Prioritized substance group: Cadmium (Cd) and Hexavalent Chromium (Cr VI)

Responsible authors: Milena Horvat (JSI) and Alessandro Alimonti (ISS)
Short name of institution: JSI and ISS

E-mail: Mr. Alimonti has since retired. Please contact new CGL Beatrice Bocca instead: beatrice.bocca@iss.it or milena.horvat@ijs.si (Milena)

Phone: +39 649 902 826 (Beatrice) / +38 615 885 335 (Milena)

Short overview of results of the activities carried out within HBM4EU in 2019 to answer the policy questions with reference to corresponding deliverables

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What is the current exposure of the European population to Cd?	Inventory of studies holding Cd exposure data was obtained through WP7 (task 7.1) with an online questionnaire which was distributed with an aim to identify existing HBM studies. Within the WP10 (task 10.4) a substance-specific research protocol has been elaborated to exploit the available datasets with an aim to assess current Cd exposure of the European population and its geographical distribution. From the datasets having information on Cd internal exposure, 37 datasets from 17 countries have so far confirmed sharing of individual or aggregated data (the majority is individual data, 33 datasets) to assess the exposure in Europe and its geographical variability. Based on the data available, we decided to look at the exposure data for the period between 2007-2017. However, the work is in progress (acquisition of individual or aggregated data from data providers) and the number of datasets is constantly being updated. Preliminary assessment of the data available from the above-mentioned datasets has been done. So far, we have 27 datasets having Cd measurements for adult general population from all 4 geographic regions (north, south, east, west). Although the preferred matrix for internal Cd assessment is Cd in blood, the majority of the datasets (23) have the measurements available for urine, while only 10 for whole blood. Additionally, we have 5 datasets for Cd in cord blood, 3 datasets for Cd in child's blood, 2 for Cd in adolescent's blood, and 10 for Cd in child's urine and 1 for Cd in adolescent's urine. Based on the concentration ranges reported for adults, the levels in urine span from below LOD to 5.34 µg/L and in blood from below LOD to 6.26 µg/L. In cord blood the levels are <LOD-2.5 µg/L, while in children/adolescents <LOD-22.9 µg/L and <LOD-0.144 µg/L in blood and urine, respectively. However, the mean values for all datasets that have this data available are all below the established

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	HBM I value of 1 µg/L urine (adults) and 0.5 µg/L urine (children/adolescents). Further assessment as described in the research protocol is on-going. Additional exposure assessment will be performed based using harmonised methodology developed and agreed in task 8.1 (aligned studies) to obtain EU-wide coverage for recent exposure (2014-2018). Cadmium will be measured in samples of identified on-going studies (200-300 participants per study). The studies selected include adults (20-39 years) from 8 countries distributed among 4 geographical areas of Europe: Denmark, Iceland, Czech Republic, Poland, Croatia, France, Switzerland, and Germany. Cadmium will be determined in urine (available in all 8 studies) or whole blood (available in 3 studies). Among others, results obtained in aligned studies will allow further evaluation of the proper use of biomarkers (urine vs. blood) at low level of exposure.
	The laboratories performing laboratory analysis have been tested through the QA/QC scheme, which has completed the third round of proficiency tests for the determination of Cd in urine and whole blood. The first list of approved laboratories is now available and includes 33 labs for urine samples and 22 labs for Cd in blood. The comparable results obtained from the aligned studies will also enable derivation of European Reference Values (ERVs) as part of task 10.3.
Does the exposure to Cd differ significantly between countries and population groups? What are the main reasons for differences in exposure?	This question will be answered once the work described in the substance-specific research protocol developed within task 10.4 is completed. The work is in progress as described above. Spatial analysis will be done according to the Statistical analysis plan (Deliverable 10.5, Section 7). Once the required data (individual or aggregated) from the available datasets will be acquired, the data will be compared statistically and visualised with respect to geographic regions (north, south, east, west), countries and the NUTS regions. In cases of individual data (which is the majority of the datasets), we'll be able to confound for the known and hypothetical determinants of Cd exposure (e.g. smoking) to reveal the geographical and/or environmental pattern(s). This will also allow as to identify the main reasons for possible differences.
Is there a significant time trend of Cd levels in existing population studies?	Only 3 datasets have been identified that have repeated Cd measurements available: German ESB and GerES (from 1986), Czech Republic (from 1996) and Belgium with limited time points (3). Therefore, data is insufficient to evaluate time-trend on the EU-wide scale. However, as described by Becker et al. (2013) no obvious trends of decreasing Cd concentrations have been observed in neither of the followed population groups in Germany. Similarly, also in Czech Republic, no significant trend was reported (Cerna et al., 2012).
Is there a link between high soil contamination with Cd and human exposure via dietary sources?	Within the work package WP5 (task 5.3) available data has been identified and applied into the mathematical models to describe the transfer from soil via fertilizers to plants (dietary source) and from plant to human via diet. Due to the scarcity of the external data available (soil, food, fertilizers, etc.), the application was limited to the region-specific case study in Slovenia. The local case study is

