



Substance report

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Chromium VI



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Authors and Acknowledgements

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First version (December 2019) by RPA consultants, based on scoping documents produced by the chemical group leader (CGL) and colleagues. The EEA has since updated this document to reflect the work developed before the conclusion of HBM4EU, with the support of the CGL and other colleagues.

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Glossary

Abbreviations	
C&L	Classification and Labelling
CLP	The 'Classification, Labelling, Packaging' Regulation Regulation (EC) No 1272/2008 on classification, labelling and packaging of substances and mixtures
EC	European Commission
ECHA	European Chemicals Agency
EFSA	European Food Safety Authority
EU	European Union
HBM	Human Biomonitoring
HBM4EU	European Human Biomonitoring Initiative
IARC	International Agency for Research on Cancer
OEL	Occupational Exposure Limits
REACH	The 'Registration, Evaluation, Authorisation and Restriction of Chemicals' Regulation Regulation (EC) No 1907/2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals
WP	Work package
WPL	Work package leader

1 Key messages

- Occupational exposure to Cr(VI) presents a high risk. A HBM exposure study in the surface coating and stainless-steel welding sectors indicated that workers in the chrome plating sector were found to have elevated Cr levels in urine, red blood cells (RBC), and exhaled breath condensate (EBC). In addition, effect marker analyses suggested that these exposure levels, although being mostly below the current occupational limit values, are not fully protective from the health risks of Cr(VI).
- For the general population, sources of Cr(VI) are mainly environmental, via ingestion of contaminated soil or food, and inhalation of ambient air. Exposure may also come from smoking tobacco and from consumer products (it may be present in some rust-proofing paints and in preserved wood).

- Overall, the knowledge on the levels of Cr(VI) in the European population is limited and further data are required to increase the understanding of the current risks posed by Cr(VI) exposure.
- A range of policy measures are in place to control the use of Cr(VI) and to protect both workers and the general population from exposure to Cr(VI).
- A multicentre study using HBM in the assessment of occupational exposure and associated health risks in occupational settings provides a model that can greatly improve risk assessment and the management at the workplaces. The data generated can be used to support the update the national limit values for Cr(VI) and to support the national enforcement programs

2 Introduction

HBM4EU is a project funded under Horizon 2020 and runs from 2017 until 2022. It generates knowledge to inform about the safe management of chemicals, and hence protect human health in Europe. HBM4EU uses human biomonitoring (HBM) to monitor the actual human exposure to chemicals and resulting health impacts to build upon existing evidence bases and improve chemical risk assessment. HBM4EU compares data from across Europe which allows an understanding of regional differences and can help to identify vulnerable groups in order to inform targeted measures to reduce exposure. The results of the HBM4EU project are aimed at supporting policy development, by providing a key evidence base in the understanding of exposure and impacts to toxic chemicals.

If you would like to read more about the project itself, please visit the HBM4EU [website](#).

2.1 How to use this document

This document provides a summary of the known and suspected adverse human health effects of hexavalent chromium – Cr(VI) – and describes the main exposure pathways for humans. It also indicates where HBM could be of value in the development of EU policy, along with the remaining challenges in determining human Cr(VI) exposure. This substance report is intended to inform policy makers and relevant stakeholders on the value of HBM to establish the EU population's exposure to Cr(VI).

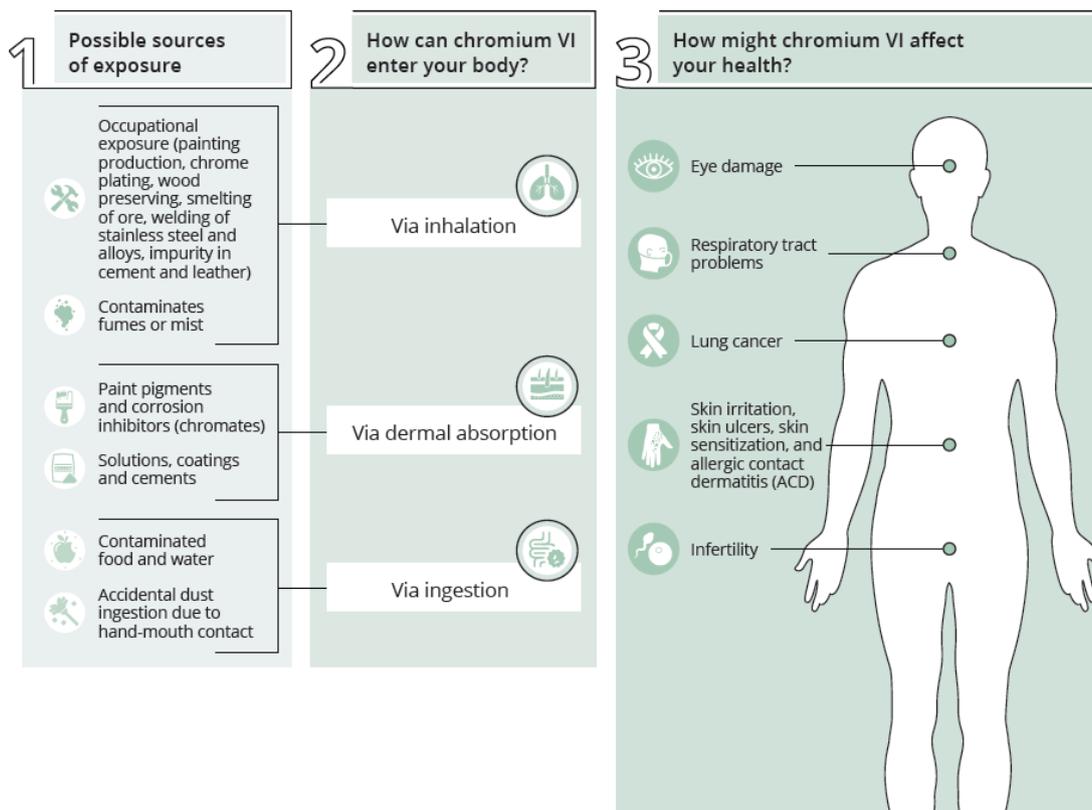
This document is based largely on the HBM4EU [scoping document](#) for Cr(VI), first draft produced in 2019 and updated regularly, as well as the accompanying reports on [legislative mapping](#) and [policy questions](#). Where necessary, additional information has been used from the European Chemical Agency (ECHA) documents including the Classification and Labelling (C&L) Inventory, and legislative text for relevant EU policy areas, have also been used for this report.

