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HBM4EU chromates study - Reflection and lessons learnt from designing and undertaking a collaborative European biomonitoring study on occupational exposure to hexavalent chromium

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ABSTRACT

The EU human biomonitoring initiative, HBM4EU, aims to co-ordinate and advance human biomonitoring (HBM) across Europe. As part of HBM4EU, we presented a protocol for a multicentre study to characterize occupational exposure to hexavalent chromium (Cr(VI)) in nine European countries (HBM4EU chromates study). This study intended to collect data on current occupational exposure and to test new indicators for chromium (Cr) biomonitoring (Cr(VI) in exhaled breath condensate and Cr in red blood cells), in addition to traditional urinary total Cr analyses. Also, data from occupational hygiene samples and biomarkers of early biological effects, including genetic and epigenetic effects, was obtained, complementing the biomonitoring information. Data collection and analysis was completed, with the project findings being made separately available. As HBM4EU prepares to embark on further European wide biomonitoring studies, we considered it important to reflect on the experiences gained through our harmonised approach. Several practical aspects are highlighted for improvement in future studies, e.g., more thorough/earlier training on the implementation of standard operating procedures for field researchers, training on the use of the data entry template, as well as improved company communications. The HBM4EU chromates study team considered that the study had successfully demonstrated the feasibility of conducting a harmonised multicentre investigation able to achieve the research aims and objectives. This was largely attributable to the engaged multidisciplinary network, committed to deliver clearly

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understood goals. Such networks take time and investment to develop, but are priceless in terms of their ability to deliver and facilitate knowledge sharing and collaboration.

1. Introduction

By revealing internal exposure and early effects in the human body, human biomonitoring (HBM) can provide an invaluable contribution for public health decision-making based on the risk assessment of chemicals. HBM has been considered as a relevant approach for the health risk management, e.g. under EU REACH regulation (Boogaard et al., 2011) and provides relevant information to support policy development by delivering better evidence of workers' exposure to chemical substances in the scope of different regulatory frameworks (Viegas et al., 2020; Jones, 2020). Both the general population (exposed through environment) and workers (occupationally exposed), can benefit from the use of data generated by HBM studies, but further harmonisation and standardization of methodologies is necessary to improve and refine such assessments (Louro et al., 2019).

The EU human biomonitoring initiative (HBM4EU) is a Joint Programme that aims to standardise and use biomonitoring to understand human exposure to chemicals (via the environment, in occupational settings or through using consumer products) and the related health risks, with the aim to improve chemical risk assessment and management as well as to support policymaking (Ganzleben et al., 2017). HBM4EU is a joint effort of 30 countries, the European Environment Agency and the European Commission, co-funded under Horizon 2020 (www.hbm4eu.eu).

Occupational health has a long tradition of using HBM to control worker's exposure. The use of HBM in occupational settings can serve as a model system to implement and improve HBM approaches that can be expanded to environmental exposure in the general population. More research in best practices and procedures for occupational HBM was to be addressed within the remit of the HBM4EU project as various challenges still present themselves. Typically, a limited number of workers can be, and are, recruited into national studies. Furthermore, the studies performed by different researchers in individual countries are usually not aligned with respect to sampling/analytical methodologies or the collection of contextual data, which complicates the comparison of the findings and use of the data in regulatory risk assessment at a European or international level. Combining national surveys using harmonised study designs, methodologies and protocols can potentially greatly improve the information collected and provide the benefit of harmonised data collected in different countries, as demonstrated in the earlier DEMOCOPHES project (Den Hond et al., 2015).

One of the most important aims of the overall HBM4EU project is the harmonisation of methodologies and standardized collection of the data useful for EU decision making. In line with this aim, we previously presented a multicentre study that intended to characterize occupational exposure to hexavalent chromium (Cr(VI)) in industrial settings across Europe (Santonen et al., 2019). Examples of industrial activities that are sources of workers exposure comprise welding, electroplating, surfaces treatment and leather tanning (Elhosary et al., 2014; Lin et al., 2018; Pan et al., 2018; Wang et al., 2012). The main exposure routes are dermal contact, inhalation of dust and mist or fumes, and ingestion due to hand-mouth contact. Despite its carcinogenicity, the use of Cr(VI) compounds (chromates, chromium trioxide and dichromium tris(chromate)) for specific purposes is still authorized under the European regulation (EC, 1907/2006) concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) and thereby still raises concern. The main adverse health outcomes due to chronic Cr(VI) inhalation are lung impairment, including pneumonia, bronchitis, asthma and lung cancer (IARC, 2012). This study also aimed to investigate the relationship between biomarkers of exposure, environmental monitoring and biomarkers of effect and to recognise how all these tools

add to occupational exposure, risk assessment and management processes. Although a number of studies has reported data on human exposure to Cr(VI), comparably fewer have included effect biomarkers analysis. As Cr(VI) is a direct and indirect (through reactive oxygen species production) genotoxic metal (Chen et al., 2019), the effect biomarkers included were associated with oxidative stress and DNA or chromosome damage (Li et al., 2014; Pan et al., 2018). In addition, following innovative methodologies development, omics-based biomarkers were also analysed. Furthermore, it is expected that the data generated may be used to address questions, for example, related to the efficacy of the regulatory measures already implemented, as well as allowing for the identification for further actions. In this contribution, we reflect on the experiences gained from undertaking this study so that lessons can be learnt and shared with the wider scientific community and can be considered when developing future occupational multicentre studies.

