1 Prioritised substance group: Hexavalent Chromium (Cr VI)

Lead author	Milena Horvat (JSI,SL) / Beatrice Bocca (ISS,IT)
Contributor	Janja Tratnik

Short overview of results of the activities carried out within HBM4EU in 2020 to answer the policy questions with reference to corresponding deliverables.

Policy Question	Short Summary of Results
1. What is the current (last 5 years) exposure of the European population to Cr(VI)?	Inventory of studies holding Cr(VI) exposure data was obtained in 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 substance; in particular, a total of 5 studies included Cr(VI) measurements: 2 of them from West European regions; 2 from South 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. A sampling frame to obtain EU recent HBM exposure data was developed by WP8 (see D8.1). 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. In AD8.1 an inventory of databases or datasets targeting occupational exposure to Cr in Europe (from WP 7.1 questionnaire) was reported. 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) (see below).
2. What is the level of exposure, environmentally and occupationally relevant to Cr(VI) in the EU population?	Cr(VI) has been identified as the first subject for a targeted occupational study under WP8 (see D8.5). Altogether 8 countries (Belgium, Finland, France, Italy, The Netherlands, Poland, Portugal, UK) volunteered to participate to the study on chromate exposure. Research plan for chromates study was published as AD8.2. After the publication of the research plan, 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 task 7.5. 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). In addition, to collect comparable data in a harmonised way, great efforts were made to develop Standard Operating Procedures (SOPs) 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, an ICI/EQUAS for Cr analysis in different biological matrices has started within WP9 in order to select candidate labs for the analysis of samples of workers exposed to Cr(VI) and the list of candidates is available in D9.3.

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	Results obtained in the chromate occupational study will allow to answer to the level of exposure to Cr(VI) in occupational settings. Analyses of samples of workers have been completed.
3. Does the exposure to Cr(VI) differ significantly between countries and population groups? What are the main reasons for differences in exposure?	In WP7 questions specific for Cr(VI) were identified to collect all the necessary information concerning countries (subdivision, GPS codes, town) 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. 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 in the statistical analyses to address Cr(VI)-specific differences among countries and population groups. Despite these protocols and procedures, the poor availability of HBM data on Cr(VI) in different countries and population groups does not allow to answer to this policy question so far. Within WP8, observations from occupational exposed cohorts could bring further evidence.
4. Is there a significant time trend of Cr(VI) levels in existing population studies?	A protocol for examination of the temporal trends of Cr has been elaborated (WP10; D10.2). However, no study was identified that have repeated Cr measurements available. Therefore, data are insufficient to evaluate time-trends on the EU-wide scale and to answer to this policy question.
5. What are the groups at risk?	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 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. Within the WP8, results of chromate study will aid to evaluate the Cr(VI) exposure in some of the most exposed classes of workers (chromium plating and welding). The main uncertainty for the evaluation of risk arises from the lack of knowledge on the relationship between Cr(VI) exposure and health effects. 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 summarised key knowledge gaps emerging from previous research. Specific activities, studies or other relevant steps to fill the missing information on Cr(VI) have been proposed in D13.5. In D5.5, it has been exemplify the inclusion of HBM data in risk assessment (RA) and health impact assessment (HIA) strategies. RA was performed also for Cr(VI). Since the data were limited to Finland and pre-authorization period, this policy question can be better answered when new, EU wide data on occupational exposure to Cr that will appear from the chromate study under WP8.

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6. Are the overall exposure levels (in different population groups) above any health-relevant assessment levels (HBM guidance values, TDI)?	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 Span, 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 (form 10 µg/L to 40 µg/L) and in erythrocye fraction of whole-blood (form 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. This policy question can be better answered when data on occupational exposure to Cr(VI) will be available under WP8.
7. Has the regulation under REACH had the favourable impact like a reduction of GM/median concentrations?	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). The current occupational biomonitoring data on Cr(VI) is scattered and its coverage is limited. Moreover, based on the very limited availability of the systematically repeated Cr(VI) exposure data available (as evidenced under the time trends policy question activities) this question cannot be answered at this stage. In future, the data from the chromate study would support not only implementation of occupational health and safety legislation but also EU chemicals legislation (REACH).
8. What are the current HBM methods for Cr(VI)?	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. In conclusion, the preferred technique for Cr determination is ICP-MS. An alternative is the speciation of Cr (VI) and Cr (III) by coupling ICP-MS to liquid chromatography. Within WP11, information about biological matrix previously used for the Cr(VI) measurement use in previuos studies and obstacle to link HBM data and health was given (D 11.1). Within WP8 and WP9 harmonised methodology for total Cr and Cr(VI) analyses including collection, conservation, transport, preparation and analysis of biological (urine, blood and exhaled breath condensate EBC) and industrial hygiene samples (air and wipes) were developed (as above reported). Moreover, SOPs were developed for any of these matrices (as above reported). In the same time, within the WP9, laboratories performing laboratory Cr analysis have been tested through QA/QC schemes for the determination of Cr in urine, whole blood and serum. The 3° round of proficiency tests has been completed. Additionally, a few laboratories have set up the methodology for the analysis of Cr(VI) in EBC (D9.7). Moreover, for EBC-Cr(VI) a small-scale interlaboratory

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	comparison to ensure the quality of the analysis has also been conducted (D8.5). The first list of approved laboratories for Cr analyses is 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).
9. Which are the appropriate biomarkers for Cr(VI)?	Regarding biomarkers of exposure, scoping document (D4.2) and deliverables (AD8.1) identified the urinary Cr levels 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 red blood cells (RBCs) were selected as the most suitable biomarker for the analysis of Cr(VI) exposure because Cr(VI) passes through cell membranes, while Cr(III) does not. The analysis of the exhaled breath condensate (EBC) was selected as a very good biomarker of occupational exposure to Cr(VI). Currently the most appropriate matrix for the determination of Cr(VI) is the analysis of RBC because only Cr (VI) can enter into them. An alternative to invasive matrices is the determination of Cr (VI) and Cr (III) in EBC to measure exposure to Cr(VI) compounds long after exposure. Furthermore, Cr-RBC correlated with Cr(VI) in exhaled breath condensate (EBC). 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 planned to be analysed in 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. Work is in progress. 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.

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