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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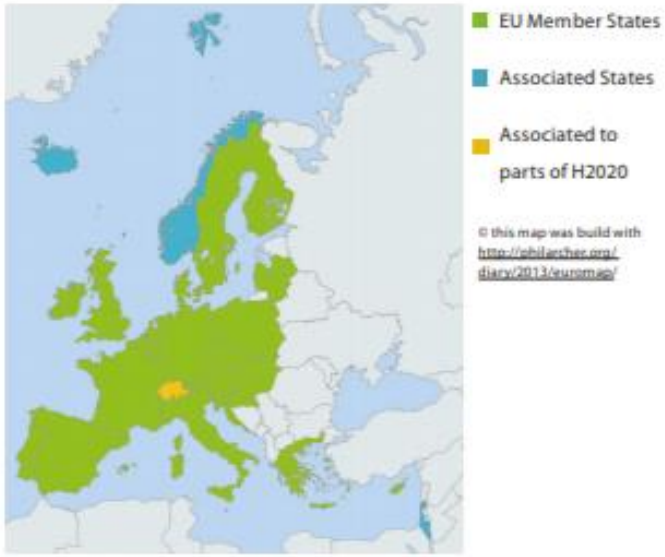
Aims of webinar

- Outline multicentre study that aimed to characterize occupational exposure to hexavalent chromium (Cr(VI)) in nine European countries (**HBM4EU chromates study**)
- Reflect and share experiences gained so that lessons can be learnt and considered when developing future occupational multicentre studies

HBM4EU - https://www.hbm4eu.eu/ https://youtu.be/fNqou9Z_VWQ

Consortium partners



Project coordination:
HBM4EU is coordinated by the
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For further information, please visit our website: www.hbm4eu.eu

About HBM4EU

People are exposed to a complex mixture of chemicals in their daily lives through the environment, consumer products, food and drinking water and at work.

HBM4EU will use human biomonitoring to assess human exposure to chemicals in Europe, to better understand the associated health impacts and to improve chemical risk assessment. At the level of the individual, human biomonitoring data can inform medical treatment or guidance on the need to reduce exposure.

HBM4EU partners will establish a dialogue with policy makers to ensure that our results can be used to support the development of policies, to evaluate existing policies and to design measures to reduce exposure to toxic chemicals.

Our results will inform the safe management of chemicals and so protect human health in Europe.

Need for harmonisation

More research in best practices & procedures for occ. HBM to be addressed within HBM4EU project.

Why?

- limited no. workers can /are recruited into national studies.
- studies performed by different researchers /countries usually not aligned (sampling / analysis, contextual data)
- complicates comparison of findings and use of data in regulatory risk assessment

Combining national surveys using harmonized study designs, methodologies and protocols can greatly improve information collected and provide benefit of harmonised data collected in different countries

Background on hexavalent chromium Cr(VI)

- *Important occupational carcinogen*
- *Effects in humans*
  - *Lung cancer*
  - *Cancer of the nose & nasal sinuses*
- *Occupational exposure to Cr(VI)*
  - *Welding activities*
  - *Cr(VI) electroplating*
  - *Other surface treatments such as paint application*

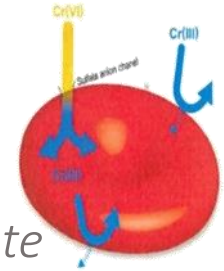
European regulation

- *Cr(VI) compounds are authorised under REACH*
- *All companies using Cr(VI) compounds have to apply for authorization for their uses*
    - *REACH does not cover process-generated fumes likes welding fumes*
- *The recent **binding occupational limit value** (BOELV) for Cr(VI) under EU Directive 2004/37/EC is 0.010 mg/m^3 for a period of 5 years after the date of transposition of the directive; after that period, **0.005 mg/m^3** will apply.*
- *There is a derogation for welding or plasma-cutting processes or similar work processes that generate fumes: the exposure limit value is 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.*
- In France and the Netherlands, an OEL of 1 µg/m^3 has been set for Cr(VI).