2. Materials and methods

The HBM4EU chromates study team participated in a number of activities to design, harmonise and undertake this multicentre study, the key ones of which are summarized in Fig. 1. It is important to highlight that whilst presented as a workflow, the events were not always sequential, with several of the activities occurring in parallel (e.g. company identification and company/worker recruitment and sample and data collection; data entry and cleaning and sample analysis). It is also essential to highlight the several overarching activities that took place across the different elements of the work program. For example, there was extensive quality assurance and quality control round robins for the biological sample analysis aspects. The HBM4EU chromates study core network expanded during the course of the study to include further researchers to collect the field samples and contextual data, additional scientists to process and analyse the collected samples, to analyse cytogenetic and molecular effect biomarkers, etc. Finally, there were extensive communications within the project team via web conferences, email correspondence to discuss the work programme as it progressed and evolved.

It was important for the HBM4EU chromates study project team to reflect on the research and evaluate both the positives and limitations of the harmonised approach that was developed and applied. Following completion of the data gathering and processing elements of the work, members of the project team were asked, via email, to provide feedback on three things that went well with the study and three things that were felt could be improved upon. The responses provided were used to facilitate an open discussion during a session at the 4th HBM4EU training school, May 14, 2020. Here, participants were able to identify any additional points that they wished to raise as well as discuss how the project team could learn from the experiences gained to inform the future HBM4EU occupational multicentre studies, focussed on diisocyanates and electronic waste management sectors. Additional input was provided during the drafting of HBM4EU deliverables, various project team meetings, with further contributions being provided by the (co-) authors during the drafting of this manuscript. The information gathered from these various initiatives is summarized in the following sections, categorized under two broad groups: (i) What were the successes and benefits? (ii) What were the issues encountered and suggested improvements? For the second group, the views are divided into several topics; these being ethics and General Data Protection Regulation (GDPR); standard operating procedures (SOPs); sample collection; sample analysis; data reporting, input and analysis; and communication.

3. Results and discussion

It should be firstly highlighted that undertaking a complex multi-centre study takes a great deal of time and effort and this should not be underestimated. The research plan for the HBM4EU chromates study was published as a HBM4EU Deliverable report in December 2017 (Ndaw et al., 2017), with development of the core chromates study team and discussions to develop the project plan taking place well in advance of this. The development of the SOPs started in February 2018 and continued until the end of 2018, since some of the SOPs were identified as requiring refining/clarification after the first site visits occurred in November 2018. The field measurement campaigns started in most countries at the end of 2018 and progressed through to the later part of 2019. Data analysis and reporting is still ongoing at the time of drafting this manuscript (end 2020) and will continue until at least early 2021.

3.1. What were the successes and benefits of the HBM4EU chromates study?

A number of successes and benefits through adopting the harmonised approach in the HBM4EU chromates study were identified. From a project perspective, that is, in terms of achieving the study aims and objectives, the harmonised approach has provided new information on the usefulness of new biomarkers for the monitoring of Cr(VI) exposure to be generated.

In the research protocol, the target sample numbers for exposed workers were 50 workers per country, aiming to collect 100 urine (two samples per worker), 25 blood and 50 exhaled breath condensate (EBC) samples (from 25 workers, each providing two samples) per country. In addition, 25 controls were to be recruited in each country, each providing one urine, blood and EBC sample (Santonen et al., 2019). In the research protocol eight countries were to participate in the HBM4EU chromates study, however the National Health Laboratory (LNS), together with the Multisectoral Occupational Health Service (STM) of

the Ministry of Health in Luxembourg were able to implement the protocol at a later stage, recruiting several welding companies, with their data being included in the HBM4EU chromates study. Thus the country participation increased to nine.

Forty-four companies were recruited for which exposed workers consented to participate in the study. Some of our control population were recruited in, for example, office staff in these companies although three additional companies were also recruited. 399 exposed workers and 203 controls were enrolled into the study, which was very close or in excess of our respective targets of 400 workers and 200 controls (>99.75% of targets achieved).

For the exposed workers, a total of 780 urine samples (397 pre-shift and 383 post-shift), 345 blood samples and 342 EBC samples were collected (167 pre-shift and 175 post-shift). Concerning the control population, a total of 143 urine, 175 blood and 98 EBC samples were obtained. Whilst the numbers of some sample types collected were slightly lower than the overall target, this was due to some countries electing to not collect particular samples (e.g., the UK did not collect blood samples, Poland and Luxembourg did not collect EBC samples, and the UK and Italy did not collect urine samples from the controls). In addition, 25 air and 25 sets of dermal wipe samples were to be collected per country (the aim being to collect these samples from half of the participating workers). 293 inhalable (218 collected outside RPE and 75 inside RPE respectively) and 155 respirable samples were collected, with 270 sets of dermal samples being collected, which exceeded our target.

The number of participants and samples collected from the nine involved countries allowed us to achieve the required statistical power for the study (Santonen et al., 2019) and obtain a more comprehensive and richer dataset to inform regulatory and policy agencies in the EU, as well as further scientific investigation when compared to (smaller) national studies. Field work also allowed us to engage workers, companies and to generate awareness for the need to control exposure to Cr(VI) in the investigated occupational sectors across Europe, as well as for the HBM4EU study at both National and International level.