2.2 Overview of Chromium VI

3 Human exposure to Chromium VI

For Cr, it is important to note that there are various forms. This substance report concerns Cr(VI) which is the most toxic form of Cr. Cr(VI) is the +6 oxidation state of Cr. Oxidation states of Cr varies from -2 to +6. The most common oxidation states of Cr are +2, +3 (which is the most stable form of this element) and +6. Chromium (0) is the elemental form and does not occur naturally ([NCDOL, 2013](#)).

Figure 3.1 Overview of exposure routes, pathways and health effects of Chromium VI



3.1 Environmental exposure

The wider environment may contribute to Cr(VI) exposure. Levels of Cr in air have been measured in the range of 5-200 ng/m³ in industrial settings and 4-70 ng/m³ in urban areas ([WHO, 2000](#)). However, these figures relate to total chromium concentrations, with the levels of Cr(VI) tending to be lower as Cr(VI) reacts in the atmosphere to form Cr(III) (USEPA, n.d.). Typical airborne concentrations of Cr(VI) are reported at 10 to 30 ng/m³ (USEPA, n.d.).

In rainwater, levels of total chromium are 0.2-1 µg/L while in groundwater they can be <1 µg/L to a few µg/L ([WHO, 2003](#)).

In soil, total Cr levels are above 100 mg/kg in 2.7 % of European soils and above 300 mg/kg in 1.1% of European soils ([Toth et al., 2016](#)). Cr(VI) in soils can react with organic matter to form Cr(III), hence reducing concentrations in soils.

3.2 Occupational exposure

The most significant exposure to Cr(VI) occurs in the workplace where the most common exposure route is inhalation. Occupational exposure to Cr(VI) substances can occur in a variety of sectors. Its uses include as corrosion inhibitors, in chemical synthesis, manufacture of pigments and dyes, metal plating and refractory production. Historically it was also present in wood preservatives, but this application is now banned in the European Union.

3.3 Consumer exposure

There is a risk of Cr(VI) being present in certain consumer goods such as leather, toys and cosmetics, despite the limits and controls already in place at a European level (see policies outlined in Section 5). For example, analysis of leather goods in Denmark found detectable levels of Cr(VI) in 21 out of 94 products tested, including in shoes for babies and children (Danish EPA, 2019). There is also a new restriction in place, with limit levels for Cr VI, related to [Commission Regulation \(EU\) 2020/2081](#) related to substances in tattoo inks or permanent make-up. Colour pigments in cosmetics and tattooing can also contain Cr(VI) impurities (Bocca et al., 2018).

Exposure in general population may also come from smoking tobacco products.

Chromium is unlikely to be present in drinking water in significant quantities with compliance data for the Drinking Water Directive indicating over 99.9 % compliance (for total chromium) (European Commission, 2016).

4 Health impacts of chromium (VI)

4.1 Overview of key health impacts from chromium (VI)

Cr(VI) is a cause of human health concern, primarily due its carcinogenic properties. It is classified as a Carc. 1B substance ([ECHA](#)) by inhalation exposure, a carcinogen Group 1 by IARC and is listed under the EU Carcinogens and Mutagens Directive ([2004/37/EC](#)).

It is also classified as a skin sensitising substance (Skin Sens. 1). Dermal exposure can also lead to skin irritation, ulceration and allergic contact dermatitis. Cr(VI) also readily penetrates cell membranes after inhalation exposure for workers and ingestion exposure for the general population.