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	described in the Deliverable 5.5. The model enables to predict an oral intake via data on Cd concentrations in soil, phosphate fertilizers and food. Using HBM and food consumption data, the oral intake will be validated using the PBPK modelling (work in progress).
Which population groups are most at risk?	Dietary intake limit values are derived based on relationship between renal tubular impairments (proteinuria) and urinary Cd for women aged above 50 years (EFSA, JEFCA, ATSDR). Also, the HBM4EU HBM guidance value (HBM-GV) has been derived for the general population based on the increase in prevalence of elevated beta-2-microgloblin urinary levels as indicator of tubular proteinuria. The HBM-GV has been set at 1 ug/g crea, similar to the value of EFSA and the German HBM-I value. The kidney dysfunction is considered as the critical effect, but there is also evidence for low dose bone effects. The EFSA evaluation (2009) of the dietary Cd exposure showed that exposure of some subgroups, such as vegetarians, children and smokers and people living in highly contaminated areas could exceed the TWI of 2.5 ug/kg bw/week by about 2-fold. However, the revised assessment (EFSA 2012) indicated that the actual risk of adverse effects for an individual at current dietary exposure in the EU was low for adults, because the TWI was established based on an early indicator of changes in kidney function suggesting possible kidney damage later in life. Within task 5.3 (Deliverable 5.5), evaluation of fractions of urinary samples exceeding the threshold value of 1 µg/g creatinine has been assessed for the available HBM data for women >50 years. The data indicated exceedance of the HBM guidance value for the higher percentile of exposure. Furthermore, attributable burden of disease related to Cd exposure was calculated in women aged > 50 years for chronic kidney disease, as a critical health effect, and osteoporosis at hip or spine. However, the estimations are preliminary and still premature for the use in policy recommendation. The main uncertainty arises from the questionable causality between Cd exposure and bone/kidney effects at low doses of exposure (below 5 µg Cd/g creatinine) that are commonly observed in the general European population. This has been outlined also in the Deliverables 13.4 and 13.5 elaborated within the task 13.2 with a purpose to establish exposure-health relationships. Variation in renal physiology is one of the main factors confounding the association at low exposure levels (co-excretion of low-molecular weight proteins and Cd). Moreover, normalisation of Cd concentrations for diuresis is also a questionable issue, therefore the health risk assessment should rely on Cd measured in blood to compensate uncertainties related to Cd in urine (Stajnko et al., 2017).
Are the overall exposure levels (in different population groups) above any health-relevant assessment	We'll be able to answer this question once the complete data from the available datasets will be acquired (work within 10.4, described above). For the time being, we have concentration ranges available from the metadata of various studies, and from the literature.

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levels (HBM guidance values, TDI)?	However, based on the EFSA evaluation of the dietary Cd exposure, mean exposure of adults across Europe is close to, or slightly exceeding the TWI of 2.5 ug/kg bw/week. The work conducted within task 5.3 (Deliverable 5.5) included evaluation of fractions of urinary samples exceeding the threshold value of 1 µg/g creatinine (critical Cd urinary level established by EFSA; HBM-I value and HBM4EU HBM guidance value) from the available HBM data (urinary Cd in women >50 years from Spain and France – BIOAMBIENT_ES and ENNS studies; and urinary Cd in women 35-45 years from 17 EU countries - DEMOCOPHES). The data indicated exceedance of the HBM guidance value for the higher percentile of exposure. These data, however, are not representative of the population at large and should be dealt with caution.
Has the regulation under REACH had the favourable impact like a reduction of GM/median concentrations?	Based on the very limited availability of the systematically repeated exposure data available (as explained under the time trends policy question activities), this question will be difficult to answer at this stage and will have to wait until repeated HBM exercises are performed in the future.
Are environmental quality standards for Cd in water sufficiently restrictive to protect human health from exposure to cadmium via the environment and via dietary sources?	Work in progress within WP10 and WP12 Following collection of HBM and dietary intake EU-wide data, and validation through the PBPK models, (drinking) water as a source of Cd will be included in exposure pathway to derive 'limit' value for Cd in water. In some of the countries (e.g. Slovenia) actual measurements in water and in population will allow direct links to be established.
What is the maximum acceptable level for Cd in food stuffs?	Work in progress within WP10 and WP12 (similarly as above)
Can input be given to the decision as to whether the exemption for Cd in quantum dots under the RoHS Directive can be scrapped?	In general, population such a relationship is difficult to establish as currently the level of exposure is rather low and the time trends not established. Moreover, at this stage studies on occupational exposure in production line are also not available.

References

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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).
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 were 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).

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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 will be completed by the end of 2019.
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 characterise 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 priority substances including Cr(VI) (see D10.2). 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.
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). 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.
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 evaluate the exposure to Cr(VI) 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 exposure and health effects.

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	This issue has been reported in D13.4 and D13.5 with a purpose to establish exposure-health relationships. WP13 did give a detailed overview of the available knowledge on AOPs for Cr(VI) (D13.4) 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 deliverable D13.5.
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/m^3 until 5 years after the transposition date and after that period the limit will be 0.005 mg/m^3. On the other hand, in France and the Netherlands, an OEL of 1 µg/m^3 has been set for Cr(VI) in all uses. These are the most stringent OELs currently set in workplace in EU. Respect to these exposure levels available, we will able to answer this question after the analysis of samples of air and HBM samples of workers within the occupational chromate study.
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 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.

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	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).
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 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) 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 now completed. Additionally, 5 laboratories have set up the methodology for the analysis of Cr(VI) in EBC. Moreover, for EBC-Cr(VI) a small-scale interlaboratory comparison to ensure the quality of the analysis has also been performed. The first list of approved laboratories is now available (see D9.3) and includes 15 laboratories for Cr measurements.
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).

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	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) by 5 countries 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.

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