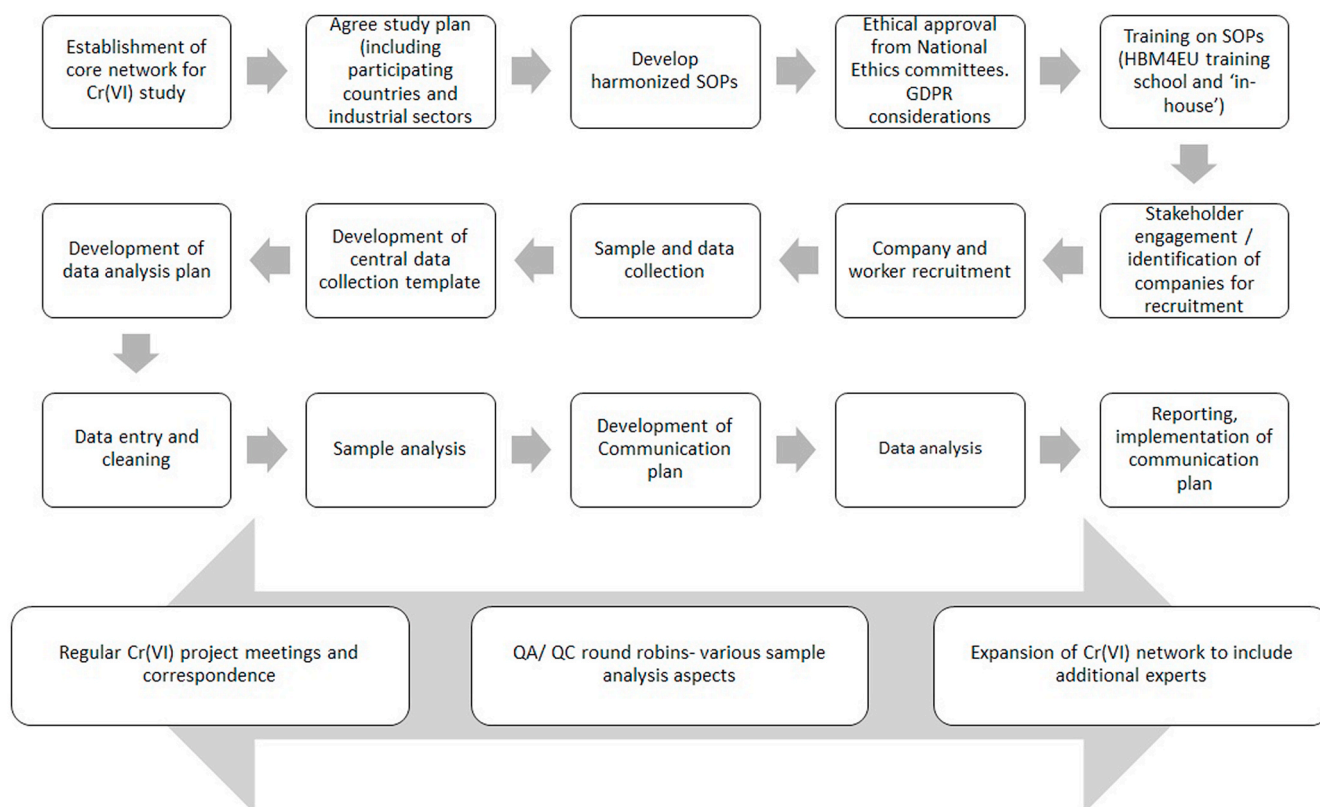


Fig. 1. Key activities in the design and implementation of the HBM4EU chromates study.

The study served also as a valued 'educational tool', where several lessons were learnt concerning the conduct of aligned, multicentre occupational studies (which will be discussed in the following sections). It was also reflected that the effort put into the development of the harmonised process in the HBM4EU chromates study would make the process easier for subsequent occupational monitoring campaigns, for example, in HMB4EU and in future projects, as many of the SOPs, materials and ethical procedures can be reused, updated or adapted if needed.

Other researchers and practitioners in the field of the scientific disciplines involved can also benefit from the harmonised efforts through applying the freely available SOPs (and materials which these contain) (Porras et al., 2019) to their own studies focused on Cr(VI). This will allow the potential of future data to be collected in the same standardized manner, thus allowing opportunities for data pooling, identify exposure trends, comparisons between different risk management measures available etc. to be undertaken. As already mentioned, this opportunity has already been exploited through the inclusion of data collected in accordance with the study protocol in Luxembourg.

It was clear that researchers involved in the study recognised that these successes were achieved through the valued and close co-operation between the participating institutions and researchers from the different countries, who were all focused and working together towards clearly defined aims and objectives. The core project network worked extremely well together and were personally committed and invested in maximizing the successes of the overall project. It was also observed that members of the HBM4EU chromates study network have continued to build and expand their relationships and networks through other biomonitoring networks (e.g., ISES-Europe working group, OECD Working Party on Hazard Assessment (WPHA) and Working Party on Exposure Assessment (WPEA)). These only serve to increase further the benefits to the scientific and regulatory communities, where exposure data and science are of paramount relevance.

3.2. What were the issues encountered and suggested improvements?

Despite adopting a harmonised and consistent approach and the time and effort that was invested into this, issues were encountered and raised. For ease, these have been grouped under several key themes, these being: ethics and GDPR; SOPs; sample collection; sample analysis; data reporting, input and analysis; and communication.

3.3. Ethics and GDPR

The management of a biobank and accompanying transport of biological samples within the context of an international occupational study is regulated by national, European and international regulation (Knudsen and Faber., 2018a). The variation and fragmentation of these biobank regulations and the consequently need for more harmonisation and standardization was the main conclusion of a report (Gottweis et al., 2012) published by the European Commission. One of such regulatory requirements concerning biobank practices is that the transport of biological samples between research partners takes place under material transfer agreements (MTAs) (Cervo et al., 2016). The restrictions and obligations of these MTAs doesn't only apply to primary sample (or parent sample), but also to (post-) interventions made using the primary sample and/or to derivative samples (Bennett et al., 2007). The fragmentation of the regulation in combination with the growing regulatory requirements, makes it more difficult to freely transfer biological samples between research partners. Therefore, international harmonisation and standardization of the biobank practices is required within the context of an international occupational study.

Ethics is a national issue. However to comply with EU regulations, special attention must be given towards data protection with respect to the GDPR regulation (Knudsen and Faber., 2018b). This regulation sets requirements for information to participants about confidentiality

measures, as well as requirements for the investigators to secure data. Data handling was organised locally with pseudonymised data forwarded to the central data management. Mutual data transfer agreements were signed and a parallel system was put in place for sample transfers. Prior to submission to National Ethics committees, a standard protocol with information sheets, consent forms etc was developed and implemented. To comply with the GDPR the participants were informed about such transfers and the protocol and information sheets provided explanations and details.