Single exposures to Cr(VI) can lead to nose irritation and irritation of the upper respiratory tract, skin damage (burns and ulceration), skin irritation and also eye damage from splashes, although indications of the exposure amount were not given in the Agency for Toxic Substances and Disease Registry (ATSDR, 2000).

Repeated or prolonged exposure to Cr(VI) has a number of serious adverse effects. At low concentrations (<2 µg/m³) workers developed smeary, crusty and atrophied septum mucosa and at higher concentrations (2-200 µg/m³) observations include nasal septum ulceration and perforation, respiratory irritation, lung cancer, renal effects and early kidney changes (at 4 µg/m³), together with reproductive system damage and mutagenic potential (ATSDR, 2000).

Table 1.1 Overview of CLP classifications for chromium VI

Substance	Properties of concern	Category according to CLP criteria	ECHA info card

4.2 Vulnerable target groups

Workers are the most vulnerable group due to occupational exposure of Cr VI. Another target group is considered to be children, with exposure through toys being controlled through relevant EU legislation. The applicable limits for Cr(VI) in toys were reduced in a revised Directive published in 2018.

4.3 Societal concerns

Some of the key societal concerns as outlined in the HBM4EU scoping report for Cr(VI) include:

- anthropogenic Cr(VI) occurrence in water, air and soils as a consequence of industrial activities despite the available limits provided by European regulations and guidelines;
- occurrence of Cr(VI) in many consumers' products such as leather, toys, cosmetics, despite the limits already in place at European level;
- some populations are at higher risk for exposure to Cr(VI), such as children (e.g., from toys, but regulated since 2018)
- occupationally exposed workers in certain industries;
- possible occurrence of adverse effects with respect to cancer, reproductive and developmental toxicity, but also skin sensitisation and allergy, in exposed and general populations.

5 EU policies on chromium (VI)

The risk posed by Cr(VI) have been recognised for some time and hence there is a relatively comprehensive legislative basis for reducing and preventing releases of Cr(VI) and for protection of both workers and the general population.

Table 2.1 Overview of EU policies relating to chromium VI

Chemicals	<ul style="list-style-type: none"> • Chromium VI is restricted in specific products under REACH (Regulation (EC) No 1907/2006) and several chromium (VI) substances are included in the candidate list of substances of very high concern. In particular Annex XVII to REACH restricts the chromium VI content of cement and leather goods (which come into contact with the skin) placed on the market. It is also part of Annex XIV of REACH and therefore requiring authorisation to be used. • Chromium (VI) is subject to EU harmonised classification and labelling under CLP (Directive on the classification, labelling and packaging of substances and mixtures) (Regulation (EC) No 1272/2008). 	Consumer
	<p><i>Food</i></p> <ul style="list-style-type: none"> • Chromium levels (all forms) in plastic material and articles intended to come in contact with food are now controlled under Regulation (EU) No 10/2011. <p><i>Cosmetics</i></p> <ul style="list-style-type: none"> • Chromium (VI) is restricted under the EU Cosmetics Regulation 1223/2009 and is prohibited from cosmetics in Germany <p><i>Toys</i></p> <ul style="list-style-type: none"> • The allowable thresholds for chromium (VI) in toys was further reduced in 2018, through an amendment of the 'Toys Directive' 2009/48/EC. 	

Environmental	<p><i>Water</i></p> <ul style="list-style-type: none"> The revised Drinking Water Directive (2020/2184) reduces the limit for chromium in drinking water to 25 µg/l. This applies from 2036 with the limit remaining at 50 µg/l until then. Chromium levels in natural mineral waters are also limited under Directive 2003/40/EC. 	<ul style="list-style-type: none"> Chromium (VI) is subject to Directive (EC) 2004/37 on the protection of workers from the risks related to exposure to carcinogens or mutagens at work. This includes the specification of an 8-hour occupational exposure limit value. 	Occupational
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Some EU countries also have established biological reference or guidance values for Cr(VI) exposure. These are summarised in Table 3-1.

Country	Reference value
Germany	0.6 µg/L for Cr(VI) compounds (inhalation) for general population of working age (DFG, 2012); biological exposure equivalent: 10 µg/L – 40 µg/L for total Cr in urine and 9 µg/L - 35 µg/L for Cr(VI) if soluble alkaline chromate of certain concentrations and/or Cr(VI) welding fumes (for urine only) inhaled over a work shift (DFG, 2015)
Spain	10 µg/L urine (total Cr) at beginning of shift and 25 µg/L urine at end of workweek (total Cr) (INSHT, 2016)
UK	10 µmol/mol creatinine in post-shift urine (HSE, 2011)
France	Biological limit value (BLV) of 2.5 µg/L or 1.8 µg/g creatinine (end of the week and end of shift) for urinary total Cr (ANSES, 2017)
Finland	Biological limit value (BLV) of 10 µg/L for urinary total Cr (STM, 2018)

6 Policy questions for chromium

6.1 Introduction

For each of the HBM priority substances stakeholders were asked to identify policy related questions that HBM4EU should address in order to contribute to the strengthening of policy ambitions on Cr(VI). Further background detail on Cr(VI) and how the policy questions were selected is available in the [scoping document](#) and the [report on stakeholder consultation and mapping of needs](#).