Biomonitoring of Cr(VI)

- *The common biomarker used is **total urinary Cr***
- *Different biological limit values have been set on a national basis in Europe*
  - *Spain: 25 µg/L*
  - *Finland: 10 µg/L*
  - *France: 2.5 µg/L*
- *The main limitation is that it is not specific for Cr(VI) since it measures exposure to both Cr(III) and Cr(VI).*
  - ➢ ***This indicates the need to develop more specific biomarkers for Cr(VI) exposure.***

Potential specific biomarkers for Cr(VI)



Cr in red blood cells (RBC)

- *Reflects mainly the exposure to Cr(VI) since Cr(VI) can easily permeate through the membrane of RBM to form a Cr-hemoglobin complex.*
- *Cr-hemoglobin complex is stable in the RBC for the cell lifetime (120 days)*

Cr(VI) in exhaled breath condensate (EBC)

- *Provides specific information on the Cr(VI) levels in the main target tissue i.e lungs*
- *Less invasive biomarker than Cr in blood*
- *Cr(VI) and Cr(III) can be analysed separately in EBC samples*

Characterization of effects biomarkers

- *To establish a relationship between the exposure to Cr (VI) and its human health impact given that they comprise sensitive endpoints that reflect early biochemical changes before the onset of disease*
- *To possibly indicate combine effects of different substances i.e Cr(VI) and Nickel in welders*
- *Potential effect biomarkers*
  - *micronuclei in human peripheral blood lymphocytes and reticulocytes*
  - *oxidative stress biomarkers*
  - *telomere length*
  - *epigenetic markers*
  - *metabolomic profiling*

Objectives of the HMB4EU chromates study

- *To provide EU **relevant data on Cr(VI) internal exposure** in occupational settings to be used as scientific evidence for regulatory risk assessment and decision-making under EU chemical legislation and under occupational safety and health legislation.*
- *To evaluate **capability and validity of different HBM parameters** for the specific assessment of Cr(VI) exposure: Cr-RBC, Cr-EBC, biomarkers of early biological effects.*
- *In addition, provide information on welders' & platers' exposure to Ni and Mn, and of chrome platers exposure to mist suppressants containing PFAS.*

Research plan

https://www.hbm4eu.eu/deliverables/
AD 8.2 Research plan for chromates study under HBM4EU

Santonen et al., *2019. Setting up a collaborative European human biological monitoring study on occupational exposure to hexavalent chromium.* Environmental Research 177, 108583