It was evident from the feedback of the various ethics committees that they would often highlight different points for further clarification and for the local project team to respond to, e.g., request to clarify what is meant by 'DNA damage' and addition of a question for the study participant to agree for unexpected results to be shared with their general practitioner. A separate ethics committee also raised the point that workers with 'elevated' results may be worried and request clarification on what is considered as being unsafe, undesirable levels. In the worker participant information sheet, we emphasised that biological monitoring is a measurement of exposure only and not a measurement of ill health and that when participants received their results, we would provide an interpretation on whether these were elevated or not. The above comments led to the ethics committee requesting to see an example results letter outlining the different example sentences for each potential scenario (e.g. within the background reference range, above the reference range but less than the guidance value and above the guidance value). Another ethics committee requested that clarification in the text on what was being studied now; in the future and to what, exactly, participants need to give their consent to. A further Ethics Board requested the information and consent forms to be provided in further languages if appropriate and necessary (e.g. Swedish as well as Finnish).

The lessons learned from an ethics and GDPR perspective are that these are two critical and essential aspects of the study design but that it can be quite time consuming to have everything put in place, submitted, reviewed, responded to and finally agreed, which clearly can then affect timescales for the following phases of the project. Different ethics committees may highlight different attributes of the application and materials for clarification and moving on it is important to synthesize these to establish what the commonalities are so that these can be addressed in subsequent ethics applications at an early stage. The approach adopted for the HBM4EU chromates study was to recruit companies to participate in the campaign and for the team to then invite their workers to participate. One ethics board highlighted that to secure a free choice by the worker to participate, the invitation to participate should be sent out by the researcher to the employees directly (without any intervention of employer) as their decision making may be influenced, either positive or negatively in this manner. This is an important point to consider for future studies, although perhaps very difficult to achieve in practice.

3.4. SOPs

Table 1 lists the SOPs prepared for the HBM4EU chromates study, which are freely available at the online library of the HBM4EU website ((Porras et al., 2019, <https://www.hbm4eu.eu/online-library/>). The SOPs used in the campaign were developed following an extensive drafting period, with input from many researchers across the project team. The procedures outlined in the SOPs were also delivered as part of a training school, which allowed participants to identify any areas of uncertainty, etc. However, in some cases, it was evident that the field researchers, those who needed to fully understand and apply the contents of the SOPs, had not been involved in these earlier discussions. This resulted in some last-minute modifications on SOPs taking place following the start of the sampling campaigns, which is not desirable.

It was highlighted that SOPs were only available in English, not in the local languages of the other European countries involved in the campaign. Whilst it was reflected that this was not an issue for the

Table 1

List of detailed SOPs prepared for the HBM4EU chromates study (Santonen et al., 2019), with additional information on the laboratory analysis undertaken for the sampling matrices.

SOP No.	Title	Topic/Sampling matrix	Laboratory analysis
1	Standard operating procedure for selection of participants and recruitment, information to the participants, informed consent	Recruitment and consent	–
2	Standard operating procedure for completion of company and worker questionnaires	Company and worker questionnaires	–
3	Standard operating procedure for blood sampling, including sample storage and transfer	Blood	RBC – Cr; Plasma – Cr, Plasma-PFAS; Whole blood - Micronucleus & Comet assays, Epigenetics; Reticulocytes - Micronucleus assay
4	Standard operating procedure for the collection of exhaled breath condensate samples	EBC	Cr(VI) and Cr(III); Ni and Mn
5	Standard operating procedure for urine sampling, including sample storage and transfer	Urine	Total Cr, Ni, Mn, oxidative stress biomarkers (e.g. malondialdehyde, 8-hydroxy-2-deoxyguanosine) and creatinine
6	Standard operating procedure for air sampling of inhalable and respirable dust fraction and (hexavalent) chromium	Air	Total Cr, Cr(VI)
7	Standard operating procedure for obtaining dermal wipe samples	Dermal	Total Cr
8	Procedure for comparing occupational hygiene measurements with exposure estimates generated using exposure models	Contextual exposure determinant information	–

HBM4EU chromates study team it may have been for some of the field researchers, particularly with some of the more technically demanding language used. It was mentioned that in at least one instance the field researchers translated a SOP into their native language themselves so that they were more comfortable with their understanding of what was needed to be done. It was also considered that sample transfer details (list of labs and contract details) should have been included in the SOPs, rather than separately.

The lessons learned from the development of the SOPs is that the time and effort involved in this activity should never be underestimated by researchers, and that, even despite this, last minute, issues do crop up. It is therefore essential that those involved in implementing the SOPs (i.e. the field researchers) are involved in the finalization of these documents. Whilst a training school was held for the HBM4EU chromates study, not all researchers involved in the fieldwork were able to participate and different local training regimes were likely to have been implemented in the various countries. More effort should be made to run centralized training sessions, for example via webinars, well in advance of the monitoring campaigns commencing so that common questions and uncertainties can be promptly addressed. If SOPs are to be translated

into local languages for future studies there would be a need to verify that the translation is indeed correct.

3.5. Sample collection

Field work to collect all the samples and contextual data was highlighted to be more time consuming than anticipated (by both the field researchers and the participating workers). It was considered that completion of the detailed worker questionnaires and collecting all of the necessary samples may have, in some instances, reduced the effective working time during the sampling day. In addition, a significant number of the participating companies were small in size, with low numbers of workers who were actually eligible to participate in each. Therefore, any reduction in working time for the participants may have a significant impact on the company's productivity. Also, due to the fact that companies were smaller in size than expected, more time and effort was spent to identify, visit and recruit additional companies reach the target number of study participants (and obtain their corresponding samples). Exposures in SMEs are important to understand and often overlooked precisely for the reasons just mentioned.