The current situation for policy questions for Cr(VI) is summarised in the next section. The responses are based on a document updated by the CGL and work package leader (WPL).

6.2 What is the current (last 5 years) exposure of the European population to Cr(VI)??

In all the EU countries the lack of studies on environmental exposure to Cr(VI) was evident, due to the very low exposure levels of Cr(VI) in the general population.

Six countries reported occupational biomonitoring data on Cr but the majority of data comes from the use of total Cr measurements. Since this is not specific for Cr(VI) it was decided to use new Cr(VI) specific biomarkers and to expand the scattered EU data on Cr(VI).

6.3 What is the level of exposure, environmentally and occupationally relevant to Cr(VI) in the EU population?

Within the targeted occupational study, workers from different occupational settings, as chrome platers, surface treatment workers and welders, have been analysed to test differences in Cr(VI) levels respect to controls and depending on the task, using HBM matrices (urine, RBC, EBC) and industrial hygiene samples (inhalable and respirable air, dermal wipes).

All of the biomarkers of exposure analysed demonstrate that workers from all the occupational settings have higher exposure to Cr and Cr(VI) when compared with control groups. Strong correlations were observed between urinary Cr levels and atmospheric concentration of Cr(VI) in platers and welders, supporting the use of urinary Cr as a primary method for the biomonitoring of Cr(VI) exposure at workplaces (Santonen et al. 2022).

In addition, workers show higher Cr(VI) levels in post-shift than in pre-shift urinary samples.

Moreover, all the occupational settings show higher values of Cr in dermal wipes samples during and in the end of the shift suggesting that hands contamination increases during the day.

6.4 Does the exposure to Cr(VI) differ significantly between countries and population groups? What are the main reasons for differences in exposure?

In all countries participating to the occupational study, the results of the targeted occupational study show higher exposure to Cr(VI) among the workers in Cr(VI)-using industries respect to controls.

The chromium plating sector shows the highest biomarkers exposure levels.

The lower biomarker values in other surface treatment workers, when compared to chrome platers, may reflect the use of respiratory protective equipment (RPE). Lower U-Cr levels in welders when compared to platers may reflect the differences in the toxicokinetics of different hexavalent chromium species.

6.5 Is there a significant time trend of Cr(VI) levels in existing population studies?

Verdonck et al. (2021) reported a decreasing time trend of urine Cr concentrations in workers, based on an unpublished biomonitoring dataset (comprising urinary levels of 3799 workers from different industries in Belgium collected during 1998 and 2018). Similar decreasing trend has been reported from Finland from biomonitoring measurements collected between 1980-2016 (Mahiout et al., 2022, *submitted*).

Data are insufficient to evaluate time-trends on the EU-wide scale.

6.6 What are the groups at risk?

The results obtained, under the Cr(VI) occupational study, reveal significant differences between subgroups of workers by industrial sector (Tables 10-12, D8.9).

In general, based on the biomonitoring data, chrome plating workers are exposed to higher internal levels than surface treatment workers and welders.

6.7 Are the overall exposure levels (in different population groups) above any health-relevant assessment levels (HBM guidance values, TDI)?

Data from Cr(VI) occupational study highlight that in the industrial hygiene measurements, the 90th percentile (P90) of inhalable Cr(VI) levels is below the binding occupational limit value of 5 µg/m³ in welding and chrome plating, whereas in surface treatment the P90 is above the transient BOELV of 10 µg/m³. Biomonitoring data supports the air measurement data showing that the exposure in most cases remains below these levels. However, the effect biomarker data suggests that even these levels may not be fully safe since increases in genotoxicity markers were observed both in platers and welders.

6.8 Has the regulation under REACH had the favourable impact like a reduction of GM/median concentrations?

Results obtained under the Cr(VI) occupational study, even with several regulatory frameworks in place (REACH and OSH), show that exposure to Cr(VI) in plating still occurs and at higher levels than in the other investigated sectors.

Although the chemical form of the chromates used and the effect of wearing RPE are likely to have an impact on this, available results also suggest that other exposure routes (ingestion due to hand-to-mouth contact) may also have a role in the total exposure.

In order to conclude whether new regulations have already had a favourable impact on Cr(VI) exposure, further evaluation and comparison to earlier data need to be performed. However, in the HBM4EU study a rich dataset has been collected, which can be used as a baseline for future research applying the same methodology.

6.9 What are the current HBM methods for Cr(VI)?

Within the Cr(VI) occupational study, new and more specific methods for biomonitoring of Cr(VI) are tested, and harmonised methodology for the collection of biological samples (urine, RBC and EBC) and environmental industrial hygiene samples (as dermal wipes and air) have been developed.

Cr(VI) in EBC has been proposed as a new biomarker. There are currently few data on the relationship between the levels in the EBC and in atmospheric air. EBC at early stages, has analytical and consistency issues making it difficult to compare.

