15: Patch test studies in humans

Subject (n)	Concentration/test material for patch test	Results	Reference
10 Cr(VI)-allergic patients + 22 controls	<u>Patch tests</u> - Cr(III) oxalate trihydrate (0.15-531µg Cr(III)/cm^2 - Potassium dichromate (0.15-53 µg Cr(VI)/cm^2 - Cr-tanned and Cr-free leather samples <u>Use tests</u> Cr-tanned of Cr-free leather bracelets	<u>Patch test:</u> - 7/10 : reaction to both Cr(III) and Cr(VI). - 1/10 reaction to Cr-tanned leather patch test. - Elicitation at lower concentration for Cr(VI) No reaction in controls Only Cr(III) released from Cr-tanned leather (0.5-59.9µg/cm2). <u>Use test</u> 4/10 had a positive response Skin deposition to Cr-tanned bracelets: 0.003-0.16µg Cr/cm2. Most probably Cr(III)	Hedberg *et al*., 2018
10 Cr(VI)-allergic patients	<u>Patch test</u> - Potassium dichromate - Coated Cr(III) or Cr(VI) disks	Elicitation of dermatitis by both Chromium(III) and Chromium(VI) disks	Bregnbak, *et al*., 2017
15 Cr(VI) allergic patients	<u>Patch test</u> - Cr(III) chloride (13%) - 0.5% potassium dichromate (Cr(VI)) - 15 leather samples (from trousers, jackets, shoes) - a control with vegetable tanned leather <u>Use test</u> Prolonged 14-day exposure to leather bracelets containing the highest Cr(VI) content (12 patients)	<u>Patch test</u> - 15/15 positive reactions to Cr(VI) - 9/15 positive reactions with Cr(III) - leather samples: 4/15 elicitided allergic reaction Analysis of leather samples content: no correlation between measured amount of Cr(VI) and soluble Cr(III) in the leather and elicitation <u>Use test</u> - 3/12 (patients with no reaction to leather patch tests)	Hansen *et al*., 2006a

2211 consecutive patients	- Cr(III) chloride (13%) - 0.5% potassium dichromate (Cr(VI)) -Leather sample patch testing (18 patients)	71 (3.2%) reacted to Cr(VI) Of the 71 Cr(VI) positive patients, 31 patients (44%) had a positive reaction to Cr(III), 18 patients (25%) had a doubtful reaction to Cr(III) and 22 patients (31%) had a negative or irritative reaction to Cr(III). Only 9/71 Cr(VI) positive patients had no other contact allergies (Cobat chloride, nickel sulfate or fragrance mix). Positive association between strength of Cr(VI) reaction and positive Cr(III) patch test. No positive reaction to Cr(III) without a concomitant positive reaction Cr(VI) Irritative response: 9 % of Cr(VI) patch tested patients and 1% of Cr(III) patch tested patients Leather patch testing and exposure: 10/18 had doubtful of positive reation. All 10 had positive or doubtful reaction to Cr(III) The authors also concluded that a positive reaction to Cr(VI) in combination with a positive reaction to Cr(III) increased the risk of foot dermatitis	Hansen *et al*., 2006b
18 Cr(VI) allergic patients	Cr(III) chloride hexahydrate (5-23350 ppm) (2-5000ppm) potassium dichromate Leather exposure	Minimal eliciting threshold for each patient for Cr(VI) : 2-221 ppm and 50 to 12675 ppm for Cr(III). Both Cr(III) and Cr(VI) are capable of eliciting dermatitis at low concentration in the same patient.	Hansen *et al.,* 2003
18 Cr-allergic patient + controls	Use Test: detergent bar	Valence state of chromium found to be Cr(III), 40-50 ppm was found in the bar. No hexavalent chromium in the detergent bar No skin reactions to the detergent bar	Iyer *et al.,* 2002
2 men	Patch test: - Chromium Chloride (0.5 to 2%) - Potassium dichromate (0.032 to 1%) Occupational exposure: tannery. Chromium(III) sulfate used in the tannery. Not possible to completely exclude Cr(VI) exposure	Increased risk in tanning due to wetness and irritancy of the work. Both men had positive patch test to trivalent and hexavalent chromium	Eslander *et al.,* 2000
56 Cr(VI) allergic patients	Patch test: Chromium(III) chloride, potassium dichromate	No positive reactions to Cr(III) up to 33 µg/Cm^2	Nethercott *et al.,* 1994
14 Cr(VI) allergic patients	Patch test: - 0.25% and 0.5% potassium dichromate - 0.045% (88.5ppm), 4.5% (8850 ppm) and 9.1% (17700ppm) chromium(III) chloride Exposure: detergent washing powders	14/14 positive to 0.5% potassium dichromate 11/14 positive to 0.25% potassium dichromate (885 ppm) 5/14 positive to chromium chloride (8850 or 17700 ppm) 1/14 reacted to 88.5 ppm chromium chloride	Allenby and Goodwin *et al.,* 1983
5 Cr-allergic patients	Chromium(III) chloride	1/5 positive to 0.187M of chromium chloride	Samitz and Shrager, 1966 (Cited in ATSDR)
Cr(VI) allergic patients	Patch test: Chromium(III) compounds (no further information)	11/17 patients reacted to Chromium(III) chloride 0.5M, 4/22 using 0.07M. Patch test activity of trivalent chromium compared with hexavalent chromium: 1/10	Fregert and Rorsman, 1964 (Cited in ATSDR 2012)

		for the oxalate, 1/100 for the chloride, 1/1000 for the acetate.	

Table 16: other mechanistic studies

Subject (n)	Concentration/test material	Results	Reference
56 Cr(VI) sensitised and 26 non-sensitised patients	Patch test - Potassium dichromate (0.5% in Vaseline) Cellular *in vitro* test : chromium-specific lymphocyte transformation test Culture stimulated with tri- or hexavalent chromium (Chromium (III) chloride hexahydrated, > 99.5% purity)	Lymphocyte transformation assay: Dose-dependant lymphocyte transformation response in volunteers with sensitisation with allergy, with sensitisation without allergy and in non-sensitised healthy controls. Both trivalent and hexavalent chromium induced proliferation and cytokine production to a similar degree. However, for comparable results a 250-fold higher concentration of chromium chloride is needed	Lindemann *et al.,* 2008

Animal data

No skin sensitisation study on chromium(III) oxide is available.

Two *in vivo* guideline studies were available on skin sensitisation properties of Chromium(III) salts, a Local Lymph Node Assay (LLNA) and a Buehler test in guinea-pigs.

The LLNA was conducted according to OECD TG TG 429 (GLP compliant). In this study, chromium(III) triacetate was found to be a skin sensitiser since the stimulation indexes (SI) above 3 were found at 10%, 25% and 50% v/v concentration (unpublished report, 2008 as reported in ECHA disseminated website). EC_3 value was not calculated in the study and was found to be < 10%. As no clear dose response was observed, EC3 value based on the extrapolation of the SI values would not be sufficiently reliable for supporting sub-categorisation.

The results of the study (Unpublished report, 2008) are reported in the table below:

Concentrations (%)	**Stimulating index**
0	-
10	7.42
25	7.25
50	13.60

The second study was a Buehler 3 application test (OECD TG 406, GLP compliant). In this study, chromium basic sulfate (containing 60% of chromium (III) sulfate) was not found to be a skin sensitiser up to a concentration of 80% (Unpublished report, 2006). This study is considered of lower weight than the LLNA as the Buehler assay has a low sensitivity.

Several old non-guideline studies, similar to OECD TG 406 M&K study (worst case conditions compare to OECD TG guideline), support the results of the positive LLNA study. Chromium(III) chloride was able to sensititize guinea-pigs following intradermal or subcutaneous injections. When a positive allergic reaction was achieved intradermally, subsequent elicitation epicutaneously has been demonstrated (Siegenthaler *et al.*, 1983; Polak *et al*., 1973; Gross *et al*., 1968).

Human data

In an old Human maximisation test, chromium(III) oxide and chromium(III) sulfate were found to be skin sensitisers (Kligman *et al*., 1989). Nevertheless, the test materials were not checked for Cr(VI) content. Therefore, the results may be unreliable.

In human patch test studies, several studies showed that chromium(III) chloride could elicite a Chrome(VI) allergic reaction. Nevertheless, there is no data showing primo-sensitisation of Chromium(III) in human.

Conclusion

Skin sensitisation potential between chromium(III) oxide and soluble chromium(III) compounds such as chromium(III) triacetate or chromium(III) chloride may differ.

Indeed, several parameters may influence skin sensitisation potential and one of the parameter is dermal absorption. Chromium(III) oxide (and other insoluble trivalent compounds) is expected to be less absorbed by skin and thus may have a lower skin sensitisation potential compare to Chromium(III) salts. Nevertheless, there are no data to confirm that less soluble compounds would not be skin sensitisers. Moreover, under certain relevant conditions (irritancy, wetness, sweat), penetration of the test material may be enhanced.

In addition, as commented by the German CA, several studies show that nanomaterials can penetrate healthy skin after repeated application (Gulson *et al*., 2010, 2012). Appendages such as hair follicles, sebaceous and sweat glands are an important routes of penetration for nanomaterial into the skin, as evidenced by the research on particle-based drug delivery systems. Moreover, hair follicles may also act as "long-term reservoirs suited for accumulation of nanomaterials" (Yoshioka *et al*., 2017). Chromium (III) oxide contains a percentage of nanoform, even if below the threshold of 50%. This nanoform can penetrate through, and accumulate in, the hair follicles and thus be available for uptake into dendritic cells. After internalisation, for instance in lysosomes, chromium (III) oxide can release chromium (III) ions.

Therefore, chromium(III) oxide should be considered as a skin sensitiser and warrant to be classified as Skin Sens. 1, H317.

7.9.3.2 Respiratory sensitisation

No data available.

7.9.5. Repeated dose toxicity

The data came from the REACH registration dossier and a literature search performed in Pubmed up to July 2019.

Table 17: Summary of repeated-dose toxicity studies - ORAL

Test method	**Results**	**Comments** (Klimisch score, test material, purity, vehicle)	**Reference**
Non-guideline repeated dose 1-year toxicity study in rats Oral: drinking water 25 ppm as Cr(III) or Cr(VI) ion	No effects	4 (secondary literature)	MacKenzie *et al.,* 1958
Non-guideline repeated dose 90-day oral toxicity study in rats (with mating) Oral: baked bread 6 males + 6 females BD rats 1, 2, 5% chromium oxide (eq. 72/75, 180/160 mg/kg in male/females at 2 and 5%) for 90 days 9 females paired with males following 60-day treatment. Limitations: - non GLP study	No effects observed Small changes in liver and spleen weight. No histopathological findings. No malformation or effects on litter size. No tumours observed in the progeny (unknown number of animals) NOAEL = 160 mg Chromium oxide/kg bw	3 (unreliable) Test material: non hydrated chromium oxide (free from chromate).	Ivankovic *et al.,* 1975

- Insufficient study protocol details - Insufficient number of animals - Unstandard diet (cooking bread)			
Non-guideline repeated dose 24-week oral toxicity study in rats Harlan Sprague-Dawley (SD) rats Oral: diet 8/group 0, 5, 25, 50, 100 ppm Sacrifice: 24-week Limitation: Gender not specified Amount of consumed food not indicated	No hematological or biochemical changes in blood (11, 17, 24 weeks) and no histopathological findings in liver or kidney Increased Cr concentration in kidney and liver was linear for both Cr Chloride and Cr picolinate	4 (secondary literature) Test material: Chromium picolinate and chromium chloride	Anderson *et al.,* 1997 (cited in EFSA, 2010)
Non-guideline repeated dose 52-week oral toxicity study in rats SD rats 6/sex/group Oral: feed 0, 25 ppm (1000 µg Cr(III)) Sacrifice: 26, 39, 52 weeks Investigation: body weight, water and food consumption, selected organ weight, physical and ocular health, hematology and clinical chemistry, hepatic lipid peroxidation and DNA fragmentation, and histopathology Limitations: - Insufficient number of animals - Only one dose tested	No significant changes NOAEL = 1 mg Cr(III)/kg	2(reliable with limitations) Test material: niacin bound chromium(III) complex Purity: no information	Shara *et al.,* 2007
Repeated dose 14-week oral toxicity study in rats and mice (similar to OECD TG 408) <u>Rat:</u> F344/N rats Oral: feed Exposure: 14-weeks 10/sex/group 0, 2000, 10000, 50000 ppm (eq to 0.8/0.7, 2.4/2.4, 19/19, 98/93, 506/507 mg/kg Cr(III) in males/females) <u>Mice</u> Oral: feed B6C3F1 mice Exposure: 14-weeks 10/sex/group 0, 2000, 10000, 50000 ppm (eq to 2/1.7, 5.9/4.8, 54/44, 274/212, 1419/1090 mg/kg Cr(III) in males/females) Limitations: - Insufficient highest dose level, as the highest dose did not produce toxicity. - Only few organs were weighted: heart, kidney, liver, lung, testis, kidney	Rats and mice No effect on survival, body weight, feed consumption or lesions No effect on sperm motility and vaginal cytology NOAEL = 506 mg/kg Cr(III) in rats and 1419/1090 mg/kg in mice males/females	2 (reliable with limitations) Test material: Chromium picolinate monohydrate Purity > 96%	NTP, 2010 (Stout MD *et al.,* 2009)
Repeated dose 90-day oral toxicity study in rats (OECD TG 408) GLP	No observed effect Chromium concentration in blood: 8.6 ng/g in	2(reliable with restriction) Test material:	Sreejayan *et al.,* 2010

SD rats 10/sex/group 0, 0.23, 2.3, and 5.7 mg/kg/day Limitations: - Only some of the results are published in detailed tables - only low dose tested - no information on purity	males and 5.6 ng/g in females at 5.7 mg/kg NOAEL ≥ 5.7 mg/kg	Chromium(III) dinicocysteinate complex (NBC or chromium nicotinate)	
Non guideline repeated acute, 7-day and 14-day toxicity study Male Wistar rats 5/group Oral: gavage 50µg/100g bw, 100µg/100g bw Male Wistar rats Biochemical study and histopathology of Kidney and brain Limitations: - Insufficient number of animals - No check for Cr(VI) content - No tabulated data and detailed of incidence and severity grade for histopathological findings	Increased LPo levels in brain and kidneys (dose-duration dependant manner) Increased MDA and depleated GHS and SOD levels Histopathological changes in kidney (congestion of renal capillaries, swollen glomeruli, focal tubular atrophy, increased eaosinophilic foci, necrosis of tubular epithelium, fibrosis at day 14) and brain (inflammation, diffuse fibrosis and neuronal vacuolisation)	3 (unreliable) Test material: chromium oxide nanomaterial Purity: not provided Vehicle: distilled water	Fatima *et al.,* 2017