Specific issues were noted with respect to the worker and company questionnaires, with these cited as being too long or short and the collection of the EBC samples were also highlighted as being time consuming. It was highlighted that, where possible, all samplings (excluding pre-shift) should be done as close as possible to the end of the shift (preferably post-shift). However it was also recognised that this may not be possible due to numbers of workers involved and their desire to leave work promptly following completion of their work shift.

Despite the SOPs, there were some variations in procedures that were followed. For example, air sampling pumps were not always switched off during the breaks and there was variability in the sample types collected, with some diversity of air samples being collected depending on the job being undertaken. In one country, only inhalable (total) dust samples were collected, whereas in the other countries both inhalable and respirable dust samples were collected (as per SOP). There was also some variability in the positioning of samples, despite instructions being given in the SOP that samples were to be collected from the breathing zone area, except for those collected from welding operators where these were to be inside the welding helmet. For example, some countries collected samples outside the RPE (in the breathing zone) whereas others collected samples both inside and outside of the RPE. In one country only pre-shift and end shift dermal wipe samples were collected although it was instructed to also collect samples before break periods.

EBC-Cr(VI) is a new biomarker-matrix combination which has been used only once before (Leese et al., 2017) in occupational biomonitoring study. Some EBC results were obtained, which were then excluded from the data analysis (high Cr(III) levels which questioned the reliability of the Cr(VI) results) which was considered to be due to different complexing (EDTA) solutions being used or contaminated glassware.

One of the lessons learned from the sample collection is that it was more time consuming than anticipated. The HBM4EU chromates study was very ambitious, collecting a wealth of biomonitoring, occupational hygiene samples and supporting questionnaire and contextual data so to achieve its aims and objectives. However, consideration must always be given to the impact that such sampling strategies have on company/worker participation and their working practices, numbers of field workers required to ensure efficient sample collection etc. Engagement and recruitment of SMEs into exposure monitoring campaigns is particularly challenging and sufficient time and resources must be allocated to this. Overall, sample and data collection were in adherence with the SOPs focussed on the biological samples, with deviations occurring in some instances with the occupational hygiene samples. Whilst the study was focussed primarily on HBM, the occupational hygiene samples served an important role in the study and so it is important that the same level of robustness and rigour be given to these samples. Ensuring that more structured training of the field researchers

takes place prior to commencing the sampling campaigns may help address this. Concerning effect biomarkers analysis, it is important to ensure that samples reach the laboratory in a short period of time after collection, particularly, if blood cells must be immediately processed upon arrival (e.g., lymphocytes cultures for micronucleus analysis in blood lymphocytes). Although it is trivial to transfer biological samples among laboratories, it must be stressed that adequate packaging and transportation conditions (e.g. temperature) have to be ensured to not compromise the success of the analyses.

3.6. Data reporting, input and analysis

A MS Excel data template was circulated for researchers to input data and results obtained from the chromates study. This data template was very large so to facilitate entry and transfer of all data collected under the HBM4EU chromates study SOPs. Templates of such a size can inadvertently lead to data entry errors occurring and so those populating the files needed a great deal of care and diligence.

In most cases, the HBM4EU chromates data template was populated according to given instructions. However, there were many exceptions, and it was evident that some researchers had difficulties with completing the template and there was variation in how this was done. For example, despite requesting that work task and overall work duration be given in hours, it was often given in minutes (sometimes with units included, other times not). On occasions entries asked for the data user to refer to other lines of information or indeed merged cells (e.g., free description of the workplace of all workers in the same company) which added time and effort to the data cleaning process. On a few occasions, it was apparent that details of the weekly work schedule were provided rather than details of the work tasks actually undertaken during the measurement period. Despite the inclusion of drop-down lists to facilitate data entry (e.g. for local exhaust ventilation and respiratory protection equipment), these were not always used and instead were manually removed by those inputting data and replaced with free text data. In response to the question, "If other tasks were done during the measuring day, please specify here (free text)", often an entry of 'yes' was assigned, with no additional information provided. Less commonly, answers were provided which did not relate to the question being responded to. Some of these issues were perhaps due to the fact that the individual SOPs developed for the study did not cover data reporting, and there was no overarching SOP covering this aspect. Whilst instructions were provided separately by email to researchers concerning many of these points (and others) to assist with the data entry process, these were not always considered. This resulted in significantly more time being taken to check, verify and clean the results before data analysis could commence.

Limit of Quantification (LOQs) varied in different laboratories, which may have had an impact on the results of, for example, EBC Cr(VI) measurements since the levels in controls and workers were often low. The verification of LOQs in each country for all sample matrices should have been done before the study began in order to assess the potential impact of possible differences and to consider options for limit the impact of these differences.

There were some deviations in the analysis undertaken for the collected occupational hygiene samples. The overarching focus of the study was on the biomonitoring sampling and analysis with extensive QC and QA procedures being put in place for this. Whilst efforts were made to harmonise the occupational hygiene sample collection, with details of the analytical methods to be used for analyzing the collected samples being included in the SOPs, the sample analysis was not part of the formal QC/QA programme. Despite the SOP stating that all air samples were to be analysed for both total Cr and Cr(VI), only five of the participating countries did so.

Some differences in the biomonitoring analysis did however occur. Although this was a chromates study, blood, urine and EBC samples were also analysed for nickel and manganese. For example, urinary

nickel levels were suggested to be analysed for welders and those platers performing nickel plating; urinary manganese levels were suggested to be analysed for stainless steel welders; however, this did not always take place.

An important but still overlooked issue is that of the control group selection. In the majority of occupational studies reporting HBM data the control group consists of administrative workers from the same industry where exposed workers were selected. It is assumed that those workers, while performing their activities in an office, are not exposed to the occupational chemicals under study. Preliminary data from this study showed, however, that some individuals from the so-called "non-exposed group" are indeed exposed to low concentrations of Cr(VI), possibly present in the ambient air or even in surfaces of common spaces like canteens. Therefore, a double lesson is learnt from this knowledge: on one hand, controls outside the industry should be included in occupational studies involving emissions of chemicals that may persist in the air and surfaces and, on the other hand, administrative workers must also be followed by the occupational health services as potentially exposed workers if HBM data is not available for them.