Participating Countries

France

Belgium

United Kingdom

Netherlands

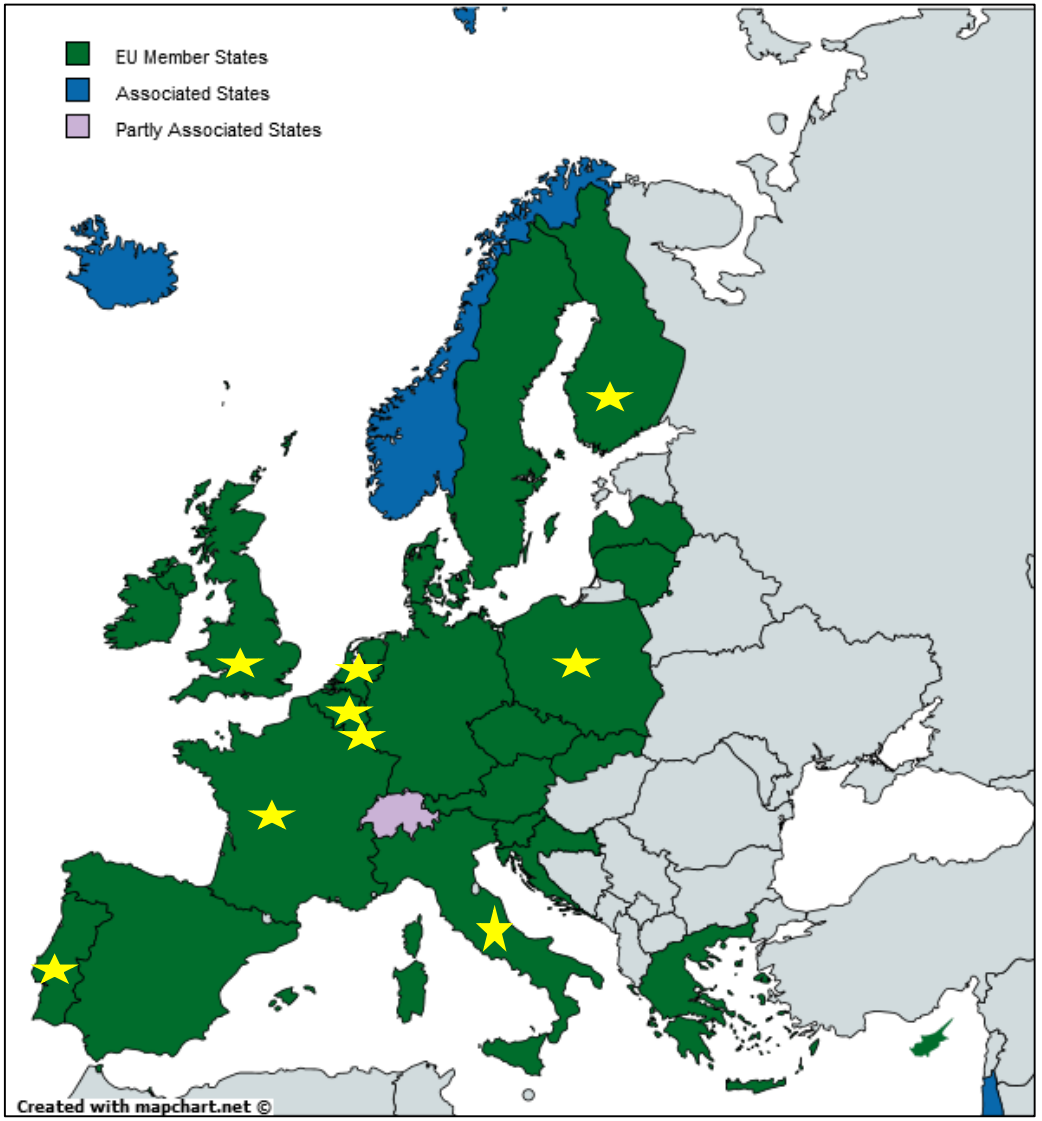
Finland

Poland

Portugal

Italy

Luxembourg



Samples collected

- Urine for total Cr analysis
- Blood for the analysis of Cr in red blood cells (RBC) and plasma
- Exhaled breath condensate (EBC) for Cr(VI) and Cr(III) analyses
- Industrial hygiene samples (air and wipe) – used for the interpretation of the HBM results
- In electrolytic Cr surface treatment when PFASs might have been used as mist suppressants => plasma samples for PFAS analyses
- Effect markers (micronuclei in PBL and reticulocytes, oxidative stress biomarkers, epigenetic markers, telomere length and metabolomic profiling)