Table 18: Summary of repeated-dose toxicity studies - INHALATION

Method	Type of effect	Remarks	Reference
90-day subchronic inhalation toxicity study in rats (similar to OECD TG 413) CDF (Fischer 344)/Crl BR VAF/Plus rats Inhalation (nose-only), dust 6h/d, 5d/w 10 rats/sex/groups + 5 rats/sex/group for 13-week recovery period 5 rats/sex/group for bronchoalveolar lavage parameters investigation 5-day exposure 0, 4.4, 15, 44 mg/m^3 chromic oxide (equivalent to 3, 10, 30 mg/m^3 chromium	**CHROMIUM OXIDE** MMAD (geometric standard deviation) (µ): 1.8 (1.93), 1.9 (1.84), and 1.9(1.78) at low, mid and high dose No effect on bw gain and bw, no clinical findings No effects on hematology, serum biochemistry and urinalysis ↓* bw (m > 54 mg/m^3 ; f≥168 mg/kg) Sperm analysis: no effect Bronchioalveolar lavage (BAL): No changes in BAL parameters. Crystalline material observed in the cells ≥ 4.4 mg/m3. Dose-related increase in percentage of affected cells and relative amount of material. LUNG: ↑*lung/trachea weights (m, 168 mg/m^3). Reversible changes. Randomly distributed foci or aggregates of pigmented macrophages filled with dense black pigment were observed within alveolar spaces adjacent to the junctions of terminal bronchioles and alveolar ducts and subjacent to the pleura (m, f ≥ 4.4 mg/m^3). Similar black pigment was also observed at the tracheal bifurcation, in the peribronchial lymphoid tissue, and within the mediastinal lymph node. The pigment stained black with hematoxylin and eosin stain and was presumed to represent the test article. The presence of	2 (reliable with limitations) Test material: chromic oxide > 99% Cr(III), <0.0001%Cr(VI) Basic chromium sulfate (25%cr(III), < 0.0003% Cr(VI). No hexavalent chromium detected in the study	Derelanko *et al.*, 1999

for chromium(III) oxide) 0, 17, 54, 168 mg/m^3 basic chromium sulfate Limitations: - Weight of the animals at the beginning of the study was not provided - no details of histopathological lung findings - Granulometry of the powder not provided (only MMAD was analysed)	the pigment corresponded to the green discoloration seen macroscopically. Trace to mild chronic interstitial inflammation of the lung, characterized by an infiltration of inflammatory cells, was observed in alveolar septa surrounding aggregates of pigmented macrophages in some mid-exposure and high-exposure level males and females. Chronic interstitial inflammation was accompanied by septal cell hyperplasia (Type II pneumocytes) in some mid-and high-exposure level males. The microscopic changes were generally associated with the pigment and corresponded to the increased lung weight observed for the males in the high- exposure-level group. Lymphoid hyperplasia of the node was also present in all exposure groups. No test article-related lesions were seen in the nasal cavities of animals exposed to chromic oxide at any exposure level. Trace to mild septal cell hyperplasia and chronic interstitial inflammation persited in males of all treatment groups and females in the mid and high exposure groups. No full recovery observed. THYROID/PARATHYROID: ↑* relative weight ,f ≥15 mg/m^3) LOAEC = 4.4 mg/m^3		
	<u>BASIC CHROMIUM SULFATE</u> MMAD (Geometric standard deviation) (µm): 4.2 (2.48), 4.2 (2.37), and 4.5 (2.50) at low, mid and high dose Sporadic labored breathing (f ≥ 168 mg/m^3) BAL parameters: ↓*total nucleated cell count. Increase amounts of cell debris and lysed cells ≥ 17 mg/m^3 ↑* Alkaline phosphatase (f≥168 mg/m^3), ↓* serum cholesterol (f≥54 mg/m^3) Sperm analysis: no effect LUNG: ↑*lung/trachea weights (m, f ≥ 17 mg/m^3) Chronic inflammation in the alveoli (m, f ≥ 17 mg/m^3). Some foci exhibited intense inflammation and thickening of alveolar walls. Chronic interstitial inflammation was usually multifocally distributed and consisted of thickened alveolar septa caused by inflammatory cell infiltration and hyperplasia of alveolar septal cells. Trace to severe, multifocal to diffuse pulmonary infiltration of alveolar macrophages with foamy or granular appearing acidophilic cytoplasm was observed in the alveolar lumens and correlated with the gray discoloration of the lungs that was observed at necropsy (type II pneumocytes). Multifocal areas of granulomatous inflammation, characterized by infiltration of macrophages and multinucleated giant cells, was observed at all exposure levels and was closely associated with foreign material seen in the lung and presumed to be the test article. BRAIN: ↑* relative weight (m ≥168 mg/m^3) KIDNEY: ↑* relative weight (m ≥168 mg/m^3) THYROID/PARATHYROID: ↑* relative weight (m,f ≥168 mg/m^3). No histopathological correlates LIVER small relative weight changes (m ≥168 mg/m^3), data not shown. No histopathological correlates SPLEEN: small relative weight changes (m, f ≥168 mg/m^3), data not shown TESTES: small relative weight changes (m ≥168 mg/m^3), data not shown		

	LOAEC = 17 mg/m³		
Non guideline repeated subchronic inhalation toxicity study in rabbits 8 male rabbits 1.2 mg/m³ 6h/d + 0.6 mg/m³ nickel or 0.5 mg/m³ colbalt and 1.2 mg/m³ $Cr(NO_3)3$, 4-month exposure Histopathological examination of the lung Limitations: - Only coexposure with other metals was tested.	Type II cell aggregation, no increase in macrophage diameter	4 (secondary literature) Test material: chromium(III) nitrate	Johanson, 1992

Summary and discussion on repeated dose toxicity

Oral route

No reliable data are available with chromium(III) oxide or chromium(III) salts by oral route in animals or humans.

Data are only available with chromium(III) complexes, used as dietary supplement, that are reported to have a higher Cr(III) bioavailability by oral route than insoluble or soluble chromium(III) compounds.
In the animal studies, no effects were identified up to 506 mg/kg Cr(III). As no critical effects were observed, no DNEL by oral route can be derived. No safety concern was identified in human at very low level (up to 250 µg/day chromium) when Cr(III) is used as a dietary supplement (chromium picolinate, nicotinate) (EFSA, 2010 a&b, 2014).

Inhalation route

In Derelanko *et al.,* 1999, inhalation exposure to chromium oxide revealed pigment-laden macrophages and lymphoid hyperplasia in all exposed animals and septal hyperplasia and interstitial inflammation in the animals at the two high exposure groups. Pigment-laden macrophages were found in the animals exposed to chromium(III) oxide even after the 13-week recovery period. By this time only partial recovery of the pathological findings had occurred. Minimal septal hyperplasia and interstitial inflammation were still observed following the recovery period. In the females of the two high exposure groups, a statistically significant increase in absolute and relative thyroid/parathyroid weights were observed. The LOAEC was set for chromium(III) oxide to 4.4 mg/m³.
No NOAEC was found. As no analysis of particle size was provided, the study may not cover potential lung effects following chromium(III) oxide exposure as nanoparticles.

In the same published study, inhalation of chromium sulfate was related to an increase in lung weight and histopathological findings in the lung. The LOAEC was 17 mg/m³ in this study.

As severe local effects were observed following inhalation, a classification STOT RE 2, H373 (lung) is warranted.

7.9.6. Mutagenicity

A summary of data available in the registration dossier are presented in the table below. Studies quoted in the following reviews were also taken into account: ATSDR, 2012;

Easmond *et al.,* 2008, De Flora *et al.,* 1990. A literature review started from 2011 to August 2019 in Pubmed database. The search terms were "trivalent chromium" or "chromium(III)" or "chromium oxide" AND genotoxicity" or "DNA damage" or DNA repair" or "micronuclei" or "micronucleus test" or "micronucleus assay" or "chromosomal aberrations" or "comet assay" or "clastogenicity" in the title, abstract and keywords.

Assays with known reported contamination of Cr(VI) and studies on hexavalent chromium were not retained.

In order to compare results found in the *in vitro* studies, some mass concentrations have been converted into molar concentrations. The following molar mass were used: 151.99 g/mol for Chromium(III) oxide; 158.36 g/mol for chromium chloride.

Table 19: Summary of in vitro and in vivo data on the genoxic potential on insoluble chromium compounds in water (e.g. chromium(III) oxide and hydroxide)

Method	**Type of effect**	**Comments** (Klimisch score, test material, purity, vehicle)	**Reference**
***In vitro* test**			
Non guideline study (DNA damage in bacteria) WP2uvrA (uvrA-)/CM571(recA-)/WP100 (uvrA-recA-) Assay: differential killing E.coli; 50 mg/ml Solvent: HCl for chromium hydroxide, not available for chromium oxide	Negative	4 (secondary literature) Test material: Chromium hydroxide, Cr_2O_3	De Flora, 1990 (cited Yagi et Nishioka, 1977)
OECD TG Guideline 471 (Bacterial reverse mutation assay) *S. typhimurium* TA 97, TA98, TA1535, TA 1537 (S9mix rat: with and without) Plate incorporation/ two independent experiments Test concentrations: 0, 8, 40, 200, 1000, 5000 µg/plate (+/- S9) Limitations: Only 2-aminoanthracene was used as positive control with S9 Unknown purity	Negative with and without metabolic activation Cytotoxicity: precipitation was observed at 1000 and 5000 µg/plate	2 (reliable with restriction) Test material: chromium hydroxyde Purity: na Solvent: water	Unpublished report, 1991
Non guideline HPRT assay (*in vitro* mammalian cell gene mutation) V79 Chinese hamster cells	Chromium oxide particles were detected in the cytoplasm of C79 cells (electron micrograph) <u>Cytotoxicity:</u> Mitotic index following treatment: 80%, 52% and 32% of control values at 50, 100 and 200 µg/ml, respectively;	2 (reliable with restriction) Cr_2O_3, powder	Elias *et al.,* 1986

50, 100, 200 µg/ml Cr_2O_3 (equivalent to 328-1316 µM) 18-h exposure Expression time: 3, 6 or 9 days Positive control: methyl methanesulfonate Negative control: Dulbecco's MEM medium Limitations: - No repeated experiment - S9 mix was not used - Only 3 concentrations - Non GLP status - No historical control data	Concentration dependant delay in cell-cycle progression with accumulation of cells in G2; <u>Mutation frequency</u>: equivocal results. Statistically significant increase in mutation frequency at all concentration tested after 9-day expression time. The increase was not dose-dependant.	Purity: 99.8% (91% < 1µm, 9 % between 1 and 3 µm), no contamination with Cr(VI), (checked for crystalline structure)	
Non guideline HPRT assay (*in vitro* mammalian cell gene mutation) Human fibroblasts 24h exposure Tested concentration: 50-250 µM 40-60 dishes/concentration Positive control: N-methyl-N'-nitro-N-nitroguanidine (MNNG) Limitations: - low level of details in the publication - only 3 tested concentrations - no GLP status - non validated cell line - no information on particle size of chromium(III) oxide - no historical control data of the laboratory	<u>Cytotoxicity</u>: assessed by surviving fraction 68%, 74% and 41% of control at 50, 100 and 250 µM. <u>Mutagenicity:</u> positive Dose-related statistically significant increase in mutation frequency at 250 µM (10-45x10^6 mutant at 250 µM *vs* <0.5-12 in control).	2 (reliable with limitations) Test material: Cr_2O_3 Purity: > 99.955%	Biederman *et al.,* 1990

<table>
<tr><td>Non guideline in vitro comet assay

Method: Singh, 1988.
A549 human lung carcinoma cells
Exposure: 6h
Electrophoresis: 30 min (24V; 300 mA)
50 cells scored per concentration
Analyse: percentage of tail DNA, tail length, olive tail moment
50-1000 µg/ml

Non-guideline block micronucleus assay
Method: Fenech et al., 2000
6h treatment</td><td>Significant induction in DNA damage ≥ 800 µg/ml (increase tail length, OTM, tail DNA)

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) results demonstrated a time and concentration dependent cytotoxicity. MTT reduction was 77.5%, 71% and 62% in 24h at 600, 800, 1000µg/ml

Increase micronucleus at ≤600µg/ml and decreased at ≥ 600µg/ml onward.</td><td>2 (reliable with limitations)

Test material: Cr_2O_3 nanoparticles
Average diameter : 248.4 nm (DLS) and 30 nm (TEM)

Purity : 99%, not checked for Cr(VI) content. Batch provided by Sigma Chemical</td><td>Senapati et al., 2015</td></tr>
<tr><td>Non guideline in vitro sister chromatid exchange assay (SCE) test</td><td>One negative study (Andersen, 1983) : Human lymphocytes (no information on particle size)

One positive study (Elias, 1983): Chinese hamster V79 cells : 653-2635µM (< 5µm particle size, vehicle: ultrasonication in water)</td><td>4 (review)

Test material: Cr_2O_3</td><td>De Flora, 1990</td></tr>
<tr><td colspan="4">In vivo test</td></tr>
<tr><td>OECD TG 474 (in vivo Mammalian erythrocyte micronucleus test)

Male and female Mice: NMRI mouse
route: intraperitoneal injection
5 mice/sex/doses per exposure duration
Single dose: 10,000 mg/kg
Sacrifice: 16, 24, 48 hours
Preliminary test performed on 2 mice/sex/doses

Positive control cyclophosphamide

Limitations:
- Purity of test material not stated
- Particle size of the test material not stated</td><td>Negative

<table><tr><th rowspan="2">Treatment</th><th rowspan="2">Sacrifice</th><th rowspan="2">Cells (#)</th><th rowspan="2">NCEs (#)</th><th colspan="2">Micronuclei (#)</th></tr><tr><th>/1000 NCEs</th><th>/1000 PCEs</th></tr><tr><td>Vehicle control</td><td>24h</td><td>10000</td><td>763</td><td>1.4</td><td>1.8</td></tr><tr><td>Test substance</td><td>16h</td><td>10000</td><td>2082*</td><td>1.0</td><td>1.1</td></tr><tr><td>Test substance</td><td>24h</td><td>10000</td><td>1537</td><td>1.3</td><td>1.9</td></tr><tr><td>Test substance</td><td>48h</td><td>10000</td><td>1497</td><td>1.3</td><td>0.6</td></tr><tr><td>Cyclophosphamide</td><td>24h</td><td>10000</td><td>675</td><td>1.8</td><td>15.7*</td></tr></table>*P<0.01

Clinical signs (apathy, stretching of body, roughened fur, staggering gait, spasm and difficulty of breathing) at this dose level.