The lessons learned from the data reporting, input and analysis were as follows. It is necessary to have a central tool in which to input data collected from such studies; however, it is essential that this tool is useable and clearly understood by those that need to populate it. It is necessary to hold training sessions with those that will be required to populate the tool so that they are familiarized with this and the data entry process (for both contextual and numerical data) that they must follow. It should be agreed by all involved that the tool will be used as per instructions and that, in the event that the template is unofficially modified or data are not input correctly, it will be returned to the provider to be corrected and amended. This agreement will ensure that the instructions are followed as well as reduce time spent on data checking and data cleaning step. The tool must be accompanied by text in each of the SOPs focused on data reporting for those particular aspects of the study. This text should detail how data entry should be undertaken, how calculations should be undertaken (where necessary), with examples given so to assist those in the tool completion and ensure that this is carried out in a standardised manner. Where possible the tool should be modified to allow for ease of entry and for key aspects to be highlighted to assist data interpretation e.g. flag data below LOQ or possible outliers. It was also apparent that much more instruction with respect to the collection and analysis of the occupational hygiene is warranted and that appropriate QA/QC for these samples should also be included within the programme of work.

3.7. Communication

The HBM4EU chromates study involved numerous companies and participants, with the project team having a wealth of knowledge and experience in undertaking occupational site work. However, challenges still presented themselves when completing the work. Some countries reported difficulties with engagement with companies and workers, with challenges being experienced with receiving clear information on the activities being undertaken from the companies' representative. There were also several instances where field researchers arrived on site to undertake sampling to discover that the workers were not undertaking any work where Cr(VI) exposure may occur, e.g. in some cases welders welded mostly mild steel instead of stainless steel; fewer workers than originally communicated were to be involved, or processes were not operating due to maintenance. These are common problems in industrial hygiene studies and the risks could be minimised by obtaining more accurate communication with the company representatives to ensure that the information they provide has reflects input from site supervisors, foreman, etc., who are more integrally involved in the activities of interest and their daily production regimes.

It was also important that due care and consideration be given to communicating the results of the study to the participating workers,

companies and wider stakeholders. Communication of the company specific data to participating companies was the responsibility of the each participating institute, according to their national/standard practises, with information leaflets also being provided.

The HBM4EU chromates study team however reflected that more consideration needed to be given to the development of a dissemination plan for the wider communications of the project findings.

Whilst the core project team itself communicated extensively via web conferences, email exchanges, training schools etc., it was still evident that communications were perhaps not always reaching those who also needed to receive it (e.g. training for all the field researchers).

The major lesson learned is that good, clear communication networks must be established with those involved in multicentre studies, whether these be the project team itself, potential and actual participants and other stakeholders. For the second European wide occupational measurement campaigns, additional SOPs focused specifically on communication, covering whom, how and when, have been put in place.

4. Conclusion

The HBM4EU chromates study team held honest and frank discussions when reflecting on the experiences gained from undertaking this study and considered it important to communicate these to the wider scientific audience so that others can learn from these too. Occupational studies such as the HBM4EU chromates study are scarce in terms of their breadth of scope and number of countries involved. Overall, even though there is room for improvements, the HBM4EU chromates study showed that it was feasible to conduct a pan-European occupational study in a consistent and concerted way and it's hoped that other research teams can draw upon these experiences. Many of the lessons learned by the HBM4EU chromates team may not be new, or may even be considered as being trivial in nature. For example, Fiddicke et al. (2015) also observed that a biomonitoring survey involving many European countries needs time for preparation and conduct and that extensive communication with potential participants (school-aged children in this instance) was necessary. For example, the importance of the method used to report non-detects was just one of the points highlighted during a workshop regarding biomonitoring study design, interpretation, and communication (Bates et al., 2005). However, this does not negate their importance or the need to reinforce to the wider scientific community that such aspects must be considered and factored into the project planning. Despite the points noted here, the data generated allows for a more robust assessment of exposure in different occupational settings and countries. The high number of data collected in turn will allow for more detailed data analysis that can provide more definitive answers to policy questions and recommendations for future studies aiming to address occupational exposure to Cr(VI). Furthermore, the findings of our study can be useful to define more adequate occupational exposure limits and support regulatory action. It is likely that the study will result in several publications that will prove useful for stakeholders, from the regulatory field to occupational health services, with any relevant limitations to the data set (e.g. due to differences in sample collection, analysis) being highlighted. The true collaborative nature of the HBM4EU chromates study team allowed for open and frank discussion on the issues encountered, has increased our awareness on additional differences in the standard practises used in different countries/laboratories, and may result in further harmonisation of practises between labs/countries. All of the points raised for improvement have been considered and taken forward to improve the SOPs for the future HBM4EU occupational studies focussed on diisocyanates and electronic waste management, which are planned to start sampling in 2021. Furthermore, additional training schools were delivered during Jan 2021 to communicate these to those researchers involved in the fieldwork, which were also recorded so that they can be made available to any additional fieldworkers.

Harmonisation is difficult to achieve and it is inevitable that it can

always be improved upon, but it can be facilitated by having a solid network in place to enable this. However, development of such a strong successful network takes effort and engagement from all and it is important to ensure that adequate time, effort, resources is given for such an initiative. It is also a continual process whereby the team learns, shares, evolves from the experiences, and lessons gained. It is very much hoped that the lessons learned highlighted in this manuscript will be of value to researchers planning similar studies.

Disclaimer

The contents, including any opinions and/or conclusions expressed of this manuscript, are those of the authors alone and do not necessarily reflect the opinions or policy of the organizations to which they are employed.