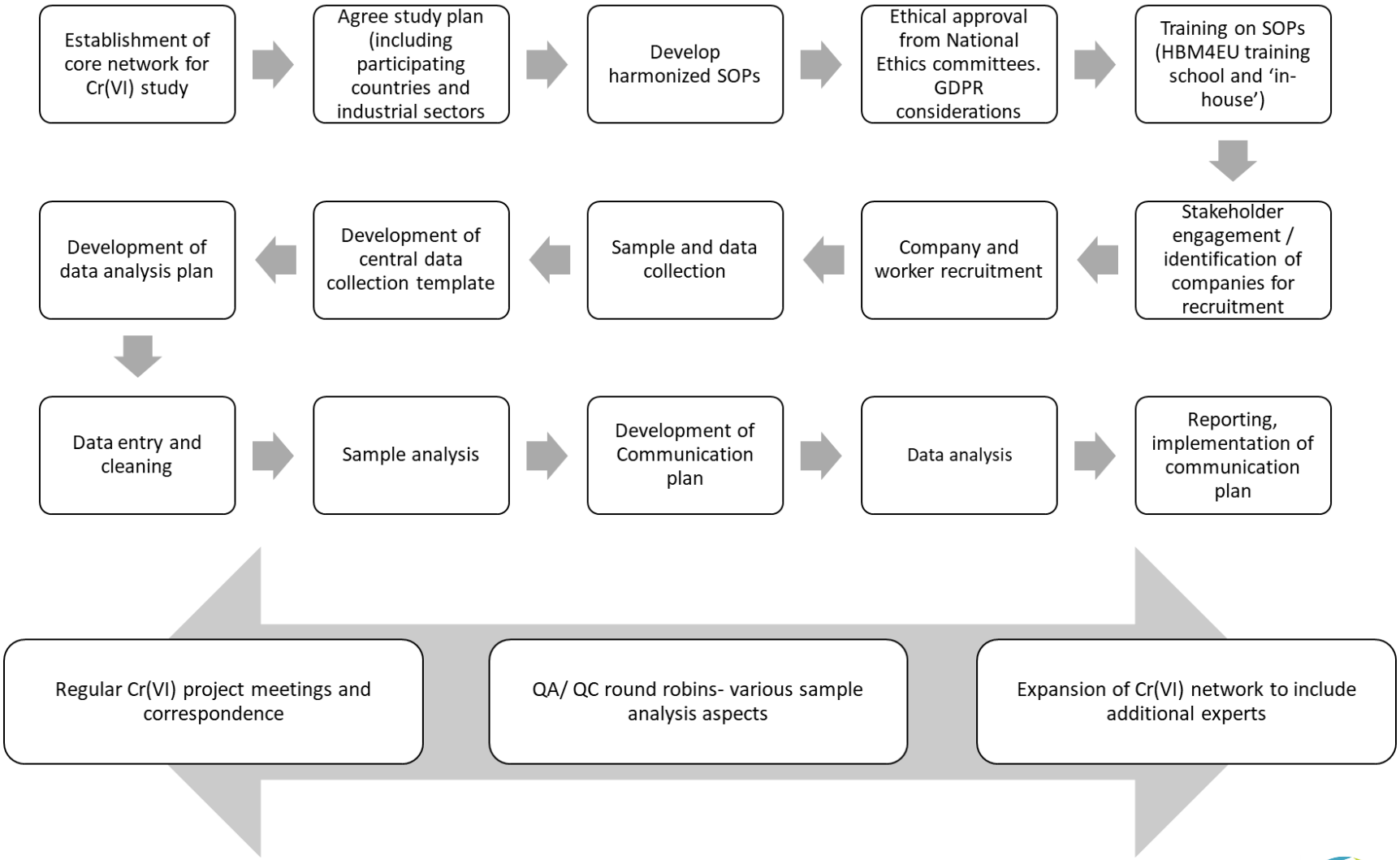
Photos: Simo Porras, FIOH

Standard operating procedures (SOPs) for samplings

SOP No.	Title	Topic / Sampling matrix
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
4	Standard operating procedure for the collection of exhaled breath condensate samples	EBC
5	Standard operating procedure for urine sampling, including sample storage and transfer	Urine
6	Standard operating procedure for air sampling of inhalable and respirable dust fraction and (hexavalent) chromium	Air
7	Standard operating procedure for obtaining dermal wipe samples	Dermal
8	Procedure for comparing occupational hygiene measurements with exposure estimates generated using exposure models	Contextual exposure determinant information

Available at: https://www.hbm4eu.eu/online-library/

Materials and methods



Key activities in the design and implementation of the HMB4EU chromates study

Important for chromates study project team to reflect on research and evaluate positives & limitations of harmonised approach

- Project team asked to provide feedback on 3 things that went well and 3 things that could be improved upon.
- Responses used to facilitate discussion during 4th HBM4EU training school, 14th May 2020.
  - Discuss how project team could learn from the experiences to inform future HMB4EU occupational multicentre studies (diisocyanates and e-waste).
- Input provided during drafting of deliverables / manuscript

Information gathered summarised under following themes

1. What were the successes and benefits?
2. What were the issues encountered and suggested improvements?
    a) ethics and General Data Protection Regulation (GDPR)
    b) SOPs
    c) sample collection
    d) sample analysis
    e) data reporting, input and analysis
    f) communication