Proof of exposure of bone marrow: statistically significant increase in NCE at 16h but not 24 or 48h.</td><td>2 (reliable with restriction)

Test material: Cr_2O_3

Purity: confidential

Vehicle: corn oil (suspension)</td><td>Unpublished report, 1992</td></tr>
</table>

- Single excessive dose tested - Only 2000 cells instead of 4000 recommended - no comparison with a positive Cr(VI) compound	Negative control: in the range of negative historical controls (using corn oil) Positive control: positive results in the range of positive historical controls		
Non guideline *in vivo* micronucleus (MN), chromosomal aberration (CA) and comet assay in rats 28-day repeated dose toxicity study Wistar rats 5/sex/group 30, 300, 1000 mg/kg Oral: gavage Positive control cyclophosphamide (ip) <u>Comet assay:</u> Method Tice *et al.,* 2000 Blood liver tissue (Miyamac, 1998) after sacrifice at different interval (not specified) 3 slides/conditions Electrophoresis: 300mA, 20min Analysis: 150 peripheal blood lymphocytes and 50 liver cells/slide/rats DNA damage: % of DNA in comet tail <u>MN assay</u> According to OECD TG 474 3 slides/animals 2000 polychromatic erythrocytes (PCE)/animals PCE/normochromatic erythrocyte (NCE) in 1000 cells for each animals <u>CA assay</u> Similar to OECD TG 475	Mean size of Cr_2O_3: - Nanoparticles (NPs): 34.39 nm - Microparticles (MPs): 3.76 µm No mortality observed in the study NP ≥ 300 mg/kg and MP at 1000 mg/kg: dullness, irritation, moribund symptoms during first week. Loss of weight (not statistically significant and no effect on feed intake). <u>Comet, CA and MN assays:</u> Dose related increased in DNA damage (measured by % tail DNA) in peripheral blood leucocytes and in liver, Micronuclei and chromosomal aberration in both males and females. The increase is statistically significant from 300 mg/kg with NPs and at 1000 mg/kg only with MPs. Cell viability was not reported in the study. <u>Biodistribution</u> Cr was biodistrisbuted in all the tissues in a dose-dependent manner. In blood, the increase was statistically significant from 300 mg/kg with NPs and 1000 mg/kg with MPs. The highest amount was found in the kidney and the lowest amount in the brain.	2 (reliable with limitations) Test material Cr_2O_3 MPs or NPs Purity: ≥98.4% for NP and 98% for MP Vehicle: suspension in miliQ water	Singh *et al.*, 2016

Ip injection of colchicine 2h prior to sacrifice 100 metaphase/animals (500/doses) Mitotic index determined for 1000 or more cells Limitations: - Non GLP - Cr(VI) content not investigated - Data on the preliminary study used for dose selection not shown - No historical negative and positive control data - No histopathological results and determination of cytotoxicity in the comet assay - Cell viability not shown in comet assay			

Non guideline *in vivo* micronucleus (MN), chromosomal aberration (CA) assays in rats Male Wistar rats Oral, gavage 1, 7 or 14-day exposure 0, 0.5, 2 mg/kg Cr2O3 Positive control cyclophosphamide Sacrifice: 24h after last treatment <u>Micronucleus test:</u> Methods : Schmid; 1975 2000 PCE per animals examined <u>Chromosomal aberration test</u> Method : Preston *et al.,* (1987) 200 metaphases scored. <u>Sperm abnormality test</u> <u>Liver anatomy examination</u> <u>Limitations:</u> - non guideline, non GLP - Cr(VI) content was not checked - number of animals not reported - no tabulated results (figures only) - low level of detail in study method - very low dose used in the study	Dose-related increased in MN, CA and sperm abnormalities. Toxicity was observed in the liver.	3 (unreliable) Test material: Cr_2O_3 NPs Vehicle: distilled water (vortex 10min for suspension)	Fatima *et al.*, 2019

Table 20 Summary of in vitro and in vivo data on the genoxic potential on soluble chromium compounds in water (e.g. chromium(III) chloride)

Method	**Type of effect**	**Comments** (Klimisch score, test material, purity, vehicle)	**Reference**
***In vitro* test: Direct damage to DNA**			
Non guideline studies (DNA damage in bacteria) induction of lambda prophage/ induction of SOS response/differential killing *E.coli/S. Typhimurium/Bacillus subtilis*	Negative in 29 assays with chromium(III)acetate, nitrate, sulfate or chloride Positive in 2 assays with chromium(III) acetate. Decreased positivity with S9mix. Positive in 6 studies using liquid micromethod in presence of phosphate, citrate or salicylate	4 (review) Chromium(III) chloride, Chromium(III) acetate, Chromium(III) nitrate or Chromium(III) sulfate or potassium sulfate	De Flora, 1990
Non guideline *in vitro* studies (DNA damage in mammalian cells)	Negative in 9 studies (dated before 1990) : Inhibition of DNA synthesis/unscheduled DNA synthesis/DNA fragmentation Positive results observed in 5 studies : - Comet in human lymphocytes (Blasiak *et al.,* 2000) : significant increase in tail moment at ≥ 600µM (no differences following EndoIII treatment). No effect at 400µM. High concentration tested. May reflect cellular death Human lymphocytes, 50-1000µM +/- treatment with EndoIII Positive control: hydrogen peroxide 1h exposure Electrophoresis (30 min, 0.73V/cm (30mA) - Positive increase in DNA SSB in J77A.1 macrophage cells; (Hassoun and Stohs, 1995) - Terpilowska *et al.,* 2018 : positive comet assay in BALB/3T3 and HepG2 cells, 24h exposure (100-1400µM) IC50 for BALB /3T3 and Hep G2 : 1200µM (MTT) and 800-900µM (LDH assay) - El Yamani *et al.,* 2011: positive comet assay in Human lymphoblastoid cell line (TK6); Treatment with FG and endo III	4 (review) Test material: Chromium(III) chloride or chromium(III) nitrate	De Flora, 1990; Eastmond, 2008; ATSDR, 2012 Terpilowska *et al*., 2018 El Yamani *et al.,* 2011 Blasiak *et al.,* 2000 Fang *et al*., 2014

	Damage induced by ChromeIII removed more rapidely than damage produced by Cr(VI) - Fang *et al.,* 2014: Increased DNA damage; Jurkat cells		
Non guideline *in vitro* studies (Gene mutation in bacteria) E.coli WP2, *S. typhimurium* TA1538, TA1535, TA100, TA98, TA1537, TA102, TA97, tA94	Negative in 24 assays Positive in one assay with chromium chloride (TA94/TA98, 0.002-2 µmole/plate ; Langerwerf, 1985) Positive in one assay with chromium acetate (E. coli Hs30R; 16-130mM; Nakamuro, 1978)	4 (review) Chromium(III) chloride, Chromium(III) acetate, Chromium(III) nitrate or Chromium(III) sulfate or Chromium(III) potassium sulfate	De Flora, 1990; Eastmond, 2008; ATSDR, 2012
OECD TG 471 (Bacterial reverse mutation assay) *S. typhimurium* TA 1535, TA 1537, TA 98 and TA 100, (S9mix rat and hamster : with and without) Test concentrations: 0, 100, 333, 1000, 3333, 10000 µg/plate (as chromium chloride) Limitations : - no historical control data; - missing GLP status - experiment was not repeated	Negative without and with metabolic activation (rat or hamster)	2 (reliable with restriction) Test material: Chromium(III) chloride Purity: Solvent: water	Whittaker *et al.,* 2005
Non guideline *in vitro* studies (gene mutation in yeast)	Negative in two studies Positive in one study: deletion mutation (Kirpnick-Sobol, 2006)	4 (review) Test material: Chromium(III) chloride	De Flora, 1990; Easmond, 2008; ATSDR, 2012
OECD TG 476 (*in vitro* mammalian cell gene mutation) L5178Y TK+/- mouse lymphoma cells (with and without rat metabolic activation)	<u>Chromium chloride</u> equivocal response at the highest dose +/- S9 (> 2-fold increase compare to negative controls, increase in mutation frequency (MF) < 126 mutants per 10^6, no dose-response but in presence of cytotoxicity)	2 (reliable with restriction) Test material: Chromium (III) chloride	Whittaker *et al.*, 2005

Test concentrations: 0, 500, 600, 650, 700, 750 µg/ml chromium chloride (-S9) (eq. to 3157-4736 µM) and 1700, 1750, 1800, 1900, 2000 µg/ml chromium chloride (+S9) (eq. to 10735-12629 µM) Exposure: 4-hour (+/-S9) Duplicate cultures Positive control: methyl methanesulphonate (-S9) and dimethylbenzanthracene (+S9) Limitations: - no GLP status - no statistical analysis was performed - no negative and positive historical controls - only short exposure duration - no confirmatory experiment was performed - only absolute cloning efficiency was provided	No increase in the number of small colonies. The proportion of small and large colonies remain constant for all compounds Cytotoxicity: Dose selection: maximum 90% reduction in the viability of cells	Purity: 99% Vehicle: distilled water	
OECD TG 476 (*in vitro* mammalian cell gene mutation) Chinese hamster ovary AA8 cells Expression time: 8 days Quadruplicate sampling Repeated experiments (5-9) Exposure: 48h Test concentration : 0.25-1mM Limitations: - no GLP status - no data on Chromium(III) purity - no negative and positive historical controls - no positive control	Cytotoxicity: no effects on % cell survival up to 1mM. Precipitation observed at higher concentration. Dose-related incease in MF; statistically significant at ≥ 0.5mM	3 (unreliable) Test material: Chromium(III) chloride hexahydrate Purity: not specified Vehicle: water	Stearns *et al.,* 2002
OECD TG 476 (*in vitro* mammalian cell gene mutation)	Surviving fraction: 66-81% (no dose-response)	2 (reliable with limitations)	Biedermann *et al.,* 1990

Human fibroblast 24h exposure Expression time: 6 days Positive control MNNG, Cr(VI) 50, 100, 250, 750µM Limitations: - no GLP status - Non validated cell line - no negative and positive historical controls	Dose related increased in mutation frequency. Statistically significant at 750 µM (mean 17, range: 15-20*10^6)	Test material: Chromium(III) chloride Purity: > 99.995% pure Vehicle: water	
OECD TG 476 (*in vitro* mammalian cell gene mutation) Chinese hamster ovary cell line (CHO) 1h exposure 10-d expression time 0.2-0.8mM Positive control Ethyl Methane Sulfonate, Cr(VI) Limitations: - no GLP status - insufficient exposure time - no negative and positive historical controls - no repeated experiment - unknown purity	Survival: 78-85% MF (x10^6 survivors): 5.5-9	3 (unreliable) Test material: chromium(III) acetate	Bianchi *et al.,* 1983
Non guideline study (*in vitro* mammalian cell gene mutation) Chinese hamster cell line V79/4 8AG induction 24h exposure 0, 20, 100, 200 µg/ml	Survival: 82-100% No statistically significant increase in MF	3 (unreliable) Test material: chromium(III) acetate	Newbold *et al.,* 1979
***In vitro* test: damage at chromosomal level (chromosome aberrations, micronuclei, SCEs)**			

Non guideline *in vitro* studies: (in vitro sister chromatid exchange test)	Negative in 21 studies Positive in 6 studies	4 (review) Test material: Chromium(III) chloride, Chromium(III) acetate, Chromium(III) nitrate or Chromium(III) sulfate or Chromium(III) potassium sulfate	De Flora, 1990 Easmond, 2008 ATSDR, 2012
Non guideline *in vitro* study (*in vitro* mammalian cell micronucleus test) Human diploid fibroblast (MRC-5) from a male foetal lung 1, 2.5, 5µM Positive control: potassium dichromate 1000 binucleated cells per treatment were scored Limitations: - no cytotoxicity - non validated cell lines - no GLP sgtatus - no repeated experiment to confirm the positive results	Statitsically significant increase at all doses tested. No dose-response.	2 (reliable with restriction) Chromium(III) chloride, hexahydrated	Seoane *et al.*, 2001
OECD TG 487 (in vitro mammalian cell micronucleus test) BALB/3T3 and HepG2 cells 24h exposure 1000 cells analysed per sample 6 independent experiments with three wells per each treatment conditions 100-1400µM Limitations: - Non validated cell line - No positive control - No GLP status	50% inhibitory concentration for BALB /3T3 and Hep G2 : 1200µM (MTT) and 800-900µM (LDH assay) Increased percentail tail DNA in BALB/3T3 clone A31 cells and in HepG2 at ≥ 400µM cells.	3 (unreliable) Test material: Chromium(III) chloride, hexahydrated	Terpilowska *et al.,* 2018

- No information on purity of test material - 0% of tail DNA and BNMN is strange			
Non guideline *in vitro* test (chromosome aberration test in mammalian cells)	Chinese hamster ovary cells Negative : 5 Positive: 4 Human peripheral blood lymphocytes Positive : 3 Negative : 3 Other studies reviewed in De Flora, 1990 with other cell lines are not included	4 (review) Test material: Test material: Chromium(III) chloride, Chromium(III) acetate, Chromium(III) nitrate or Chromium(III) sulfate or Chromium(III) potassium sulfate	De Flora, 1990 Easmond, 2008 ATSDR, 2012
***In vivo* test: damage at chromosome level (Chromosome aberrations, micronuclei, SCE's)**			

Non guideline *in vivo* study (micronucleus assay in bone marrow) Mouse BDF1 (males and females) 7-month exposure Oral, Drinking water 140/165mg Cr(III)/kg bw in M/F	Negative No effect on NCE/PCE ratio	Test material: Chromium potassium sulfate dodecahydrate CrK(SO4)2.12H2O	De Flora *et al.*, 2006
Non guideline *in vivo* study (micronucleus assay in bone marrow) Male Slc:ddY mice Intraperitoneous administration Once a day for 2 days 5 mice/group 65.5, 125 mg/kg Maximum dose chosen according to maximal survival dose (data not shown) 1000 PCE scored for MN and MNPCE Positive control mitomycin C Limitations: - No GLP status - No data on purity of test material - Only 2 concentrations tested - No historical control data	No increase in MN	2 (reliable with limitations) Test material chromium(III) chloride hydrated Vehicle: physiological saline	Itoh *et al.*, 1995
Non guideline *in vivo* study: chromatin and DNA binding Male SD rats Ip injection, 80 mg/kg 4, 24 and 40h exposure liver and kidney nuclei Intraperitoneal exposure Limitations: - Unknown number of animals	Negative: DNA crosslinks, DNA-protein crosslinks, DNA strand breaks Chromium(III) chloride enter the liver and kidney at a slower rate than sodium dichromate. No DNA-protein cross-links, DNA interstrand cross link and DNA-protein cross-link. no DNA damage in liver or kidney nuclei (alkaline elution method)	3 (unreliable) Test material: chromium(III) chloride hexahydrate Purity: not stated	Cupo *et al.,* 1985

- No GLP status			

Table 21: Summary of *in vitro* and *in vivo* data on the genoxic potential on soluble chromium complexes (e.g. chromium(III) picolinate)