Declaration of competing interest

No conflicts of interest are declared.

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References

- Bennett, A.B., Streitz, W.D., Gacel, R.A., 2007. Specific issues with material transfer agreements. In: Krattiger, A., Mahoney, R.T., Nelson, L., et al. (Eds.), *Intellectual Property Management in Health and Agricultural Innovation: A Handbook of Best Practices*. MIHR: Oxford, UK and PIPRA, Davis, USA. Available from: URL: http://www.iphandbook.org/handbook/ch07/p03/index_print.html. (Accessed 14 November 2020).
- Bates, M.N., Hamilton, J.W., LaKind, J.S., Langenberg, P., O'Malley, M., Snodgrass, W., 2005. Workgroup Report: biomonitoring study design, interpretation, and communication—lessons learned and path forward. *Environ. Health Perspect.* 113, 1615–1621.
- Boogaard, P.J., Hays, S.M., Aylward, L.L., 2011. Human biomonitoring as a pragmatic tool to support health risk management of chemicals - examples under the EU REACH programme. *Regul. Toxicol. Pharmacol.* 59, 125–132.
- Cervo, S., De Paoli, P., Mestroni, E., Perin, T., Escoffier, L., Canzonieri, V., Steffan, A., 2016. Drafting biological material transfer agreement: a ready-to-sign model for biobanks and biorepositories. *Int. J. Biol. Markers* 31 (2), 211–217.
- Chen, Q.Y., DesMarais, T., Costa, M., 2019. Metals and mechanisms of carcinogenesis. *Annu. Rev. Pharmacol. Toxicol.* 59, 537–554.
- Den Hond, E., Govarts, E., Willems, H., Smolders, R., Casteleyn, L., Kolossa-Gehring, M., Schwedler, G., Seiwert, M., Fiddicke, U., Castaño, A., Esteban, M., Angerer, J., Koch, H.M., Schindler, B.K., Sepai, O., Exley, K., Bloemen, L., Horvat, M., Knudsen, L.E., Joas, A., Joas, R., Biot, P., Aerts, D., Koppen, G., Katsonouri, A., Hadjipanayis, A., Krskova, A., Maly, M., Mörck, T.A., Rudnai, P., Kozepesy, S., Mulcahy, M., Mannion, R., Gutleb, A.C., Fischer, M.E., Ligočka, D., Jakubowski, M., Reis, M.F., Namorado, S., Gurzau, A.E., Lupsa, I.R., Halzlova, K., Jajcay, M., Mazej, D., Tratnik, J.S., López, A., Lopez, E., Berglund, M., Larsson, K., Lehmann, A., Crettaz, P., Schoeters, G., 2015. First steps toward harmonized human biomonitoring in Europe: demonstration project to perform human biomonitoring on a European scale. *Environ. Health Perspect.* 123 (3), 255–263.
- EC, 1907/2006. Regulation. (EC) No. 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorization and Restriction of Chemicals (REACH), Establishing the European Chemicals Agency, Amending Directive 1999/45/EC and Repealing Regulation (EEC) No. The 793/93 and Regulation (EC) No. No 1488/94, as well as Council Directive 76/769/EEC and Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC. Available from: <https://eur-lex.europa.eu/leg-content/PT/TXT/?uri=CELEX%3A02006R1907-20140410>. (Accessed 15 February 2021).
- Elhosary, N., Maklad, A., Soliman, E., El-Ashmawy, N., Oreby, M., 2014. Evaluation of oxidative stress and DNA damage in cement and tannery workers in Egypt. *Inhal. Toxicol.* 26, 289–298.
- Fiddicke, U., Becker, K., Schwedler, G., Seiwert, M., Joas, R., Joas, A., Biot, P., Aerts, D., Casteleyn, L., Dumez, B., Castaño, A., Esteban, M., Angerer, J., Koch, H.M., Schoeters, G., Den Hond, E., Sepai, O., Exley, K., Knudsen, L.E., Horvat, M.,