Pre Dec 2017	• Development of project team and ideas
Dec 2017	• Publication of research plan
Feb – end 2018	• SOP development
End 2018 – end 2019	• Field measurements, analysis and data input
2020 - present	• Data cleaning, analysis and reporting

Successes and benefits

	Achieved
Countries	9
Population	399 workers / 203 controls
Urine samples	780 workers / 143 controls
Blood	345 workers / 175 controls
EBC	342 (workers) / 98 controls
Air sample sets	293 inhalable / 155 respirable
Wipe sample sets	270

Achieved the required statistical power for the study (Santonen et al., 2019) and obtain more comprehensive and richer dataset

Successes and benefits

- Study served also as a valued ‘educational tool’
- Others can benefit from the harmonized efforts through applying freely available SOPs to their own studies focused on Cr(VI)
    - standardized manner, allowing opportunities for data pooling, identify exposure trends, comparisons between different risk management measures available etc. to be undertaken.
- Many of the development SOPs, materials and ethical procedures can be reused (updated or adapted) for other campaigns
- Successes achieved through valued and close co-operation between participating institutions / researchers, who were all working towards clearly defined aims and objectives
- Network have continued to build and expand networks through other biomonitoring initiatives (e.g., ISES-Europe working group

Ethics and GDPR

- Transport of biological samples between research partners takes place under material transfer agreements (MTAs).
- Restrictions and obligations of these MTAs also apply to (post-) interventions made using the primary sample and/ or to derivative samples.
- Fragmentation of regulations in combination with the growing regulatory requirements, makes it more difficult to freely transfer biological samples between research partners.
- International harmonization and standardization of the biobank practices required within the context of an international occupational study.

Ethics and GDPR

- To comply with EU regulations, special attention must be given towards data protection with respect to the GDPR.
- Prior to submission to National Ethics committees, a standard protocol with information sheets, consent forms etc developed and implemented.
- Data handling organised locally with pseudonymised data forwarded to central data management. Mutual data transfer agreements signed and parallel system in place for sample transfers.
- Participants informed about such transfers and the protocol and information sheets provided explanations and details.
- Various ethics committees that they would often highlight different points for further clarification and for the local project team to respond.

Very time consuming, impacts on further project activities

Establish commonalities with differing ethic committees views

Lessons learned – Ethics and GDPR

Very important and can't be overlooked

Further consideration on how to obtain consent without *any perceived influence* from the company

Standard Operating Procedures (SOPs)

- SOPs freely available at library of the HBM4EU website, https://www.hbm4eu.eu/online-library/).
- Developed following extensive drafting period, delivered as part of training school
- Evident that not all field researchers had been involved in earlier discussions, resulting in last minute modifications on SOPs.
- SOPs in English, not in the local languages of the European countries involved in the campaign - may have been for some of the field researchers, particularly with some of the more technically demanding language used.
- Sample transfer details (list of labs and contract details) should have been included in SOPs, rather than separately.

Time and effort involved should never be underestimated

Essential that those involved in implementing the SOPs are involved in finalization of documents

Lessons learned – SOPs

More effort should be made to run centralized training sessions,

If SOPs are translated into local languages, need to verify translation correct

Sample collection

- More time consuming than anticipated (by both the field researchers and the participating workers).
  - Completion of detailed worker questionnaires
  - Collection of EBC samples
  - Collection of wipe samples
- Significant number of participating companies small in size, with low numbers of eligible.
  - Any reduction in working time may have a significant impact on the company's productivity.
- Due to companies being smaller in size than expected, more time and effort spent to identify, visit and recruit additional companies reach the target number of study participants.
- Not always possible to collect samples close to end of work shift due to numbers of workers involved and wish to leave work promptly following completion of their work shift.

Sample collection

- Despite SOPs, some variations in procedures, e.g.:
  - Air sampling pumps not always switched off during breaks
  - Variability in inhalation sample type collected

- Some EBC results were 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.