The HBM4EU chromate study demonstrated a high correlation between chromium urinary levels (U-Cr) and air Cr(VI) or dermal total Cr exposure. U-Cr showed its value as a first approach for the assessment of total, internal exposure. The results showed correlations between U-Cr and Cr(VI)

air concentrations that can be used to set human biomonitoring guidance values (HBM-GVs) for U-Cr corresponding to air limit values for Cr(VI) (Viegas et al., 2022). The data supports other studies carried out in occupational settings that have reported measurements of U-Cr and their correlations with Cr(VI) air concentrations in Cr plating activities (ANSES, 2017). Correlations between U-Cr and Cr(VI) in EBC and Cr in RBC were instead low, probably due to differences in kinetics and indicating that these biomonitoring approaches may not be interchangeable but rather complementary. RBC-Cr and plasma (P-) Cr in chrome platers showed, however, a high correlation with Cr(VI) in inhalable dust measured outside respiratory protective equipment (RPE) (Ndaw et al., 2022). Cr-blood-based biomarkers can provide information on how workplace exposure translates into systemic availability of Cr(III) (extracellular, P-Cr) and Cr(VI) (intracellular, RBC-Cr). Further studies are, however, needed to fully appreciate their use in an occupational health and safety context (Ndaw et al., 2022).

6.10 Which are the appropriate biomarkers for Cr(VI)?

Even though urinary total Cr is a non-specific biomarker, it has shown its value as the first approach to assess the total, internal exposure to Cr. The biomarkers RBC and EBC were the most specific to Cr(VI) and can be used to provide complementary data.

The biomarkers more specific to Cr(VI) exposures are the RBC-Cr(VI) and EBC-Cr. Both are indeed significantly elevated in workers compared to controls.

The HBM4EU chromate study also includes collection of the samples for several effect biomarkers analyses which can be used as an indirect exposure biomarker. Analysis of the results of the effect markers reveal that workers in electrolytic bath plating display the highest levels of DNA and chromosomal damage in leukocytes and the second highest level of chromosomal damage in reticulocytes. Welders exhibited the highest frequency of micronuclei in reticulocytes despite the lower U-Cr levels, suggesting recent exposure to Cr(VI) that might not have been captured by post-shift U-Cr levels. The frequency of micronucleated lymphocytes was significantly related to the U-Cr level with the highest exposed group (3rd tercile of U-Cr level) displaying the highest frequency of micronucleated cells. Likewise, the micronucleus frequency in reticulocytes was also related to the U-Cr level. The data of genotoxicity biomarkers also evidenced that controls recruited within the industries (e.g., office workers) displayed levels of genotoxic damage much higher than those of controls recruited outside the companies. The comparison of global DNA methylation levels between the exposed and the control groups revealed a statistically significant decrease in levels observed in workers, suggesting deregulation of gene expression. The changes in abundance of urinary excreted metabolites observed in workers reflected fatty acid and monoamine neurotransmitter metabolism, oxidative modifications of the amino acid residues, excessive formation of abnormal amino acids metabolites and changes in steroid and thyrotropin-releasing hormone.

7 HBM4EU results

7.1 Categorisation

Substances under HBM4EU have been categorised depending on availability of HBM data. The categorisation indicates the information gaps allowing the development of targeted activities to fill the knowledge gaps. Substances will pass from Category E over D, C, B towards Category A as more information becomes available. Fully characterised substances should end up as category A substances.

Table 4.1 HBM4EU categorisation for chromium (VI)

Category	Priority substance(s)
HBM data exist but not for across Europe	Chromium (VI)

7.2 Key outputs

An inventory of studies holding Cr(VI) exposure data was performed under WP7 with an online questionnaire which was distributed with the aim to identify existing HBM studies. Data available and data gaps are summarised in a report (see D7.1). Among all the priority substances Cr(VI) was one of the least studied substances; in particular, a total of 5 studies included Cr(VI) measurements: 2 of them from Western European regions; 2 from Southern European regions and 1 in Israel. Although the preferred matrix for internal Cr assessment was blood, measurements were also available for blood erythrocytes, plasma, serum and urine spot random samples.

WP7 has produced a variety of materials to provide the groundwork for a harmonised approach to study and conduct HBM surveys in Europe. In WP7 some specific questions for Cr(VI) were identified as useful to collect information concerning countries and population characteristics (sociodemographic, dietary, occupational, lifestyle, environmental and health factors) with the aim to characterize possible differences among EU populations (see D7.3). In particular, exposure to metallic dust, type of work (surface treatment, handling metals, etc.), body modifications (piercings, tattoos), metallic jewellery on the skin, type of food and drink consumed before the sampling, have been identified as the main possible reasons for differences in Cr(VI) exposure. For Cr(VI), questionnaires for adults are available as well as specific questionnaires that can be used in occupational studies.