- Bloemen, L., Katsonouri, A., Hadjipanayis, A., Cerna, M., Krsková, A., Jensen, J.F., Nielsen, J.K., Rudnai, P., Kőzepésy, S., Gutleb, A.C., Fischer, M.E., Ligočka, D., Kamińska, J., Reis, M.F., Namorado, S., Lupsa, I.R., Gurzau, A.E., Halzlová, K., Mazej, D., Tratnik, J.S., Rivas, T.C., Gómez, S., Berglund, M., Larsson, K., Lehmann, A., Crettaz, P., Dewolf, M.C., Burns, D., Kellegher, A., Kolossa-Gehring, M., 2015. Lessons learnt on recruitment and fieldwork from a pilot European human biomonitoring survey. *Environ. Res.* 141, 15–23.
- Ganzleben, C., Antignac, J.P., Barouki, R., Castaño, A., Fiddicke, U., Klánová, J., Lebre, E., Olea, N., Sarigiannis, D., Schoeters, G.R., Sepai, O., Tolonen, H., Kolossa-Gehring, M., 2017. Human biomonitoring as a tool to support chemicals regulation in the European Union. *Int. J. Hyg Environ. Health* 220, 94–97.
- Gottweis, H., Kaye, J., Bignami, F., Rial-Sebbag, E., Lattanzi, R., Macek, M., 2012. Biobanks for Europe: a challenge for governance. In: *Inhaled Particles VII*. Elsevier Science, Oxford, pp. 77–81, 978-92-79-22858-2. Available from: URL: https://ec.europa.eu/research/science-society/document_library/pdf_06/biobanks-for-euro_pe_en.pdf. (Accessed 15 November 2020).
- IARC, 2012. Chromium(VI) compounds. In: *A Review of Human Carcinogens: Arsenic, Metals, Fibers, and Dusts*. International Agency for Research on Cancer, Lyon, pp. 147–167 (IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 100C; Available from: URL: <https://publications.iarc.fr/Book-And-Report-Series/Iarc-Monographs-On-The-Identification-Of-Carcinogenic-Hazards-To-Humans/Arsenic-Metals-Fibers-And-Dusts-2012>. (Accessed 15 February 2021).
- Jones, K., 2020. Human biomonitoring in occupational health for exposure assessment. *Port J Public Health* 38, 2–5. <https://doi.org/10.1159/000509480>.
- Knudsen, L.E., Faber, B., 2018a. HMB4EU deliverable 1.5 legal and ethics policy paper. September 2018. Available from: URL: <https://www.hbm4eu.eu/deliverables/>. (Accessed 15 November 2020).
- Knudsen, L.E., Faber, B.A., 2018b. HMB4EU Legal and ethics policy document. August 2018 Available at: URL: <https://www.hbm4eu.eu/mdocs-posts/hbm4eu-legal-and-ethics-policy-document/>. (Accessed 9 November 2020).
- Leese, E., Morton, J., Gardiner, P.H.E., Carolan, V.A., 2017. The simultaneous detection of trivalent & hexavalent chromium in exhaled breath condensate: a feasibility study comparing workers and controls. *Int. J. Hyg Environ. Health* 220 (2), 415–423.
- Li, P., Gu, Y., Yu, S., Li, Y., Yang, J., Jia, G., 2014. Assessing the suitability of 8-OHdG and micronuclei as genotoxic biomarkers in chromate-exposed workers: a cross-sectional study. *BMJ Open* 4, e005979. <https://doi.org/10.1136/bmjopen-2014-005979>.
- Lin, C.-H., Lai, C.-H., Peng, Y.-P., Wu, P.-C., Chuang, K.-Y., Yen, T.-Y., Xiang, Y.-K., 2018. Comparative health risk of inhaled exposure to organic solvents, toxic metals, and hexavalent chromium from the use of spray paints in Taiwan. *Environ. Sci. Pollut. Res. Int.* 26, 33906–33916.
- Louro, H., Heinälä, M., Bessems, J., Buekers, J., Vermeire, T., Woutersen, M., van Engelen, J., Borges, T., Rousselle, C., Ougier, E., Alvito, P., Martins, C., Assunção, R., Silva, M.J., Pronk, A., Schaddelee-Scholten, B., Gonzalez, M., Alba, M., Castaño, A., Viegas, S., Humar-Juric, T., Kononenko, L., Lampen, A., Vinggaard, A.M., Schoeters, G., Kolossa-Gehring, M., Santonen, T., 2019. Human biomonitoring in Health Risk Assessment in Europe: current practices and recommendations for the future. *Int. J. Hyg Environ. Health* 222, 727–737.
- Ndaw, S., Santonen, T., Duca, R.C., Robert, A., Hanser, O., Porras, S., Galea, K., Jones, K., Godderis, L., Viegas, S., Göen, T., Scheepers, P., Schaddelee, B., Pronk, A., Halldorsson, T., Iavicoli, I., El Yamani, M., Fréry, N., 2017. Research Plan for Chromates Study under HBM4EU. HMB4EU Deliverable Report 8.2 WP 8 - Targeted field work surveys and alignment at EU level. Available at: URL: <https://www.hbm4eu.eu/deliverables/>. (Accessed 11 November 2020).
- Pan, C.-H., Jeng, H.A., Lai, C.-H., 2018. Biomarkers of oxidative stress in electroplating workers exposed to hexavalent chromium. *J. Expo. Sci. Environ. Epidemiol.* 28, 76–83.
- Porras, S., Ladeira, C., Ribeiro, E., Viegas, S., Uuksulainen, S., Santonen, T., Galea, K., Cherrie, J., Louro, H., Ventura, C., Silva, M.J., Leese, E., Jones, K., Hanser, O., Ndaw, S., Robert, A., Duca, R.C., Poels, K., Godderis, L., Kiilunen, M., Norppa, H., Veijalainen, H., Parshintsev, E., Tuomi, T., Ruggieri, F., Alimonti, A., Koch, H., Bousoumah, R., Antoine, G., Jacoby, N., Musgrove, D., 2019. HBM4EU occupational biomonitoring study on hexavalent chromium and other harmful chemicals. Standard Operating Procedures (SOPs). Available at: <https://www.hbm4eu.eu/online-library/>. (Accessed 20 November 2020).
- Santonen, T., Alimonti, A., Bocca, B., Corneliu Duca, R., Galea, K.S., Godderis, L., Göen, T., Gomes, B., Hanser, O., Iavicoli, I., Janasik, B., Jones, K., Kiilunen, M., Koch, H.M., Leese, E., Leso, V., Louro, H., Ndaw, S., Porras, S.P., Robert, A., Ruggieri, F., Scheepers, P.T.J., Silva, M.J., Viegas, S., Wasowicz, W., Castano, A., Sepai, O., 2019. Setting up a collaborative European human biological monitoring study on occupational exposure to hexavalent chromium. *Environ. Res.* 177.
- Viegas, S., Zare Jeddi, M., Hopf, N.B., Bessems, J., Palmén, N., Galea, K.S., Jones, K., Duca, R.-C., Kujath, P., Verhagen, H., Santonen, T., Pasanen-Kase, R., 2020. Biomonitoring as an underused exposure assessment tool in Occupational Safety and Health context – challenges and way forward! *Int. J. Environ. Res. Publ. Health* 17, 5884.
- Wang, T.C., Song, Y.S., Wang, H., Zhang, J., Yu, S.F., Gu, Y.E., Chen, T., Wang, Y., Shen, H.Q., Jia, G., 2012. Oxidative DNA damage and global DNA hypomethylation are related to folate deficiency in chromate manufacturing workers. *J. Hazard Mater.* 213–214, 440–446.