More time consuming than anticipated (recruitment and also site activities)

Full consideration given to impact that sampling strategies has on company / worker participation and working practices

Lessons learned – Sample collection

Reinforce importance of OH sample collection and their QA/QC in BM studies

Collection to lab: Ensure correct storage, transportation and time conditions are met

Data reporting, input and analysis

- A large MS Excel data template developed to input data and results
- Large templates can in advertently lead to data entry errors occurring.
- Evident some researchers had difficulties, e.g.
  - Work task and overall work duration given in minutes, rather than hours
  - Entries asked to refer to other lines of information or merged cells
  - Details of weekly work schedule provided rather than that during measurement period
  - Included drop-down lists 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 further info.
- Instructions provided separately by email concerning many of these points (and others) to assist, these were not always considered.
- More time being taken to check, verify and clean the results before data analysis could commence.

Data reporting, input and analysis

- Limit of Quantification (LOQs) varied in different laboratories, which may have had an impact on the results of, eg, EBC Cr(VI) measurements since levels in controls and workers were often low.
- Verification of LOQs in each country for all sample matrices should have been done before study began to assess potential impact of possible differences etc.
- There were some deviations in the analysis undertaken for the collected occupational hygiene samples.
- Overarching focus of study was on biomonitoring sampling and analysis with extensive QC and QA procedures being put in place. Whilst efforts made to harmonise the occupational hygiene sample collection, 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.
- Urine and EBC samples were also too analysed for Ni and Mn. E.g., urinary Ni levels suggested to be analysed for welders / platers performing Ni plating; urinary Mn levels suggested to be analysed for stainless steel welders; however, this did not always take place.

Data reporting, input and analysis

- 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, assuming they are not exposed to the occupational chemicals under study.
- Preliminary data showed some individuals from the so-called “non-exposed group” are exposed to low concentrations of Cr(VI), possibly present in the ambient air or even in surfaces of common spaces like canteens.

Lessons learned – Data reporting, input and analysis

Control groups may indicate cross contamination in workplaces

Necessary to have a central data entry tool that is usable and clearly understood

Hold training sessions with those required to populate the tool so persons familiarized with data entry process.

Tool accompanied by text in relevant SOPs focused on data reporting (e.g. example calculations

Template must not be unofficially modified or data input incorrectly. Tool should be officially modified to allow for greater ease of entry and for key aspects to be highlighted to assist data interpretation e.g. flag data below LOQ or possible outliers

More instruction to be provided on collection and analysis of the occupational hygiene is warranted and that appropriate QA / QC for these samples should also be included within programme of work

Communication

- Project team had wealth of knowledge and experience in undertaking occupational site work.
- Difficulties with engagement with companies and workers, challenges experienced with receiving clear information on activities being undertaken from company representative.
- Due care and consideration to be given to communicating results of study to the participating workers, companies and wider stakeholders.
- Whilst core project team communicated extensively, evident communications were perhaps not always reaching those who also needed to receive it e.g. field researchers.

Additional SOP to be developed focused specifically on communication (who, what, how, where and when)

Communications not always reaching those who also needed to receive it (e.g. field researchers)

Lessons learned – Communication

Liaise with those integrally involved in activities of interest and daily production regimes

Carefully plan worker and company feedback (feeds into ethics)

Conclusion

- Occupational studies such as HBM4EU chromates study are scarce.
- HMB4EU chromates study showed feasible to conduct a pan-European occupational study in a consistent and concerted way
- Many of the lessons learned may be considered trivial, however does not negate their importance or need to reinforce to the wider scientific community that such aspects must be considered when project planning.
- Data generated allows for a more robust assessment of exposure in different occupational settings and countries, providing more definitive answers to policy questions and recommendations for future studies aiming to address occupational exposure to Cr(VI).
- True collaborative nature of the HBM4EU chromates study team allowed for open discussion on issues encountered and may result in greater harmonisation of practises between labs/countries.
- Points raised for improvement considered and taken forward to improve SOPs for future HBM4EU occupational studies (diisocyanates & e-waste)
- Harmonisation difficult to achieve but facilitated by having a solid network in place, which takes time, effort and engagement.

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Companies and workers who participated in the HBM4EU chromates study

Any questions?

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