Method	Type of effect	Remarks	Reference
In vitro test: direct DNA damage			
Non guideline *in vitro* studies (DNA damage in mammalian cells)	positive in two studies DNA SSB in J77A.1 macrophage cells; (Hassoun and Stohs, 1995)	Chromium picolinate, chromium nicotinate	Eastmond, 2008; ATSDR, 2012
Similar to OECD TG Guideline 471 (Bacterial reverse mutation assay) *S. typhimurium* TA98, , TA100, and E. coli WP2 uvrA/pKM101, (rat liver S9: with and without) Test concentrations: 0, 100, 500, 1000, 5000, 10000 µg/plate Limitations: - No historical control data	Negative without and with metabolic activation.	2 (reliable with restriction) Test material: Chromium picolinate monohydrate Purity: >95% (CrVI < 0.025%), same purity as 2-year NTP study Solvent: not reported	NTP, 2010
Similar to OECD TG 471 (Bacterial reverse mutation assay) *S. typhimurium* TA102, TA104, TA100, TA1535, TA97, TA98 (rat and hamster liver S9: with and without) Test concentrations: 0, 100 to 10000 µg/plate Limitations: - No historical control data	Negative without and with metabolic activation.	2 (reliable with restriction) Test material: Chromium picolinate Purity: na Solvent: not reported	NTP, 2010
Similar to OECD TG 471 (Bacterial reverse mutation assay) *S. typhimurium* TA 1535, TA 1537, TA 98 and TA 100, (S9mix rat and hamster : with and without)	Negative without and with metabolic activation (rat or hamster) for chromium chloride, chromium picolinate and picolinic acid.	2 (reliable with restriction) Test material: Chromium(III) chloride, picolinic acid, chromium picolinate	Whittaker *et al.,* 2005

Test concentrations: 0, 100, 333, 1000, 3333, 10000 µg/plate (as chromium chloride, chromium picolinate or picolinic acid) Limitations (no impact expected on the results of the study): - excessive concentration were used (> 5000 µg/plate) - no historical control data; - missing GLP status - only one experiment		Purity: n.a. Solvent: water	
OECD TG 476 (*in vitro* mammalian cell gene mutation) L5178Y TK+/- mouse lymphoma cells (with and without rat metabolic activation) Test concentrations: 0, 50, 150, 500, 1000 µg/mL (+/-S9) Exposure: 4-hour (+/-S9) Duplicate cultures Positive control: methyl methanesulphonate (-S9) and dimethylbenzanthracene (+S9) Limitations: -no GLP status - No statistical analysis was performed - no negative and positive historical controls - only short exposure duration - no confirmatory experiment was performed - only absolute cloning efficiency was provided	<u>Chromium picolinate</u> Positive response with and without S9 mix (>2-fold increase, increase in MF > 126 mutants per 10^6, similar results as positive controls, dose-reponse). <u>Picolonic acid</u> Positive response with S9 at the 2 highest dose (> 2-fold increase compare to negative controls, dose-related reponse, increase in MF < 126 mutants per 10^6) No increase in the number of small colonies. The proportion of small and large colonies remain constant for all compounds Cytotoxicity: Dose selection: maximum 90% reduction in the viability of cells	2 (reliable with restriction) Test material: picolinic acid, chromium picolinate Purity: 99% Vehicle: distilled water	Whittaker *et al.,* 2005
OECD TG 476 (*in vitro* mammalian cell gene mutation)	Cytotoxicity: visible precipitate observed at 500 µg/mL. Relative cloning efficiency : 78% and 68% in the highest tested concentration –S9 or +S9, respectively.	3 (unreliable)	Slesinski *et al.*, 2005

CHO-K1 cells (with and without rat metabolic activation) Test concentrations: 15.6, 31.3, 62.5, 125, 250, and 500 µg/mL (+/-S9) Exposure: 4-hour (+/-S9) or 48-hour (-S9) Duplicate cultures/ independent repeat assay following 4-hour exposure Positive control were used Limitations: - No statistical analysis was performed - no negative and positive historical controls - only short exposure duration - no confirmatory experiment was performed - The use of dimethyl sulfoxide (DMSO) is questionable due to potential scavenging effect	Mutagenicity: negative	Test material: Chromium picolinate Purity: not specified (Cr3+ : 12.18-12.68%) Vehicle: DMSO (rapid precipitation in other solvent including water)	
Non guideline *in vitro* assay (*in vitro* mammalian cell gene mutation) CHO AA8 cells 1mM CrPic in DMSO or 80µg/cm^2 CrPic in acetone 48h exposure Limitation - No GLP status - Only one concentratin tested - Purity not specified - No positive control	Statistically significant increase in mutant frequency in acetone and in DMSO.	3 (unreliable) Test material: Chromium picolinate Purity: not specified Vehicle: DMSO or acetone	Coryell *et al.,* 2006
In vivo test: Damage at chromosomal levels (Micronucleus, SCE, chromosomal aberration)			

OECD TG 474 (*in vivo* Mammalian erythrocyte micronucleus test) B6C3F1 mouse Peripheral blood Oral: feed 10m/10f Exposure: 14 weeks Tested dose: 0, 14/17, 40/50, 370/450, 1775/2300, 9140/11900 mg/kg in m/f, respectively 1000 polychromatic erythrocyte Limitations: - No historical controls - Only 1000 PCE scored	Increased in MN frequency was observed in females at very high dose (> 2000 mg/kg). Negative in males. Proof of exposure: no	2 (reliable with restriction) test material: Chromium picolinate monohydrate Purity: > 96% Vehicle: none	NTP, 2010a
OECD TG 474 (Mammalian erythrocyte micronucleus test) F344/N rat Oral: gavage 5/group Exposure: 3 times at 24-hour intervals Tested dose: 0, 156 to 2500 mg/kg in m/f, respectively 1000 polychromatic erythrocyte Positive control: cyclophosphamide Limitations: - No historical controls - only 1000 PCE scored	Negative Proof of exposure: no	2 (reliable with restriction) test material: Chromium picolinate Purity: > 96% Vehicle: corn oil	NTP, 2010b
In vivo non guideline chromosomal aberration assay Sprague-Dawley rats	Negative in 2 studies	4 (review) Chromium picolinate	Easmond, 2008

***In vivo* test: direct DNA damage**			
Non-guideline *in vivo* Alkaline comet assay 18 female Wistar rats Peripheral blood lymphocytes (10 animals) Electrophoresis: 300mA, 0.56V/cm, 30 min Positive control Cr(VI) : potassium dichromate (10 mg/kg) Dietary exposure: 4 weeks Concentration : 1000 mg Cr(III)/kg bw (equivalent to 100 mg Cr/kg bw) Sacrifice: 12-h following end of exposure period Analysis: 50 comet per slides Limitations: - No GLP status - No historical control range - Only one concentration tested - Missing information on animal selection (18 animals in the study whereas peripheral blood lymphocytes were obtained in 10 animals)	General toxicity: Decreased bw gain in Cr(VI) positive control Comet assay results: negative	*3 (non reliable)* test material: chromium (III) propionate cation Purity: unknown Vehicle: none	Staniek *et al.,* 2009
In vivo non guideline comet and micronucleus test Single ip injection (3 mg/kg) positive control: cyclophosphamide Limitations: - Only one very low dose tested (due to solubility difficulties) - Comet performed 16h following treatment may be too late as damage may have already been repaired	Negative micronucleus No increase in tail moment in the comet assay in peripheral lymphocytes and liver cells 16 hour after single exposure. Positive *in vitro* comet (high concentration 500µM, without serum)	4 (review) Chromium picolinate	Easmond, 2008 (cited Andersson, 2007)

Non guideline *in vivo* DNA damage study 9 male SD rats 4 control, 4 treated with chromium picolinate and one male treated with hexavalent chromium 600 µM chromium picolinate Injection in tail vein	No DNA binding Increase urinary level of 8-oxo-2-desoxyguanidine (8-OHdG) and lipid peroxidation *in vivo*	4 (mechanistic study) Chromium picolinate Vehicle: water	Hepburn *et al.,* 2003

Table 22: human data

Method	Type of effect	Remarks	Reference
Chromosomal aberration in workers Tannery, Iraq Lymphocytes cultures 17 healthy chromium exposed workers 13 controls 100 metaphases examined minimum Chromium concentration in air: 15-47 µg/m^3 Urinary exposure of workers: 0.014µg/100mL and 0.115 µg/L in plasma	No effects No differences in plasma Cr concentration between exposed and unexposed workers	Chromium alum	Hamamy *et al*., 1987
Tannery drum workers lymphocytes	Negative for micronuclei	Trivalent chromium	Migliore *et al.,* 1991
Tannery workers lymphocytes	No effects for chromosomal aberration or micronuclei	Trivalent chromium	Gonzalez, Cid *et al.,* 1991
Tannery workers Lymphocytes biological measures of chromium in blood, urine and plasma Limitations: no athmospheric samplings	Increased DNA–protein-crosslink and micronuclei (< 2-fold) Significant correlation between urine and plasma Cr concentration and DNA-protein-crosslink	Trivalent chromium	Medeiros *et al.,* 2003
Tannery workers	Significant associations between DNA damage and blood and urinary chromium levels were observed; blood chromium levels ranged from 13.10 to 68.30 µg/L (median of 22.95 µg/L) and urinary chromium levels ranged from 1.50 to 42.20 µg/L (median of 10.60 µg/L) in the high-exposure group (tanning place) and 4.30–64.3 µg/L (median of 22.95 µg/L) and 1.50–18.00 µg/L (median of 2.25 µg/L), respectively, in the low-exposure group (finishing place). Short time sampling (15 min) was performed to measure atmospheric concentrations of **total air chromium** (0.054 and 0.016 mg/m^3 in tanning and finishing places resp.). Although it is well known that CrIII is mainly used in tanning industry (chromium sulfate as the basic tanning agent), there is a lack of data on atmospheric chromium species measured for both exposure groups. The data observed cannot be attributed to an exposure to Cr(VI) or Cr(III)	Trivalent chromium	Zhang *et al.,* 2008

Comet assay and oxidative stress analysis Population living near tanning industry, North India 100 exposed and unexposed people	Exposed group showed significantly higher Cr concentration than in the unexposed group. The blood Cr concentration in female population was found to be higher than male population in both groups. Increase DNA damage (comet assay) Increased oxidative stress Correlation with Cr level but not with duration of exposure.	Trivalent chromium	Khan *et al*., 2012
Comet assay and oxidative stress analysis Tannery workers, North India 100 males exposed and 10 healthy males without exposure to Cr	Increased Cr level in blood: Exposed group showed significantly ($p < 0.0001$) higher Cr concentration (tanners: 167.58 + 23.44 mg/l) than in controls (22.09+ 3.78 mg/l). Increase DNA damage (comet assay) Increased oxidative stress Correlation with Cr level (total concentration in blood) and duration of exposure No measure of air concentration	Trivalent chromium	Ambreen *et al.,* 2014

Summary and discussion

Table 23: Summary of WOE attributed to studies available with chromium(III) insoluble in water compounds (Chromium(III) oxide or hydroxide data)

	Negligible/low weight	Moderate weight	High weight
Direct DNA damage			
DNA damage *in bacteria in vitro*	1		
Gene mutation in bacteria *in vitro*	1		
Gene mutation mammalian *in vitro*		1 | 1	
Gene mutation *in vivo*		1	
Damage at chromosomal levels			
Micronuclei or CA *in vitro*			
SCE *in vitro*	1 | 1		
Micronuclei and CA *in vivo*		1 | 1	

Green: negative; orange: equivocal and red: positive results

Table 24: Summary of WOE attributed to studies available with chromium(III) soluble in water compounds (e.g. Chromium(III) chloride)

	Negligible/low weight	Moderate weight	High weight
Direct DNA damage			
DNA damage *in bacteria*	29 | 2		
DNA damage in mammalian cells (including comet)	9 | 5		
Gene mutation in bacteria *in vitro*			25 | 2
Gene mutation mammalian *in vitro*		1 | 1	
Gene mutation *in vivo*			
Damage at chromosomal levels			
Micronuclei and CA *in vitro*		8 | 8	
SCE *in vitro*	21 | 6		
Micronuclei and CA *in vivo*			2

Green: negative; orange: equivocal and red: positive results

Table 25: Summary of WOE attributed to studies available with chromium(III) complexes (e.g. Chromium(III) picolinate)

	Negligible/low weight	Moderate weight	High weight
Direct DNA damage			
DNA damage *in bacteria*			
DNA damage in mammalian cells (including comet)	2		
Gene mutation in bacteria *in vitro*			3
Gene mutation mammalian *in vitro*		1	
Gene mutation *in vivo*		1	
Damage at chromosomal levels			
Micronuclei and CA *in vitro*			
SCE *in vitro*			
Micronuclei and CA *in vivo*			5 1
SCE *in vivo*			

Green: negative; orange: equivocal and red: positive results

Subcellular systems

Cr(III) ions are able to bind to DNA resulting in DNA strand breaks, oxidized DNA bases (8-OHdG). When bound to DNA, Cr(III) is able to interfere with DNA replication. Cr(III) possibly damages DNA if it is able to access intracellular DNA at a sufficient concentration (ATSDR, 2012).

In vitro assays

No Ames assays were available with chromium(III) oxide. Nevertheless, Ames assays were available with insoluble chromium hydroxide, soluble chromium salts and chromium complexes. Concentration up to 10000 µg/plate were tested. All the recommended TG strains were used. Focusing on recommended OECD TG strain, all the assay (around 30 assays) were negatives. Only one test using TA98 with chromium(III) chloride (Langerwerf *et al.,* 1985) was positive. In this study negative results were observed with chromium(III) sulfate. As negative results were observed in acceptable studies up to 10000 µg/plate with this strain and this compound, this single positive result is considered of low concern. Overall, Chromium(III) compounds are considered negative in Ames assays. Nevertheless, as commented by the German CA, it is worth considering the validity of the *in vitro* gene mutation test in bacteria for non-soluble compounds such as chromium(III) oxide. It is known that bacteria cannot internalise particles. According to OECD TG 471, it has to be demonstrated that the concentrations tested do not induce precipitation. As stated in the study report (Unpublished report, 1991), only the two highest concentrations tested with chromium hydroxide exhibited precipitation of the substance, the three lowest did not. The test is therefore considered valid. Nevertheless, being a concern of mutagenicity related to nanoform of chromium(III) oxide, and being the insoluble fraction of the substance not internalised in bacteria, the Ames assay cannot assess the effect under investigation and was classified as negligible/low weight in table 23.

Regarding gene mutation and cytogenicity in mammalian cells, chromium(III) oxide was found to be equivocal in an HPRT assay in V79 chinese hamster cells (Elias *et al*., 1986) as an increase in mutation frequency was observed but no dose-response was observed. Equivocal results were also observed with chromium(III) chloride in a gene mutation assay in mouse lymphoma cells in presence of cytotoxicity (Whittaker *et al.,* 2005). In non-

guideline chromosomal aberration studies, 7 positive tests *vs* 6 negative studies were published with chromium(III) salts.