The strategy to collect HBM data EU wide in a harmonised way to get comparable HBM data all over Europe was reported in D8.1. In AD8.1 an inventory of databases or datasets targeting occupational exposure to Cr in Europe (from WP 7.1 questionnaire) was reported.

To evaluate the levels of Cr(VI) exposure in occupational settings, a targeted occupational study in the surface treatment and stainless-steel welding sectors started in 2018 under WP8 (see D8.5), with samples taken in France, Netherlands, Finland, Belgium, Poland, Portugal, UK, Italy and Luxembourg. This study included ca. 40 companies and a total of 602 workers comprising 203 controls (not occupationally exposed to Cr(VI)) and 399 workers exposed to Cr(VI) in several occupational activities using different HBM matrices as exhaled breath condensate (EBC) and red blood cell (RBC), as well as Cr in urine.

The research plan for chromates study was published as AD8.2 and in the publication of Santonen et al. 2019 that describes methods, harmonised questionnaires and SOPs applied for the collection of occupational hygiene and HBM samples in different countries (Santonen et al., 2019). In addition, Cr(VI) information sheet, information leaflets to the participating companies and to workers as well as informed consent forms for companies and workers have been prepared in collaboration with WP7. These were translated for local languages (French, Italian, Portuguese, Polish, German, Dutch and Finnish). In order to collect relevant background information on possible confounding exposures and operating conditions and risk management measures in place at the workplace, a questionnaire for data collection was prepared (Annex1, D8.5).

Moreover, to collect comparable data across EU, Standard Operating Procedures (SOPs) were developed for the collection, handling, sample storage and transfer for each of the biological and industrial hygiene samples covered within the Cr(VI) occupational study. SOPs for each specific matrix have been published in the HBM4EU on-line library.

In the same time, within the WP9, an inventory of available methods and matrices suitable for Cr measurements have been reported (see D9.2). This inventory, covering articles published in the years 2010-2017, revealed the presence of 16 references in total, but only 8 fulfilled the analytical requirements. Chromium is analysed in urine, whole blood, exhaled breath condensate (EBC) and red blood cells (RBC). All described methods use ICP-MS, GF-AAS, EAAS and AAS, and the most frequent sample preparations are: liquid extraction, centrifugation and clean up using strong acid.

Moreover, an ICI/EQUAS for Cr analysis in different biological matrices was performed within WP9 in order to select candidate laboratories for the determination of Cr in urine, whole blood and serum. Additionally, a few laboratories have set up the methodology for the analysis of Cr(VI) in EBC (D9.7) and a small-scale interlaboratory comparison to ensure the quality of the analysis was conducted (D8.5). The list of approved laboratories for Cr analyses was made available (see D9.3) and all qualified laboratories were asked for information on price, capacity, time frames and technical details of Cr analyses (AD9.3).

Regarding the most appropriate biomarkers of exposure, scoping document (D4.9) and deliverables (AD8.1) identified the urinary Cr levels (U-Cr) as a measure of total Cr exposure as Cr(VI) is reduced within the body to Cr(III). The analysis of plasma is indicative of total Cr exposure because Cr(VI) may be reduced to Cr(III) in the plasma. Cr measurements in RBC were selected as a suitable biomarker for the analysis of Cr(VI) exposure because Cr(VI) passes through cell membranes, while Cr(III) does not. The analysis of EBC was selected as a potentially good biomarker of occupational exposure to Cr(VI).

In addition, WP10 has developed a substance-specific statistical analysis plan for Cr(VI) (see D10.2, D10.5 and D10.6), including the definition and harmonisation of the variables, the statistical test to be applied, the specific procedure for calculating EU reference values, uncertainty analyses, data descriptions, and visualisations. Variables on general exposure levels, geographic comparisons and exposure determinants were defined in relation to Cr(VI) exposure (like SES, education, type of area of residence, density of traffic in the residential area, smoking, passive smoking, cotinine, local food, seafood, tattoo, jewellery, nutrients). These variables were mandatory included in the statistical analyses to address Cr(VI)-specific differences among countries and population groups.

Concerning the population most at risk of Cr(VI) exposure, the literature review within the framework of the scoping document (D4.2) and of deliverable AD8.1 has identified occupations with potentially elevated exposure to Cr(VI). In the EU the estimated number of Cr(VI)-exposed workers in 2012 was ~786,000, with the largest numbers exposed to welding. Other major uses of Cr(VI) include metal plating, manufacture of pigments and dyes, corrosion inhibitors, chemical synthesis, refractory production, leather tanning, and wood preservation.