Overall, there is evidence that chromium(III) compounds are able to induce damage at chromosomal level and gene mutation *in vitro* in particular conditions (e.g. high concentration).

In vivo assays

Two *in vivo* studies were available with chromium(III) oxide: one negative micronucleus study (Unpublished report, 1992) and one positive published study (Singh *et al.,* 2016).

In the positive published study (MN, CA and comet assays), Singh *et al.,* 2006, chromium oxide was tested in forms of microsize particles or nanosize particles. The tests were similar to OECD TG. Rats were administered chromium(III) oxide for 28-day by gavage. A positive result in the micronucleus assay was observed at 1000 mg/kg with Cr(III) oxide microsize particles (MPs) and from 300 mg/kg with synthetised Cr(III) oxide nanosize particles (NPs). Positive results at the same dose levels were also observed in this study in an *in vivo* comet assay and in an chromosomal aberration assay with MPs and NPs. It may be noted that at these dose levels, severe toxicity was observed as loss weight and moribund symptoms were noted by the authors. In this study higher concentrations of Cr were measured in tissues rats treated with NPs compared to rats treated with MPs. The major limitation of the study is the **absence of information on the purity of Chromium(III**). Indeed, the presence of chromium(VI) as an impurity in the tested batch could impact the results. In addition, there were an inconsistency between the age of animals and the weight of animals at the beginning of the study. Therefore, the animals may have been underweighted or in bad health condition. In addition, the negative control range was very high compare to expected range for comet assay. This leads also to uncertainties on the validity of the study. Therefore, a potential concern have been identified for Chromium(III) oxide containing a fraction of nanoparticles. Nevertheless, additional data would be needed to confirm the results provided by Singh *et al.,* 2006. Due to the above uncertainties, this in vivo study was considered of moderate weight rather than high weight in table 23.

In the study report (1992), the *in vivo* micronucleus study with chromium(III) oxide gave negative results in male and female NRMI mice following a single intraperitoneal administration (10 g/kg). The ratio of polychromatic to normochromatic erythrocyte was markedly decreased at this excessive dose. As stated by the Health risk assessment report on chromium published in 2006 (Riihimäki and Luotamo*,* 2006) "the decrease indicates a cytotoxic effect on bone marrow which hamper the detection of a positive effects". No information on Cr(VI) content and particle size distribution was available in the study report. Moreover, as chromium(III) may possibly accumulate in the cells during long-term exposure leading to increase Cr(III) in blood (Riihimäki and Luotamo, 2006), a single administration of the test material may be insufficient. Thus, although negative results were obtained, there are some uncertainties on the results. Due to these uncertainties, this in vivo study was considered of moderate weight rather than of high weight in table 23.

Micronucleus assays with Chromium(III) salts or chromium(III) complexes gave, in the majority of the studies, negative results. *In vivo* Micronucleus studies performed according to OECD TG and rated Klimish score 2 are detailed in the table below: chromium(III) oxide (Internal report, 1992), chromium(III) picolinate monohydrate in mice (NTP, 2010a) and with chromium(III) picolinate in rats (NTP, 2010b).

Table 26: Overview of the four available micronucleus studies performed according to OECD TG and scored Klimish 2

Study	Study report, 1992	Singh *et al.,* 2016		NTP, 2010b	NTP, 2010a
Test material	Cr2O3	Cr2O3	Cr2O3	Chromium picolinate	Chromium picolinate, monohydrate
Cr(VI)	na	na	na	Not expected	Not expected

Form (mean size of particles)	Solid (No information on size)	Solid (34.89± 2.65 nm)	Solid (3.76 ± 3.41 µm)	Liquid	Liquid
Purity	99,8%	≥ 98,4%	≥ 98%	> 96%	> 96%
Species (no of animals)	NMRI mice (5M+5F)	Wistar rats (5M+5F)		F344/N rats (5M)	B6C3F1 mice (5M+5F)
Vehicule	Corn oil	Water + ultrasonication		Corn oil	None
Method	Single IP	28-day gavage		3-day gavage	90-day diet
Dose	3283 mg Cr(III)/kg	9.9, 99, 329 mg Cr(III)/kg		19-310 mg Cr(III)/kg	2- 1500 mg Cr(III)/kg
Proof of exposure	Excessive bone marrow cytotoxicity	↑*Cr(III) in blood	↑*Cr(III) in blood	No information	Cr(III) found in plasma
Results	Negative	positive M/F at mid and high dose	Positive M/F Top dose	Negative	Males: negative Females: equivocal (↑1500 mg/kg)

na: not available; * dose converted to Cr(III) using molecular weight correction

The *in vivo* micronucleus performed in rats using chomium picolinate gave negative results at 2500 mg/kg chromium picolinate. The test material was administered 3-times at 24-hour intervals by gavage.

In the 90-day feeding experiment, chromium picolinate monohydrate did not increase micronuclei in male mice, but for females, an increase in micronuclei was noted at the highest dose of 11900 mg/kg chromium picolinate monohydrate and a positive trend was noted. The result obtained in female mice was thus considered equivocal.

The following studies were also found positive *in vivo*:

- Increased deletion mutations with chromium picolinate (Kirpnick-Sobol *et al.*, 2006);
- increased 8-OHdG in SD rats with chromium picolinate (Hepburn, 2003);

Nevertheless, the study design used in these studies was not robust and reliable.

In conclusion, taken together as a weight-of-evidence of all available information, *in vivo* data on complexes were mostly negative for Cr(III) compounds.

Further information is needed on the gene mutation and clastogenic potential of the test material containing nanoparticles of chromium(III) oxide. Nevertheless, Chromium(III) oxide is currently not registered as nanoparticles as the boundary composition of chromium(III) oxide is less that 50%. Moreover, the positive results performed by Singh *et al.,* 2016 were of limited value due to the major uncertainty on Chromium(VI) content. Therefore, there is currently insufficient concern to request a new *in vivo* comet assay to clarify this concern.

Human data

Because the exposure may not have been specific to chromium(III) compounds, the human studies cannot be used for the assessment of the genotoxic potential of the substance.

Overall, a concern has been identified on nanoforms of chromium(III) and further reseach would be need to confirm the concern. Nevertheless, based on the mostly negative assays with chromium(III) soluble compounds, the available data do not allow to classify the substance as germ cell mutagen.

7.9.7. Carcinogenicity

Table 27: Summary of carcinogenicity studies using oral route

Method	Results	Remarks	Reference
OECD TG 451 (carcinogenicity study) Rat (F344/N) Oral: diet 50/sex/group 105-week 0, 2000, 10000, 50000 ppm (eq to 90/100, 460/510, 2400/2630 mg/kg in males/females, respectively) Limitations: - Highest dose level is not the MDT	No effects on bw and survival ↑* preputial gland adenomas in males at 10000 ppm > Historical control (1/50, 1/50, 7/50, 4/50 at 0, 2000, 10000 and 50000 ppm respectively)	2 (reliable with restriction) Test material: Chromium picolinate monohydrate Purity: > 96%	NTP, 2010 Stout *et al.,* 2009
OECD TG 451 (carcinogenicity study) Mice (B6C3F1) Oral: diet 50/sex/group 105-week 0, 2000, 10000, 50000 ppm (eq to 250/240, 1200/1200, 6565/6100 mg/kg in males/females, respectively) Limitations: Highest dose level is not the maximum tolerable dose (MTD)	No effect on survival Decreased bw in females (10% less than control following one year, similar following 2 year) No neoplastic findings	2 (reliable with restriction) Test material: Chromium picolinate monohydrate Purity: > 96%	NTP, 2010 Stout *et al*., 2009
Non guideline carcinogenicity study BD bred rats 60 males and females Oral: diet 5d/w, 2-year 1, 2 or 5% Cr_2O_3 Limitations: - Low number of animals - No GLP status - Examined organs are not listed (brain and nervous systems were not examined) - Only histopathology was investigated in the study	No effect on survival or body weight No increase in neoplastic findings	2 (reliable with limitation) Test material: non hydrated pure chromium oxide Purity: not available but free from chromate (< Limit of detection 1µg $CrO4^{2-}$) Vehicle: test material was baked into bread	Ivankovic *et al.*, 1975

Table 28 Summary of carcinogenicity studies_inhalation

Method	Results	Remarks	Reference
Non guideline carcinogenicity study C54BL/6 mice Inhalation (whole body) of dust 5.5h/d, 5d/week 6, 12 or 18-month 15 mice/group 0, 17 mg/m^3 air (50 ppm) Histopathological investigation only Pre-treatment: X-ray or virus or no pre-treatment Positive control: gasoline fumes Negative control: air and non chamber experiment Limitations: - no GLP status - Low number of animals - no details on general health of the animals - List of examined organs not provided, brain was not examined	No effects on lung tumour incidence. No additive or potentiating effects following pretreatment with virus or X-ray	3 (unreliable) Test material: Cr_2O_3 Purity: not stated, bulk particle size of 0.85µ Vehicle: none	Nettesheim, 1970
Non guideline carcinogenicity study Bethesda black rats Inhalation: dust (whole body) 5-6h/d, 4d/week for 24 months Dose concentration not reported 72 rats/sex	Twenty six rats survived to 24 months. Histopathology revealed bronchiectasis, inflammation and hyperplasia of the bronchial epithelium. No lung tumours were detected.	3 (unreliable) Test material: Cr_2O_3 Vehicle: none	IARC, 1990 (cited Hueper, 1961)

Table 29: Summary of carcinogenicity studies_other route

Method	Results	Remarks	Reference
Non guideline carcinogenicity study Wistar rats 10, 20, 50 mg chromic oxide Injection into the trachea, pleural interstitial space or the abdominal cavity Limitations: - poor reporting of the methods and the results - Use of a control not reported	Malignant tumours (observed at 11-12 month): At 20 mg: 7/34 (including 4 lung sarcoma) At 50 mg: 6/ 18 animals (including 5 lung sarcomas) Local tumours including reticulosarcomas were seen at the injection sites in 13.5% of the treated animals.	4 (secondary literature) 4 (secondary literature, article not in english) Test material: Cr_2O_3 Vehicle: air	IARC, 1990 (cited Dvizhkov & Fedorova, 1967)
Non guideline carcinogenicity study Rats Intrabronchial implantation of pellets	No lung tumours were observed in rats administered chromium (III) oxide. In contrast, tumours were observed in animals treated with some chromium (VI) compounds.	4 (secondary literature) Test material: Cr_2O_3	IARC, 1990 (cited Laskin *et al.*, 1970)

100 treated rats+ 24 controls 3-5 mg in a 50:50 mixture of chromic oxide with a chrolesterol binder 136 week exposure		Vehicle: cholesterol	
Non guideline carcinogenicity study Rats Intrabronchial pellets (implantation) 2 mg 48 males + 52 females	Survival to 24 months exceeded 90% in both groups. Examination of the treated bronchus revealed squamous epithelial metaplasia in 11% of controls and 6% of the test animals. No evidence of carcinogenicity was seen. Positive results obtained with Cr(VI)	4 (secondary literature) Test material: Cr_2O_3 (99-100% pure) Vehicle: cholesterol	IARC, 1990 (cited Levy, 1986)

Table 30: Summary of other studies

Method	Results	Remarks	Reference
Non guideline *in vitro* cell transformation mouse or hamster cells	Anchorage independent test : Negative in two test (Bianchi, 1983; Hansen, 1985) Morphological transformation: positive in one test (Raffetto, 1977)	4 (review) Test material: Cr_2O_3	De Flora, 1990
Non guideline *in vitro* cell transformation assay Syrian hamster BHK fibroblasts Soft agar assay 100-1600 µg/ml	Negative (Alias, 1984 reported positive results in Syrian hamster embryo primary cells, abstract only, concentration not reported)	4 (review) Test material: Cr_2O_3	De Flora, 1990 (citing Hanse and stern, 1985)
Non guideline *in vitro* cell transformation assay Human fibroblasts Anchorage independance 10 dishes /group 48h exposure Examination 12-day following treatment No positive control but Cr(VI) compounds were positive	Positive, dose-related increase in Anchorage independence 1000-fold higher concentration than Cr(VI) concentration	4 (mechanistic studies) Test material: Cr_2O_3 Purity: > 99.955%	Biederman *et al.,* 1990
Hypomethylation in sperm with in mice.	Positive	4 (secondary litterature) chromium chloride	Esmond *et al.,* 2008 (cited Shiao, 2005)
Non guideline transgenerational carcinogenicity study 5 male mice Single ip injection 52 mg Cr^{3+}/kg After 2-week period, treated males are mated with untreated females Sacrifice: 20-week Analyse: lung tumour incidence	No signs of toxicity in treated males Increase number of lung tumours in offspring of chromium exposed males (8 offsprings/119 in 6 litters vs 1/144 in controls)	4 (secondary literature) Chromium chloride hexahydrate	Riimaki and Marita, 2006 (cited Anderson *et al.,* 1994)
Non guideline transgenerational carcinogenicity study	Increased in adrenal, thyroid and harderian gland tumours	4 (secondary literature)	Riimaki and Marita, 2006

Single ip injection 52 mg Cr^{3+}/kg Male rats	and reproductive organ tumours in offsprings	Chromium chloride hexahydrate	(cited Yu *et al.,* 1999)

Summary and discussion on carcinogenicity

Following oral route of exposure, chromium(III) picolinate was not carcinogenic in rats and mice (NTP, 2010). Chromium(III) oxide had also no carcinogenic potential in a 2-year oral study in BD rats (Ivankovic and Preusmann, 1974). The authors provided no information on the particle size of the substance tested in the study.

No reliable data are available with chromium(III) oxide by inhalation.

7.9.8. Toxicity to reproduction (effects on fertility and developmental toxicity)

Table 31: Summary of toxicity studies on fertility and sexual organ toxicity

Method	Type of effect	Remarks	Reference
2-generation study SD rats Oral: diet 30/dose/sex Doses / Concentrations: 0, 4, 15 and 60 ppm (ca. 0, 0.5, 2 and 8 mg/kg bw/d chromium nicotinate F0 parental generation received feed containing NBC for a period of 10 weeks before mating, throughout mating until their termination; F1: received feed containing NBC for a period of 10 weeks before mating, throughout mating until their termination After mating males were killed and were subject to necropsy, all females were killed after weaninig. One male and female pup (F1) from each litter were selected for second-generation parents (F1). Weaned pups (F1) not selected for second generation were killed on lactation day 21. Second generation (F2): all weaned pups (F2a) were killed. One male and female pup from each litter (F2b) were selected for teratology study. Limitations: - Weight of the animals at the beginning of the study was not provided - Highest dose was chosen to be 100 times the maximum recommended dose in human. MTD was not reached.	No effects observed on bw, food consumption and clinical signs No reproductive or developmental toxicity The only finding was a statistically significant increase in brain weight at ≥ 4 ppm in F2a males. No correlated histopathological changes. NOAEL ≥ 8 mg/kg chromium nicotine	2 (reliable with limitations) Test material: ChromeMate CM-100 M powder (chromium nicotinate) Vehicle: none	Deshmukh *et al.,* 2009
Non guideline study on Spermatogenesis in mouse BALB/C Swiss mice Oral: feed 35-day treatment	Reduced sperm count Increased number of morphologically abnormal sperms and degeneration of the outer cellular layer of the seminiferous tubules	3 (unreliable) Test material: chromium di sulfate powder	Zahid *et al.,* 1990

15, 30, 60 chromium(III) mg/ kg bw 7 (6?, 11?) animals/group Oral: diet Limitations - Amount of substance in feed was not analytically verified - no information on GLP status - Contradictory information on treatment period, i.e. 7 weeks vs. 35 days - Animal number per group too low, conflicting summaries of the actual group sizes throughout the report - Selection of dose levels not justified - Very limited examination of the animals during study conduct and termination, i.e. missing information on clinical signs, mortality, gross pathology, accessory sex organs not sufficiently examined - individual data or historical control were not provided by the authors		Purity: no information	
Non guideline fertility study in male rats Male Sprague-dawley rats Oral: drinking water 12-weeks 100 ppm equivalent to 24 mg (Cr(III)/kg bw) 10 males Limitations: - low number of animals per groups - No information on GLP status - animal examination not reported, i.e. confirmation and timepoint of mating/pregnancy unknown - Actual water consumption was apparently not monitored, thus the actual dose received cannot be determined - Accessory sex organs were not examined, neither during necropsy nor via histopathology - Single dose administration does not allow to derive a dose-response relationship	Decreased body weight (30%), absolute weight of testes, seminal vesicles and preputial glands	3 (unreliable) Chromium chloride Purity: not available	Bataineh, 1997

Non guideline study on effect of long-term ingestion of chromium compounds on agression, sex behavior and fertility <u>Exp I:</u> Male swiss mice 82, 204 mg/kg Cr(III) 12-week exposure prior to mating Number of animals: 20 in controls, 9-10 in treated groups <u>Exp II:</u> Female swiss mice 12-week exposure 85, 212 mg Cr(III)/kg bw Number of animals: 18 in controls, 12-14 in treated groups Sacrifice: 140 days Limitations: - Number of animals per group unequally distributed - no information on GLP status - animal number per group too low, which significantly reduces the statistical power - animal examination not reported, i.e. confirmation and timepoint of mating/pregnancy unknown - contradictory dose levels reported - reduced water consumption was mentioned by authors, but without specifying how far this influenced the dose - actual water consumption was apparently not monitored, thus the actual dose received cannot be determined - accessory sex organs were not examined, neither during necropsy nor via histopathology	No mortality or clinical signs observed in the study Exp I Significant decreased in bw No histopathological changes Decreased preputial gland weight, increase testis weight Decreased fertility at 204 mg/kg ExpII Decreased uterus and ovary weight Decreased number of implantation and viable foetuses Decreased pregnancy at 212 mg/kg	3 (unreliable) Test material: chromium chloride Purity: no information	Elbetieha and Al-Hamood, 1997
Non guideline testicular toxicity study Male Wistar rats 8 males/group Intraperitoneal injection 5-day treatment 1, 2, 4 mg/kg Cr(III) Sacrifice: 7 and 60 days after administration.	No clinical signs observed in the study; No changes observed with trivalent chromium (relative testicular weight, testicular histopathology, epididymal sperm number).	3 (unreliable) Test material: chromium chloride or hexavalent chromium Vehicle: saline	Ernst *et al.,* 1990

Limitations: - no data on purity of test material - No information on GLP status - low number of animals - justification of the dose not provided - insufficient level of details in study results and method			
Non guideline study on effects of chromium on aggressiveness and fertility Tuck ordinary male mice Oral: drinking water 1000, 5000 ppm chromium chloride (aggression test) 5000 ppm (fertility) 8 treated male + 16 females/group 90-day exposure Agression tests, fertility and body and male reproductive organ weight were determined Limitations: - Actual dose not provided; - Drinking water and food consumption of the animals not reported; - Justification of the dose used in the study not provided; - No information on GLP status; - Low number of animals; - Individual data not provided; - No historical control data;	No statistically significant changes in body weight, testis, preputial gland or seminal vesicle weight. Male mice exposed to chromium chloride (at 1000 or 5000 ppm) significantly augmented social aggression. The fertility of male mice exposed to chromium chloride was unaffected as compared to control group.	3 (unreliable) Test material: chromium chloride Vehicle: water	Hussain *et al.,* 2000

Table 32: Summary of developmental toxicity studies

Method	Type of effect	Remarks	Reference
Placental transfer Pregnant rats	No radioactivity into the litter	4 (study not available) Chromium chloride	Riimaki and Marita, 2006 (quoted Mertz, 1969)
Non guideline developmental toxicity study in mice CD-1 mouse Oral: diet Gestation day (GD)6-17 Sacrifice: GD17 0, 200 mg/kg chromium picolinate (equivalent to 25 mg Cr/kg bw), 174 mg/kg picolinic acid, 200 mg/kg chromium chloride (eq. to 39 mg Cr/ kg bw) Examination: resorption, dead and live foetuses, weight, gross and skeletal anomalies. Limitations: - GLP status not stated - Only one concentration per test material - The highest dose was not the MTD - Data obtained in the preliminary study not shown - Only gross malformation and skeletal examination were performed - Low number of litter/fetuses examined following chromium chloride treatment (136 foetuses, 14 litters) - Number of animals used in the study not stated - No contextual information on the historical control of the laboratory (date, number of studies, number of animals, study protocol…)	*Preliminary study* Increased in cervical arch defect with Chromium picolinate at 200 mg/kg *Main study* **Maternal toxicity** : none (bw; food consumption, clinical signs) **Developmental toxicity** Statistically significant increase in bifurcated cervical arch in Chromium picolinate dose group outside historical control of the laboratory. No effects with chromium chloride. This defect was also increased in picolinic acid dose group but the increase was not statisticaly significant.	3 (unreliable) Test material: Chromium picolinate, chromium chloride, picolinic acid Purity: not stated Vehicle: none	Bailey *et al.,* 2006
Non guideline developmental toxicity study in mice CD-1 mouse Oral: diet	**Maternal toxicity** No effect on maternal weight gain or food consumption. No signs of maternal toxicity.	3 (unreliable)	Bailey *et al.*, 2008

Exposure: GD 6-17 Sacrifice: GD17 0, 15, 120 mg/kg Cr3 (eq. to 0, 3.3, 25 mg Cr/kg bw) or 200 mg/kg chromium picolinate (eq. to 26 mg Cr/kg/d) Examination: resorption, dead and live foetuses, weight, gross and skeletal anomalies. Chromium potassium sulfate content in the diet : 0.48 mg/kg of diet. Limitations: - GLP status no specified - Purity not specified - Only one concentration for chromium picolinate and on only two concentrations for Cr3. The highest dose was not the MTD. - No data on picolinic acid - Number of animals/dose not stated - Only gross malformation and skeletal examination were performed - Majority of the data not shown	**Developmental toxicity** None No effect on cervical arch defects (4.65% in control, 6.26% in Cr(pic)3, 5.18% at 15 mg/kg Cr3, 3.98% at 120 mg/kg Cr3). Control outside historical control of the laboratory: 0-2.09%.	Test material: Chromium picolinate, Cr3, picolinic acid Purity: not stated Vehicle: none	
In vitro embryotoxicity test Mouse ES cell line D3 Mouse BALB/3T3 clone A31 fibroblast cell line Limitations: - No information on purity - No valid positive control - No negative control	Cr(III) was non embryotoxic whereas Cr(VI) was classified as strongly embryotoxic. MeHg was classified as non-embryotoxic but was considered embryotoxic following refinement of the classification criteria.	3 (unreliable) Test material: chromium chloride hexahydrate Purity: no information	Stummann, 2007
Teratogenicity study as part of a 2-generation toxicity study	**See table above**	See above	Deshmukh, 2009

Non guideline developmental toxicity study Wistar rats Oral diet 20 male + 20 females GD0-GD21 Concentration of Cr in diet: 0.020 or 7.2 mg Cr/kg bw Examination: blood, uteri, live foetuses, weight of foetuses and gross examination. Limitations: - Purity was not provided - GLP status not provided - Low number of dams - Only one low dose tested	**Dams** No effect on feed intake and body mass Increased liver and kidney Cr levels (177% and 455% compare to control) and decreased Cu and Zn by 9 and 12% respectively. Decreased total protein concentration No effects on Fe, glucose, cholesterol, TAG, ALT, AST, creatinine and urea concentration **Foetuses** No gross abnormalities in organ morphology (heart, liver, kidney, lungs)	2 (reliable with limitations) Test material: Chromium(III) propionate cation in form of nitrate salt Purity: no information Vehicle: none	Staniek, 2009

Discussion and conclusion

No screening reproduction toxicity study or EOGRTS were available with chromium(III) oxide. With soluble salts, several non-guideline studies were available on fertility but the studies were not considered reliable. With chromium complex, no effects were observed in a 2-generation study. Nevertheless, as only low doses were studied, the potential hazard related to chromium(III) has not been sufficiently investigated. An EOGRTS is recommended by the eMSCA to be requested under CCH on a representative substance of chromium(III) compounds.

No developmental toxicity study is available with chromium(III) oxide or salts. One developmental toxicity study is available with chromium(III) propionate. Nevertheless, in view of the low dose tested, the potential hazard related to chromium(III) has not been sufficiently investigated. A developmental toxicity study is recommended by the eMSCA to be requested by ECHA under CCH on a representative substance of chromium(III) compounds.

7.9.9. Hazard assessment of physico-chemical properties

No hazard identified.

7.9.10. Selection of the critical DNEL(s)/DMEL(s) and/or qualitative/semi-quantitative descriptors for critical health effects

No DNEL was proposed in the dossier as the substance is not classified by the lead registrants. No exposure scenarios were thus provided. A classification for skin sensitisation and repeated toxicity is however considered justified. In particular, in view of the severe local toxicity induced by chromium oxide by inhalation, a local long-term DNEL would be necessary. It is recommended that the lead registrant build a local long-term DNEL.

7.9.11. Conclusions of the human health hazard assessment and related classification and labelling

Cr(III) is intrinsically considered as a skin sensitiser. As discussed in section 7.9.4, chromium(III) oxide should be considered as a skin sensitiser and should be classified as Skin Sens. 1, H317. In addition, a classification as STOT RE for pulmonary effect is also warranted.

7.10. Assessment of endocrine disrupting (ED) properties

ED properties were not assessed due to insufficient data on the Substance and therefore unable to conclude. However, an EOGRTS study and a prenatal developmental study are recommended by the eMSCA to be requested under CCH if initiated.

7.11. PBT and VPVB assessment

Not assessed.

7.12. Exposure assessment

7.12.1. Human health

According to the information submitted in all the registration dossiers, the aggregated tonnage of the substance manufactured or imported in the EU is more than 10,000 tonnes per year.

Three main industrial application and uses are identified in the registration dossiers: manufacture of pigments, catalyst and refractories materials. In addition, the use of the articles/products by workers and consumers is described (article service life).

During manufacture of chromium(III) oxide, process categories (PROC), PROC 4 "Chemical production where opportunity for exposure arises" and PROC 5 "mixing of blending in batch process" were identified. Therefore worker exposure may arise from this use.

Chromium(III) oxide pigments are produced and used at industrial site in paints, plastics, building products, artist colours, ceramics, glass, etc.

Chromium(III) oxide pigments are also used in finger paints, cosmetic and personal care products. PROC 4 and 5 are also identified in the pigments formulation and manufacture at industrial sites. According to Riihimäki and Luotamo, (2006), exposure to chromium(III) oxide during these uses can occur during weighing the pigment, mixing and milling as well as packing. Therefore worker exposure may arise from this use.

Chromium(III) oxide is also used as a starting material (intermediate) for catalyst manufacture. The most common use is in high shift reactions in the petroleum industry. PROC 0 is proposed (PROC0: other) for this scenario in the registered dossier. According to Riihimäki and Luotamo, (2006), the process is a continuous semi-automated closed process and most of the work-tasks involve low exposure levels to chromium(III) oxide.

Chromium(III) oxide is also used as a refractory material in high temperature and corrosive environments in many industries. PROC 0 is also proposed (PROC0: other) for this scenario. According to Riihimäki and Luotamo, (2006), during this operation, workers could be exposed to fairly high dust levels of chromium(III) oxide.

Therefore, exposure of workers cannot be excluded.

As described in the CSR, consumers could be exposed to chromium(III) oxide when they use stone, plaster, cement, glass, ceramic articles, brake, linings, enamel, cosmetics, household products. The substance is not intended to be released from any of these articles. Indeed, according to the registrant, the substance is bound in material and/or articles. Therefore, the exposure of consumers is expected to be low from most of the products/articles. Nevertheless, eMSCA notes that some products/articles (e.g. cosmetic products, metal products), for which migration and possibility of prolonged skin contact are relevant, are used by consumers. Therefore, eMSCA considers that exposure to consumers cannot be excluded.

Nevertheless, although potential worker or consumer exposure was identified by the registrant of the substance no exposure scenario were provided in the CSR as the substance was not classified by the lead registrant.

As the substance warrant to be classify STOT RE 2 (H373) and Skin Sens. 1 (H317), FR-MSCA is of the opinion that respective exposure scenario should be provided.

7.12.2. Environment

Not assessed.

7.13. Risk characterisation

Not assessed.

7.14. References

Adam, C., Wohlfarth, J., Haußmann, M., Sennefelder, H., Rodin, A., Maler, M., Martin, S.F., Goebeler, M., Schmidt, M., 2017. Allergy-Inducing Chromium Compounds Trigger Potent Innate Immune Stimulation Via ROS-Dependent Inflammasome Activation. J. Invest. Dermatol. 137, 367–376. https://doi.org/10.1016/j.jid.2016.10.003

Allenby, C.F., Goodwin, B.F., 1983. Influence of detergent washing powders on minimal eliciting patch test concentrations of nickel and chromium. Contact Derm. 9, 491–499.

https://doi.org/10.1111/j.1600-0536.1983.tb04470.x

Ambreen, K., Khan, F.H., Bhadauria, S., Kumar, S., 2014. Genotoxicity and oxidative stress in chromium-exposed tannery workers in North India. Toxicol Ind Health 30, 405–414. https://doi.org/10.1177/0748233712457447

Anderson, R.A., Bryden, N.A., Polansky, M.M., 1997. Lack of toxicity of chromium chloride and chromium picolinate in rats. J Am Coll Nutr 16, 273–279.