The results under the WP8 occupational chromate study aid to evaluate the Cr(VI) exposure in some of the most exposed classes of workers (chromium plating, welding and other surface treatment activities). The findings were reported in deliverables D8.9 and D8.13, and in the scientific publication by Santonen et al., 2022 (Santonen et al., 2022). The data obtained revealed significant differences between subgroups of workers by industrial sector (Tables 10-12, D8.9). In all countries participating to the occupational study, the results of the targeted occupational study showed higher exposure to Cr(VI) among the workers in Cr(VI)-using industries respect to controls, and the chromium plating sector shows the highest biomarkers exposure levels respect to surface treatment workers and welders. Elevated U-Cr levels in exposed workers were observed not only in post-shift samples but also in pre-shift samples supporting the hypothesis of extended elimination/excretion of Cr from the body. In addition, potential bystanders' exposure among the office workers of the companies handling Cr(VI) were found. In addition, results showed the strong correlation between inhalable Cr(VI) levels and urinary total Cr levels and a significant association

between dermal total Cr contamination and internal exposure to Cr in all worker groups. On the contrary, low correlations between U-Cr and the other tested biomarkers (RBC-Cr and EBC-Cr(VI)) have been found.

Moreover, in the paper of Viegas et al. 2022 (Viegas et al., 2022), some exposure determinants that contribute to Cr exposure have been identified and the actions to reduce and control such exposure. In particular, the automation of Cr electroplating dipping explained lower exposure levels in platers. Use of respiratory protective equipment resulted in lower U-Cr levels in welding, bath plating and painting. Lower U-Cr levels were also observed in machining for workers wearing gloves, and in welding where local exhaust ventilation was available and previous training was provided.

Additionally, a paper on the lessons learned from designing and conducting a collaborative EU biomonitoring occupational study have been published (Galea et al., 2021) and some practical aspects have been highlighted for improvement in future HBM4EU studies, e.g., implementation of SOPs, more training on the use of the data entry template, improved company communications (Galea et al., 2021).

The paper by Ndaw et al 2022 showed a high correlation between RBC-Cr and plasma (P-) Cr in chrome platers with Cr(VI) in inhalable dust measured outside respiratory protective equipment (RPE) (Ndaw et al., 2022). Cr-blood-based biomarkers can provide information on how workplace exposure translates into systemic availability of Cr(III) (extracellular, P-Cr) and Cr(VI) (intracellular, RBC-Cr). Further studies are, however, needed to fully appreciate their use in an occupational health and safety context (Ndaw et al., 2022, in press).

The paper by Leese et al., (“HBM4EU chromates study – the measurement of hexavalent and trivalent chromium in exhaled breath condensate samples from occupationally exposed workers across Europe”, submitted to Toxicology Letters) explored further the applicability of EBC-Cr(VI) analyses as biomarker of exposure to Cr(VI) and concluded that EBC has the potential to be a valid, non-invasive biological matrix to assess occupational exposure to Cr(VI) and Cr(III) for biological monitoring assessment, with the ability to detect low level inhalation exposures.

This issue has been reported in WP13 with a purpose to establish exposure-health relationships. WP13 give a detailed overview of the available knowledge on AOPs for Cr(VI) (D13.4 and D13.5) and subsequently summarized key knowledge gaps emerging from previous research. Specific activities, studies or other relevant steps to fill in the missing information on Cr(VI) have been proposed in D13.5. In D5.5, the inclusion of HBM data in risk assessment (RA) and health impact assessment (HIA) strategies has been exemplified. RA was performed also for Cr(VI).

Relevant HBM guidance values for the exposure to Cr have been reported on a national basis, but not at EU level. In the scoping document (D4.2) all the available limits have been reviewed. In Spain, a biological limit value (BLV) for total Cr concentration of 10 µg/L in urine measured during a shift and 25 µg/L at the end of the workweek has been reported (INSHT, 2016). In the UK, a biological monitoring guidance value (BMGV) of 10 µmol/mol creatinine in post shift urine was established (HSE, 2011). In Germany, DFG established the DFG-EKA values (biological exposure equivalents for carcinogenic substances) for Cr(VI) that set the range of total Cr in urine (from 10 µg/L to 40 µg/L) and in erythrocyt fraction of whole-blood (from 9 µg/L to 35 µg/L) if soluble alkaline chromate of a certain concentration and/or hexavalent welding fumes (only for urine) were inhaled over a work shift (DFG, 2015). Management of Cr(VI) formed during the welding process is achieved by compliance with occupational exposure limit values (OELs). The recent binding OEL set under EU Directive 2004/37/EC on the protection of workers from the risks related to exposure to carcinogens or mutagens at work is 0.010 mg/m³ for a period of 5 years after the date of transposition of the directive; after that period a limit of 0.005 mg/m³ will apply. For welding or

plasma-cutting processes or similar work processes that generate fumes, there is a derogation, with an OEL value of 0.025 mg/m³ until 5 years after the transposition date and after that period the limit will be 0.005 mg/m³. On the other hand, in France and the Netherlands, an OEL of 1 µg/m³ has been set for Cr(VI) in all uses. These are the most stringent OELs currently set in workplace in EU. Data from Cr(VI) occupational study highlighted that in the industrial hygiene measurements, the 90th percentile (P90) of inhalable Cr(VI) levels is below the binding occupational limit value of 5 µg/m³ in welding and chrome plating, whereas in surface treatment the P90 is above the transient BOELV of 10 µg/m³. However, genotoxicity marker analyses showed that even these levels may not be fully safe (Tavares et al., 2022, "HBM4EU Chromates Study – genotoxicity and oxidative stress biomarkers for the biomonitoring of hexavalent chromium occupational exposure", *submitted*).

In HBM4EU an HBM-ELCR for Cr(VI) has been derived of 8 µg/g cr based on an OEL of 5 µg/m³ corresponding to an excess lifetime risk of 20 cases of lung cancer per 1000 workers exposed 8 hours a day for 40 years. Cr(VI) is one of the most important occupational carcinogens, which has been shown to cause lung cancer in humans. It is currently an issue in the EU since some Cr(VI) compounds are authorised under Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH). Within WP11, information about biological matrix previously used for the Cr(VI) measurement use in previous studies and obstacle to link HBM data and health was given (D11.1).

Concerning biomarkers of effects, in WP8 (task 8.5) the chromate study includes also the collection and analysis of samples for several effect biomarkers analyses. Effect markers analysed in the chromate study (see D8.5) were reticulocyte micronuclei (MN), MN in peripheral blood lymphocyte (in collaboration with WP14), comet assay in leukocytes, global methylation analysis (and specific epigenetic markers), telomer length in blood, metabolomics studies (urine), oxidative stress biomarkers in urine. Moreover, a literature survey was performed for Cr(VI) in order to create an inventory of available biomarkers of effects for this specific exposure. The results of that survey are presented in AD14.5. The traditional effect biomarkers included oxidative stress (e.g., malondialdehyde) and genotoxicity (e.g., micronucleus analysis) markers. Among the novel effect biomarkers, those relying on gene expression and epigenetic effects (e.g., DNA methylation analysis) were identified as the most informative ones.

In addition, in the paper of Kozłowska et al. nontargeted metabolomics was applied to investigate changes in metabolic pathways in response to Cr(VI) exposure (Kozłowska et al., 2022). The study population consisted of 220 male workers with exposure to Cr(VI) and 102 male controls from Belgium, Finland, Poland, Portugal and The Netherlands within the HBM4EU Chromates Study. Findings highlighted clustering by industrial chromate application, such as welding, chrome plating, and surface treatment, distinct from controls and not explained by smoking status, age, body mass index or alcohol use. Significant differences in abundance of metabolites primarily associated with fatty acid and monoamine neurotransmitter metabolism, oxidative modifications of the amino acid residues, excessive formation of abnormal amino acids metabolites and changes in steroid and thyrotropin-releasing hormone were observed in workers exposed to Cr(VI) in comparison to the control group.

The manuscript by Tavares et al., 2022 describes the results of the genotoxicity marker analyses (Reticulocyte and peripheral blood lymphocyte micronuclei, comet assay and oxidative stress) showing that even the measured exposure levels may not be fully safe (Tavares et al., 2022, "HBM4EU Chromates Study – genotoxicity and oxidative stress biomarkers for the biomonitoring of hexavalent chromium occupational exposure", *submitted*).

7.3 Key data gaps

HBM4EU provided EU relevant data on occupational Cr(VI) exposure to support the regulatory risk assessment and decision-making. In addition, the usefulness of new and specific different biomarkers (RBC and EBC) for the assessment of Cr(VI) exposure are evaluated, and specific methods have been developed for detecting Cr(VI) additionally to total Cr.

Further follow up need to be performed to:

- evaluate time-trends of Cr(VI) exposure on the EU-wide scale
- conclude if regulations have had a favourable impact on the Cr(VI) exposure.

However, in the HBM4EU study a rich dataset has been collected, which can be used as a baseline for future research applying the same methodology.

Based on the policy questions (please insert hyperlink to scoping document here, linked to “policy questions”), ongoing work to address the knowledge gaps is summarised in the table below. If you would like to read more about the work packages (WP), please visit the HBM4EU [website](#).

8 Future recommendations

The HBM4EU provided EU relevant data on occupational Cr(VI) exposure to support the regulatory risk assessment and decision-making. In addition, the capability and validity of different biomarkers for the assessment of Cr(VI) exposure were evaluated in a harmonised way and under quality assurance/quality control measures.

Some practical recommendations and aspects for the future are the followings:

- the use of human biomonitoring in conjunction with air measurements in the control of Cr(VI) exposure at workplaces
- the use of U-Cr as primary, first tier approach for the assessment of total, internal exposure
- the collection and analysis of paired U-Cr samples (pre-shift in the beginning of the workweek – post-shift in the end of the workweek) are recommended
- the usefulness of collecting and analyzing dermal samples from workers
- the determinations of U-Cr and other tested biomarkers (RBC-Cr and EBC-Cr(VI)) may not be interchangeable but rather complementary
- potential bystander exposure of workers should be considered
- standard operating procedures for field researchers, training on the use of the data entry template, as well as improved company communications should be implemented in future studies
- to identify exposure determinants that contribute to Cr exposure and to evaluate the influence of RMMs (Risk Management Measures) at workplaces, contextual information by dedicated questionnaires should be always collected.

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