Andersson, M.A., Petersson Grawé, K.V., Karlsson, O.M., Abramsson-Zetterberg, L.A.G., Hellman, B.E., 2007. Evaluation of the potential genotoxicity of chromium picolinate in mammalian cells in vivo and in vitro. Food and Chemical Toxicology 45, 1097–1106. https://doi.org/10.1016/j.fct.2006.11.008

Bagchi, D., Hassoun, E.A., Bagchi, M., Stohs, S.J., 1995. Chromium-induced excretion of urinary lipid metabolites, DNA damage, nitric oxide production, and generation of reactive oxygen species in Sprague-Dawley rats. Comp. Biochem. Physiol. C, Pharmacol. Toxicol. Endocrinol. 110, 177–187. https://doi.org/10.1016/0742-8413(94)00093-p

Bailey, M.M., Sturdivant, J., Jernigan, P.L., Townsend, M.B., Bushman, J., Ankareddi, I., Rasco, J.F., Hood, R.D., Vincent, J.B., 2008a. Comparison of the potential for developmental toxicity of prenatal exposure to two dietary chromium supplements, chromium picolinate and [Cr3O(O2CCH2CH3)6(H2O)3]+, in mice. Birth Defect Res B 83, 27–31. https://doi.org/10.1002/bdrb.20140

Bailey, M.M., Sturdivant, J., Jernigan, P.L., Townsend, M.B., Bushman, J., Ankareddi, I., Rasco, J.F., Hood, R.D., Vincent, J.B., 2008b. Comparison of the potential for developmental toxicity of prenatal exposure to two dietary chromium supplements, chromium picolinate and [Cr3O(O2CCH2CH3)6(H2O)3]+, in mice. Birth Defect Res B 83, 27–31. https://doi.org/10.1002/bdrb.20140

Bataineh, H., Al-Hamood, M.H., Elbetieha, A., Hani, I.B., 1997. Effect of Long-Term Ingestion of Chromium Compounds on Aggression, Sex Behavior and Fertility in Adult Male Rat. Drug and Chemical Toxicology 20, 133–149. https://doi.org/10.3109/01480549709003875

Bianchi, V., Celotti, L., Lanfranchi, G., Majone, F., Marin, G., Montaldi, A., Sponza, G., Tamino, G., Venier, P., Zantedeschi, A., Levis, A.G., 1983. Genetic effects of chromium compounds. Mutation Research/Genetic Toxicology 117, 279–300. https://doi.org/10.1016/0165-1218(83)90128-3

Bianchi, V., Zantedeschi, A., Montaldi, A., Majone, F., 1984. Trivalent chromium is neither cytotoxic nor mutagenic in permeabilized hamster fibroblasts. Toxicology Letters 23, 51–59. https://doi.org/10.1016/0378-4274(84)90009-2

Biedermann, K.A., Landolph, J.R., 1990. Role of valence state and solubility of chromium compounds on induction of cytotoxicity, mutagenesis, and anchorage independence in diploid human fibroblasts. Cancer Res. 50, 7835–7842.

Blasiak, J., Kowalik, J., 2000. A comparison of the in vitro genotoxicity of tri- and hexavalent chromium. Mutat. Res. 469, 135–145. https://doi.org/10.1016/s1383-5718(00)00065-6

Bregnbak, D., Thyssen, J.P., Jellesen, M.S., Zachariae, C., Johansen, J.D., 2017. Experimental patch testing with chromium-coated materials: PATCH TESTING WITH CHROMIUM-COATED MATERIALS. Contact Dermatitis 76, 333–341. https://doi.org/10.1111/cod.12747

Buters, J., Biedermann, T., 2017. Chromium(VI) Contact Dermatitis: Getting Closer to Understanding the Underlying Mechanisms of Toxicity and Sensitization! J. Invest. Dermatol. 137, 274–277. https://doi.org/10.1016/j.jid.2016.11.015

Coryell, V.H., Stearns, D.M., 2006. Molecular analysis of hprt mutations induced by chromium picolinate in CHO AA8 cells. Mutation Research/Genetic Toxicology and Environmental Mutagenesis 610, 114–123. https://doi.org/10.1016/j.mrgentox.2006.06.020

Cupo, D.Y., Wetterhahn, K.E., 1985. Binding of chromium to chromatin and DNA from liver

and kidney of rats treated with sodium dichromate and chromium(III) chloride in vivo. Cancer Res. 45, 1146–1151.

Danish competent authoriry for REACh, 2012. Annex XV report. Proposal for a restriction : chromium (VI) compounds.

David R. Lide, ed., CRC Handbook of Chemistry and Physics, Internet Version 2005, <http://www.hbcpnetbase.com>, CRC Press, Boca

De Flora, S., Bagnasco, M., Serra, D., Zanacchi, P., 1990. Genotoxicity of chromium compounds. A review. Mutation Research/Reviews in Genetic Toxicology 238, 99–172. https://doi.org/10.1016/0165-1110(90)90007-X

De Flora, S., Iltcheva, M., Balansky, R.M., 2006. Oral chromium(VI) does not affect the frequency of micronuclei in hematopoietic cells of adult mice and of transplacentally exposed fetuses. Mutation Research/Genetic Toxicology and Environmental Mutagenesis 610, 38–47. https://doi.org/10.1016/j.mrgentox.2006.06.011

Derelanko, M.J., Rinehart, W.E., Hilaski, R.J., Thompson, R.B., Löser, E., 1999. Thirteen-week subchronic rat inhalation toxicity study with a recovery phase of trivalent chromium compounds, chromic oxide, and basic chromium sulfate. Toxicol. Sci. 52, 278–288. https://doi.org/10.1093/toxsci/52.2.278

Deshmukh, N.S., Bagchi, M., Lau, F.C., Bagchi, D., 2009. Safety of a novel oxygen-coordinated niacin-bound chromium(III) complex (NBC): I. Two-generation reproduction toxicity study. Journal of Inorganic Biochemistry 103, 1748–1754. https://doi.org/10.1016/j.jinorgbio.2009.07.014

Eastmond, D.A., MacGregor, J.T., Slesinski, R.S., 2008. Trivalent Chromium: Assessing the Genotoxic Risk of an Essential Trace Element and Widely Used Human and Animal Nutritional Supplement. Critical Reviews in Toxicology 38, 173–190. https://doi.org/10.1080/10408440701845401

Elbetieha, A., Al-Hamood, M.H., 1997. Long-term exposure of male and female mice to trivalent and hexavalent chromium compounds: effect on fertility. Toxicology 116, 39–47. https://doi.org/10.1016/S0300-483X(96)03516-0

Elias, Z., Poirot, O., Schneider, O., Danière, M.C., Terzetti, F., Guedenet, J.C., Cavelier, C., 1986. Cellular uptake, cytotoxic and mutagenic effects of insoluble chromic oxide in V79 Chinese hamster cells. Mutation Research/Genetic Toxicology 169, 159–170. https://doi.org/10.1016/0165-1218(86)90095-9

Elias, Z., Schneider, O., Aubry, F., Daniere, M.C., Poirot, O., 1983. Sister chromatid exchanges in Chinese hamster V79 cells treated with the trivalent chromium compounds chromic chloride and chromic oxide. Carcinogenesis 4, 605–611. https://doi.org/10.1093/carcin/4.5.605

El-Yamani, N., Zúñiga, L., Stoyanova, E., Creus, A., Marcos, R., 2011. Chromium-Induced Genotoxicity and Interference in Human Lymphoblastoid Cell (TK6) Repair Processes. Journal of Toxicology and Environmental Health, Part A 74, 1030–1039. https://doi.org/10.1080/15287394.2011.582282

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. https://doi.org/10.1016/0378-4274(90)90069-x

Estlander, T., Jolanki, R., Kanerva, L., 2000. Occupational allergic contact dermatitis from trivalent chromium in leather tanning. Contact Derm. 43, 114.

Fang, Z., Zhao, M., Zhen, H., Chen, L., Shi, P., Huang, Z., 2014. Genotoxicity of Tri- and Hexavalent Chromium Compounds In Vivo and Their Modes of Action on DNA Damage In Vitro. PLoS ONE 9, e103194. https://doi.org/10.1371/journal.pone.0103194

Fatima, R., Ahmad, R., 2019. Hepatotoxicity and chromosomal abnormalities evaluation due to single and repeated oral exposures of chromium oxide nanoparticles in Wistar rats. Toxicol Ind Health 35, 548–557. https://doi.org/10.1177/0748233719863632

Fatima, R., Akhtar, K., Hossain, M.M., Ahmad, R., 2017. Chromium oxide nanoparticle–

induced biochemical and histopathological alterations in the kidneys and brain of Wistar rats. Toxicol Ind Health 33, 911–921. https://doi.org/10.1177/0748233717735266

Fregert, S., Rorsman, H., 1964. ALLERGY TO TRIVALENT CHROMIUM. Arch Dermatol 90, 4–6. https://doi.org/10.1001/archderm.1964.01600010010003

Gammelgaard, B., Fullerton, A., Avnstorp, C., Menné, T., 1992. Permeation of chromium salts through human skin in vitro. Contact Derm. 27, 302–310. https://doi.org/10.1111/j.1600-0536.1992.tb03284.x

Gross, P.R., Katz, S.A., Samitz, M., 1968. Sensitization of guinea pigs to chromium salts. The Journal of Investigative Dermatology 50.

Gulson B, McCall M, Korsch M, Gomez L, Casey P, Oytam Y, Taylor A, McCulloch M, Trotter J, Kinsley L, Greenoak G. Small amounts of zinc from zinc oxide particles in sunscreens applied outdoors are absorbed through human skin. Toxicol Sci. 2010 Nov;118(1):140-9. doi: 10.1093/toxsci/kfq243. Epub 2010 Aug 12.

Gulson B, Wong H, Korsch M, Gomez L, Casey P, McCall M, McCulloch M, Trotter J, Stauber J, Greenoak G. Comparison of dermal absorption of zinc from different sunscreen formulations and differing UV exposure based on stable isotope tracing. Sci Total Environ. 2012 Mar 15;420:313-8. doi: 10.1016/j.scitotenv.2011.12.046.

Hamamy, H.A., Al-Hakkak, Z.S., Hussain, A.F., 1987. Chromosome aberrations in workers at a tannery in Iraq. Mutation Research/Genetic Toxicology 189, 395–398. https://doi.org/10.1016/0165-1218(87)90048-6

Hansen, M.B., Johansen, J.D., Menné, T., 2003. Chromium allergy: significance of both Cr(III) and Cr(VI). Contact Derm. 49, 206–212. https://doi.org/10.1111/j.0105-1873.2003.0230.x

Hansen, Malene Barré, Menné, T., Johansen, J.D., 2006a. Cr(III) reactivity and foot dermatitis in Cr(VI) positive patients. Contact Derm. 54, 140–144. https://doi.org/10.1111/j.0105-1873.2006.00802.x

Hansen, Malene Barré, Menné, T., Johansen, J.D., 2006b. Cr(III) reactivity and foot dermatitis in Cr(VI) positive patients. Contact Derm. 54, 140–144. https://doi.org/10.1111/j.0105-1873.2006.00802.x

Hansen, Malene Barre, Menne, T., Johansen, J.D., 2006. Cr(III) and Cr(VI) in leather and elicitation of eczema. Contact Derm. 54, 278–282. https://doi.org/10.1111/j.0105-1873.2006.00824.x

Hedberg YS, Erfani B, Matura M, Lidén C. Chromium(III) release from chromium-tanned leather elicits allergic contact dermatitis: a use test study. *Contact Dermatitis*. 2018;78(5):307-314. doi:10.1111/cod.12946

Hepburn, D.D.D., Marcel Burney, J., Woski, S.A., Vincent, J.B., 2003. The nutritional supplement chromium picolinate generates oxidative DNA damage and peroxidized lipids in vivo. Polyhedron 22, 455–463. https://doi.org/10.1016/S0277-5387(02)01369-4

Hussain, H., Homady, M., Tarawneh, K., 2000. Effects of chromium Compounds on Incidence of social agression and fertility in prepubertal male mice. Pakistan Journal of Biological sciences 1519–1524.

International Agency for Research on Cancer, , 1990. Chromium, nickel and welding: views and experts opinions of an IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, which met in Lyon 5 - 13 June 1989, IARC monographs on the evaluation of carcinogenic risks to humans. Lyon.

Itoh, S., Shimada, H., 1996. Micronucleus induction by chromium and selenium, and suppression by metallothionein inducer. Mutat. Res. 367, 233–236. https://doi.org/10.1016/s0165-1218(96)90082-8

Ivankovic, S., Preussman, R., 1975. Absence of toxic and carcinogenic effects after administration of high doses of chromic oxide pigment in subacute and long-term feeding experiments in rats. Food Cosmet Toxicol 13, 347–351.

Iyer, V.J., Banerjee, G., Govindram, C.B., Kamath, V., Shinde, S., Gaikwad, A., Jerajani,

H.R., Raman, G., Cherian, K.M., 2002. Role of different valence states of chromium in the elicitation of allergic contact dermatitis. Contact Derm. 47, 357–360. https://doi.org/10.1034/j.1600-0536.2002.470608.x

James Speight, Ph.D. Lange's Handbook of Chemistry, Sixteenth Edition (McGraw-Hill Education: New York, Chicago, San Francisco, Lisbon, London, Madrid, Mexico City, Milan, New Delhi, San Juan, Seoul, Singapore, Sydney, Toronto, 2005). https://www.accessengineeringlibrary.com/content/book/9780071432207

Jansen, L.H., Berrens, L., 1968. Sensitization and partial desensitization of guinea-pigs to trivalent and hexavalent chromium. Dermatologica 137, 65–73.

Johansson, A., Curstedt, T., Jarstrand, C., Camner, P., 1992. Alveolar macrophages and lung lesions after combined exposure to nickel, cobalt, and trivalent chromium. Environmental Health Perspectives 97, 215–219. https://doi.org/10.1289/ehp.9297215

Khan, F.H., Ambreen, K., Fatima, G., Kumar, S., 2012a. Assessment of health risks with reference to oxidative stress and DNA damage in chromium exposed population. Science of The Total Environment 430, 68–74. https://doi.org/10.1016/j.scitotenv.2012.04.063

Khan, F.H., Ambreen, K., Fatima, G., Kumar, S., 2012b. Assessment of health risks with reference to oxidative stress and DNA damage in chromium exposed population. Science of The Total Environment 430, 68–74. https://doi.org/10.1016/j.scitotenv.2012.04.063

Kirpnick-Sobol, Z., Reliene, R., Schiestl, R.H., 2006. Carcinogenic Cr(VI) and the Nutritional Supplement Cr(III) Induce DNA Deletions in Yeast and Mice. Cancer Res 66, 3480–3484. https://doi.org/10.1158/0008-5472.CAN-05-3944

Kligman, A.M., 1989. The identification of contact allergens by human assay. III. The Maximization Test: a procedure for screening and rating contact sensitizers. J. Invest. Dermatol. 92, 151S; discussion 152S. https://doi.org/10.1038/jid.1989.44

Langerwerf, J.S., Bakkeren, H.A., Jongen, W.M., 1985. A comparison of the mutagenicity of soluble trivalent chromium compounds with that of potassium chromate. Ecotoxicol. Environ. Saf. 9, 92–100. https://doi.org/10.1016/0147-6513(85)90039-9

Lejding, T., Mowitz, M., Isaksson, M., Bruze, M., Pontén, A., Svedman, C., Zimerson, E., Engfeldt, M., 2018. A retrospective investigation of hexavalent chromium allergy in southern Sweden. Contact Derm. 78, 386–392. https://doi.org/10.1111/cod.12969

Levis, A.G., Majone, F., 1981. Cytotoxic and clastogenic effects of soluble and insoluble compounds containing hexavalent and trivalent chromium. Br. J. Cancer 44, 219–235. https://doi.org/10.1038/bjc.1981.173

Li, F.P., Fine, W., Jaffe, N., Holmes, G.E., Holmes, F.F., 1979. Offspring of patients treated for cancer in childhood. J. Natl. Cancer Inst. 62, 1193–1197.

Mackenzie, R.D., Byerrum, R.U., Decker, C.F., Hoppert, C.A., Langham, R.F., 1958. Chronic toxicity studies. II. Hexavalent and trivalent chromium administered in drinking water to rats. AMA Arch Ind Health 18, 232–234.

Mali, J.W., Malten, K., van Neer, F.C., 1966. Allergy to chromium. Arch Dermatol 93, 41–44.

Medeiros, M.G., 2003. Elevated levels of DNA-protein crosslinks and micronuclei in peripheral lymphocytes of tannery workers exposed to trivalent chromium. Mutagenesis 18, 19–24. https://doi.org/10.1093/mutage/18.1.19

Migliore, L., Parrini, M., Sbrana, I., Biagini, C., Battaglia, A., Loprieno, N., 1991. Micronucleated lymphocytes in people occupationally exposed to potential environmental contaminants: the age effect. Mutation Research/DNAging 256, 13–20. https://doi.org/10.1016/0921-8734(91)90028-A

Mishra, M., Sharma, A., Negi, M.P.S., Dwivedi, U.N., Chowdhuri, D.K., 2011. Tracing the tracks of genotoxicity by trivalent and hexavalent chromium in Drosophila melanogaster. Mutation Research/Genetic Toxicology and Environmental Mutagenesis 722, 44–51. https://doi.org/10.1016/j.mrgentox.2011.02.010

Moretto, A., 2015. Hexavalent and trivalent chromium in leather: What should be done?

Regul. Toxicol. Pharmacol. 73, 681–686. https://doi.org/10.1016/j.yrtph.2015.09.007

Morris, G.E., 1958. Chrome dermatitis: a study of the chemistry of shoe leather with particular reference to basic chromic sulfate. AMA Arch Derm 78, 612–618.

National Toxicology Program, 2010. NTP toxicology and carcinogenesis studies of chromium picolinate monohydrate (CAS No. 27882-76-4) in F344/N rats and B6C3F1 mice (feed studies). Natl Toxicol Program Tech Rep Ser 1–194.

Nethercott, J., Paustenbach, D., Adams, R., Fowler, J., Marks, J., Morton, C., Taylor, J., Horowitz, S., Finley, B., 1994. A study of chromium induced allergic contact dermatitis with 54 volunteers: implications for environmental risk assessment. Occup Environ Med 51, 371–380. https://doi.org/10.1136/oem.51.6.371

Nettesheim, P., Hanna, M.G.J., Doherty, D.G., Newell, R.F., Hellman, A., 1970. Effects of chronic exposure to artificial smog and chromium oxide dust on the incidence of lung tumors in mice (Technical Report No. CONF-691001-). Oak Ridge National Lab., Tenn.

Newbold, R.F., Amos, J., Connell, J.R., 1979. The cytotoxic, mutagenic and clastogenic effects of chromium-containing compounds on mammalian cells in culture. Mutat. Res. 67, 55–63. https://doi.org/10.1016/0165-1218(79)90099-5

Polak, L., Frey, J.R., 1973. Studies on contact hypersensitivity to chromium in the guinea pig. Inhibition of the migration of macrophages by chromium salts. Int. Arch. Allergy Appl. Immunol. 44, 51–61. https://doi.org/10.1159/000230917

Riihimäki, V., & Luotamo, M. (2006). Health risk assessment report for metallic chromium and trivalent chromium. *Otavan kirjapaino, Keuruu*, 25-31.

Samitz, M.H., Shrager, J., 1966. Patch test reactions to hexavalent and trivalent chromium compounds. Arch Dermatol 94, 304–306.

Santonen, T., International Programme on Chemical Safety (Eds.), 2009. Inorganic chromium (III) compounds: first draft, Concise international chemical assessment document. World Health Organization, Geneva.

Sbrana, I., Caretto, S., Battaglia, A., 1991. Chromosomal aberration analysis of workers in tannery industries. Mutation Research/Genetic Toxicology 260, 331–336. https://doi.org/10.1016/0165-1218(91)90018-H

Senapati, V.A., Jain A.J, Gupta, G.S., Panday, A.K, Dhawan, A., 2015. Chromium oxide nanoparticle-induced genotoxicity and p53-dependent apoptosis in human lung alveolar cells: Chromium oxide nanoparticles toxicity in human lung cells. J. Appl. Toxicol. 2015; 35: 1179-1188.

Seoane, A.I., Dulout, F.N., 2001. Genotoxic ability of cadmium, chromium and nickel salts studied by kinetochore staining in the cytokinesis-blocked micronucleus assay. Mutat. Res. 490, 99–106. https://doi.org/10.1016/s1383-5718(00)00145-5

Shara, M., Kincaid, A.E., Limpach, A.L., Sandstrom, R., Barrett, L., Norton, N., Bramble, J.D., Yasmin, T., Tran, J., Chatterjee, A., Bagchi, M., Bagchi, D., 2007. Long-term safety evaluation of a novel oxygen-coordinated niacin-bound chromium (III) complex. J. Inorg. Biochem. 101, 1059–1069. https://doi.org/10.1016/j.jinorgbio.2007.03.015

Siegenthaler, U., Laine, A., Polak, L., 1983. Studies on contact sensitivity to chromium in the guinea pig. The role of valence in the formation of the antigenic determinant. J. Invest. Dermatol. 80, 44–47. https://doi.org/10.1111/1523-1747.ep12531034

Singh, S.P., Chinde, S., Kamal, S.S.K., Rahman, M.F., Mahboob, M., Grover, P., 2016. Genotoxic effects of chromium oxide nanoparticles and microparticles in Wistar rats after 28 days of repeated oral exposure. Environ Sci Pollut Res 23, 3914–3924. https://doi.org/10.1007/s11356-015-5622-0

Slesinski, R.S., Clarke, J.J., C. San, R.H., Gudi, R., 2005. Lack of mutagenicity of chromium picolinate in the hypoxanthine phosphoribosyltransferase gene mutation assay in Chinese hamster ovary cells. Mutation Research/Genetic Toxicology and Environmental Mutagenesis 585, 86–95. https://doi.org/10.1016/j.mrgentox.2005.04.001

Sreejayan, N., Marone, P.A., Lau, F.C., Yasmin, T., Bagchi, M., Bagchi, D., 2010. Safety

and toxicological evaluation of a novel chromium(III) dinicocysteinate complex. Toxicol. Mech. Methods 20, 321–333. https://doi.org/10.3109/15376516.2010.487880

Staniek, H., Kostrzewska-Poczekaj, M., Arndt, M., Szyfter, K., Krejpcio, Z., 2010. Genotoxicity assessment of chromium(III) propionate complex in the rat model using the comet assay. Food and Chemical Toxicology 48, 89–92. https://doi.org/10.1016/j.fct.2009.09.020

Staniek, H., Krejpcio, Z., 2009. The effects of tricentric chromium(III) propionate complex supplementation on pregnancy outcome and maternal and foetal mineral status in rat. Food and Chemical Toxicology 47, 2673–2678. https://doi.org/10.1016/j.fct.2009.07.035

Stearns, D.M., Silveira, S.M., Wolf, K.K., Luke, A.M., 2002. Chromium(III) tris(picolinate) is mutagenic at the hypoxanthine (guanine) phosphoribosyltransferase locus in Chinese hamster ovary cells. Mutat. Res. 513, 135–142. https://doi.org/10.1016/s1383-5718(01)00301-1

Stout, M.D., Nyska, A., Collins, B.J., Witt, K.L., Kissling, G.E., Malarkey, D.E., Hooth, M.J., 2009. Chronic toxicity and carcinogenicity studies of chromium picolinate monohydrate administered in feed to F344/N rats and B6C3F1 mice for 2 years. Food Chem. Toxicol. 47, 729–733. https://doi.org/10.1016/j.fct.2009.01.006

Stummann, T.C., Hareng, L., Bremer, S., 2007. Embryotoxicity hazard assessment of methylmercury and chromium using embryonic stem cells. Toxicology 242, 130–143. https://doi.org/10.1016/j.tox.2007.09.022

Terpilowska, S., Siwicki, A.K., 2018. Interactions between chromium(III) and iron(III), molybdenum(III) or nickel(II): Cytotoxicity, genotoxicity and mutagenicity studies. Chemosphere 201, 780–789. https://doi.org/10.1016/j.chemosphere.2018.03.062

Thyssen, J.P., 2009. The epidemiology of contact allergy. Allergen exposure and recent trends. G Ital Dermatol Venereol 144, 507–514.

Unpublished report, 1992. In vivo micronucleus test with Chromoxid-Grün GN (study report).

Unpublished report, 1991. Chromiumhydroxide salmonella/microsome test (study report).

Unpublished report, 1992. Micronucleus test on the mouse (Study report).

Unpublished report, 2008. Local lymph node assay (study report).

Unpublished report, 2006. Mixture of basic chromium (III) sulfate with sodium sulfate and water: study for the skin sensitization effect in guinea pigs (Buehler Patch Test).

Whittaker, P., San, R.H.C., Clarke, J.J., Seifried, H.E., Dunkel, V.C., 2005. Mutagenicity of chromium picolinate and its components in Salmonella typhimurium and L5178Y mouse lymphoma cells. Food and Chemical Toxicology 43, 1619–1625. https://doi.org/10.1016/j.fct.2005.05.003

Yoshioka Y, Kuroda E, Hirai T, Tsutsumi Y, Ishii KJ. Allergic Responses Induced by the Immunomodulatory Effects of Nanomaterials upon Skin Exposure. Front Immunol. 2017 Feb 16;8:169. doi: 10.3389/fimmu.2017.00169.

Yu, W., Sipowicz, M.A., Haines, D.C., Birely, L., Diwan, B.A., Riggs, C.W., Kasprzak, K.S., Anderson, L.M., 1999. Preconception urethane or chromium(III) treatment of male mice: multiple neoplastic and non-neoplastic changes in offspring. Toxicol. Appl. Pharmacol. 158, 161–176. https://doi.org/10.1006/taap.1999.8692

Zahid, Z.R., Al-Hakkak, Z.S., Kadhim, A.H.H., Elias, E.A., Al-Jumaily, I.S., 1990. Comparative effects of trivalent and hexavalent chromium on spermatogenesis of the mouse. Toxicological & Environmental Chemistry 25, 131–136. https://doi.org/10.1080/02772249009357512

Zeiger, E., Anderson, B., Haworth, S., Lawlor, T., Mortelmans, K., 1992. Salmonella mutagenicity tests: V. Results from the testing of 311 chemicals. Environ. Mol. Mutagen. 19 Suppl 21, 2–141. https://doi.org/10.1002/em.2850190603

Zeiger, E., Haseman, J.K., Shelby, M.D., Margolin, B.H., Tennant, R.W., 1990. Evaluation

of four in vitro genetic toxicity tests for predicting rodent carcinogenicity: confirmation of earlier results with 41 additional chemicals. Environ. Mol. Mutagen. 16 Suppl 18, 1–14. https://doi.org/10.1002/em.2850160502

Zhang, M., Chen, Z., Chen, Q., Zou, H., Lou, J., He, J., 2008. Investigating DNA damage in tannery workers occupationally exposed to trivalent chromium using comet assay. Mutation Research/Genetic Toxicology and Environmental Mutagenesis 654, 45–51. https://doi.org/10.1016/j.mrgentox.2008.04.011

7.15. Abbreviations

8-OHdG	8-oxo-2-desoxyguanidine
AC	Article category
ALF	Artificial lysosomal fluid
ANSES	*Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail* [French agency for food, environmental and occupational health & safety]
ASW	Artificial sweat
ATSDR	Agency for toxic substances and disease registry
BAL	Bronchoalveolar lavage
BW	Body weight
CA	Chromosomal aberration
CCH	Compliance check
CHO	Chinese hamster ovary
CLP	Classification, labelling, packaging
CORAP	Community rolling action plan
DD	Draft decision
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DMEL	Derived minimal effect level
DNEL	Derived no effect level
ECHA	European chemical agency
ED	Endocrine disrupting
EOGRTS	Extended one-generation reproductive toxicity study
FCA	Freuds complete adjuvant
FIOH	Finnish Institute of Occupational Health
ERC	Environmental release categories
EU	European union
GD:	Gestation day
GLP	Good laboratory practice
HPRT	Hypoxanthine-guanine phosphoribosyl transferase
IARC	International Agency for Research on Cancer
IP	intraperitoneal
JS	Joint submission
LD50	Median lethal dose
LLNA	Local lymph node assay
LOAEL	Low observed adverse effect level
MF	Mutation frequency
MMAD	Median mass aerodynamic diameter
MN	Micronucleus
MNNG	N-methyl-N'-nitro-N-nitroguanidine
MSCA	Member state competent authority
MP	Microparticles
MTD	Maximum tolerable dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide
n.a.	Not available
NCE	Normochromatic erythrocytes
NOAEL	No observed adverse effect level
NP	Nanoparticles
OECD TG	Organisation for economic co-operation and development
NTP	National Toxicology Program

PBT	Persistent, bioaccumulative and toxic
PC	Product categories
PCE	Polychromatic erythrocytes
PROC	Process categories
QSAR	Quantitative structure-activity relationship
REACH	Registration, authorisation, restriction of chemicals
SCE:	Sister chromatid exchange
SD	Sprague-Dawley
SLS	Sodium lauryl sulfate
STOT RE	Specific target organ toxicity, repeated-exposure
SU	Sector of end-use
SVHC	Substance of very high concern
TG	Technical guidance
VPVB	Very persistent very bioaccumulative
WHO	Workd health organisation
WOE	Weight of evidence