



science and policy  
for a healthy future

HORIZON2020 Programme  
Contract No. 733032 HBM4EU

## **SOP 1:**

**Standard operation procedure for selection of  
participants and recruitment, information to the  
participants, informed consent**

## **WP 8**

## **Task 8.5**

## Table of Contents

Authors and Acknowledgements.....	2
1 Introduction.....	2
2 Study design and participants.....	2
2.1 Target population.....	2
2.2 Sample size.....	2
3 Selection of sampling locations.....	3
4 Selection of participants, their recruitment and information.....	3
5 Informed Consent form.....	4

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## 1 Introduction

Standard Operation Procedure 1 (SOP 1) is focussing on the selection of participants and recruitment, information to the participants and informed consent.

## 2 Study design and participants

Considering the information provided above, potentially exposed workers and controls from companies performing chrome plating, surface treatment with chromates or stainless steel welding will be recruited from eight countries, namely: Belgium, Finland, France, Italy, Poland, Portugal, The Netherlands and United Kingdom (UK).

### 2.1 Target population

The target population will be workers from primary sampling units, which are defined as companies performing surface treatment with Cr(VI) - chrome plating in baths, surface treatment by spraying or painting, and stainless steel welding. The exact welding and surface treatment techniques used in the companies and by the workers will be specified during the collection of contextual information. In addition, a control population of workers not involved in the above activities will also be recruited.

Both genders will be eligible, with ages ranging from 18-70 years. For some analyses, e.g., effect biomarkers characterization, specific inclusion/exclusion criteria may be defined because those biomarkers are affected by several confounding factors that should be reduced to avoid results misinterpretation.

### 2.2 Sample size

The target population size for this project is 50 workers *per* country and 3-5 companies *per* country. The sample size is indicative and may need further adjustment for the specific chemical group because of expected population variability of the biomarker. Additionally, 25 workers (controls) *per* country will be also engaged in the study.

### 3 Selection of sampling locations

Sampling will be conducted in 3-5 previously identified companies located in eight countries, namely: Belgium, Finland, France, Italy, Poland, Portugal, The Netherlands and United Kingdom (UK). Companies will be contacted, informed about the study aims and invited to participate. The same approach will be followed for the workers' engagement in the study.

### 4 Selection of participants, their recruitment and information

Recruitment and information provision will be undertaken in the local language. For this purpose, a two-step approach will be undertaken, the first one for the company itself and the second one related to workers contact and information within the company:

1. Establishment of phone contact with the Company responsible. Upon company decision to participate in the study, the information leaflet "INFORMATION FOR PARTICIPATING COMPANIES" will be delivered. An authorised representative of the Company should complete the "EMPLOYER CERTIFICATE OF INFORMED CONSENT". Then, request to the company responsible of a list of names of those involved in activities that can implicate Cr(VI) exposure so that we can approach them to explain the project and invite them to participate. Also request a list of suitable controls to approach.
2. Establishment of a contact with the worker, which is recommended to be done through a direct face-to-face meeting. Information on the study scope and actions to be developed (sample collection and filling in a questionnaire) will be provided to the workers. The information leaflet on the study "*INFORMATION FOR PARTICIPATING WORKERS*" will be distributed and discussed during the first contact with the workers. Within this contact, a period to clarify all workers queries regarding the project is mandatory. The workers should fill in the "WORKER CERTIFICATE OF INFORMED CONSENT" if they are willing to give their informed consent to participate in the study. The same approach will be followed for controls.

Therefore, and following workers' acceptance to participate in the study, we will recruit workers in jobs that are likely to result in occupational exposure to Cr(VI), for example, direct involvement in tasks such as chrome plating, surface treatment with chromates or stainless steel welding. In addition, we will recruit a group of unexposed (control) subjects, individually matched to the subjects for age (plus/minus 5-years), gender and smoking status (current smoker/ex-smoker/non-smokers). These control subjects will be selected from companies in the same geographical area with no known occupational exposure to Cr(VI).

Subjects should be in good health and present at work during the planned period of the study.

Blood samples to be used in in genotoxicity biomarkers study should only be obtained from workers that, in addition to the above criteria, should fulfil the following inclusion criteria: i) are under the age of 50; ii) are non-smokers or ex-smokers for more than six months; iii) have not been subjected to a medical exam such as a medical X-ray or Computerised Axial Tomography (CAT) scan in the last 3-months; iv) do not suffer or have suffered from cancer.

## 5 Informed Consent form

Prior to the workers contact, the company should analyse and sign the Informed Consent Form (“EMPLOYER CERTIFICATE OF INFORMED CONSENT”). After the signature, the workers can be contacted directly by the researcher(s) coordinating the study.

The workers and controls accepting to participate in the study must sign the consent form (“WORKER CERTIFICATE OF INFORMED CONSENT”) before the collection of any information or samples.

The Informed Consent form can be signed after receiving information explaining the aims of the study and all details required by the appropriate ethical regulations in each country. The researcher must be available for clarification during the reading and analysis of the consent form by the participants (workers and controls).

The Informed Consent forms should be archived and kept during all the study duration in each institution that participate the sample collection (not less than 5 years). Identification of companies and workers will not be use in all the process of samples handling and storage to guarantee the confidentiality needed.

Therefore, a standardised convention will be used to assign unique identification codes for all samples collected. The identification code convention is as follows:

Country ID (XX) - Participant ID (XX) - Sample ID (BXX/UXX/EXX/AXX/WXX)

Country ID 'XX' is the country code, using the ISO Alpha-2 country codes for the participating countries<sup>1</sup>.

Country	ISO Alpha-2 country codes
Belgium	BE
Finland	FI
France	FR
Italy	IT
Poland	PL
Portugal	PT
The Netherlands	NL
United Kingdom	UK

Participant ID 'XX' is a two-digit running number of participants in each country (e.g. 01 for the first participant recruited, 02 the second and so forth).

Sample ID 'UXX' is one letter (B/U/E/A/W) to identify the type of sample collected, followed by a two-digit identifier (XX) to identify the running number of each type of sample for that worker (e.g. 01 for

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the first sample, 02 for the second and so forth). The letter code applied for the sample types is as follows:

Type of sample collected	Sample type code
Air	A
Blood	B
Exhaled breath	E
Urine	U
Wipe	W



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**SOP 2:**  
**Standard operating procedure for  
completion of company and worker questionnaires**

**WP 8**  
**Task 8.5**

## Table of Contents

Authors and Acknowledgements.....	2
1 General introduction.....	2
2 Instructions to fill in the workplace questionnaire (self-administered by company representative).....	3
3 Instructions for completion of the workers post-shift questionnaire (interviewed by researcher).....	4
4 Storing of gathered information.....	11
Appendix 1: Statistical Classification of Economic Activities in the European Community, NACE Rev 2. (2008).....	12
Appendix 2: Structure of the international standard classification of occupations (ISCO-08).....	17
Appendix 3: Flash Card 1: PPE (personal protective equipment) used for chrome plating and spray painting.....	20
Appendix 4: Flash Card 2: PPE (personal protective equipment) used for welding.....	21
Appendix 5: Flash Card 3: LEV (local exhaust ventilation) used for welding.....	22

## Authors and Acknowledgements

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## 1 General introduction

This SOP 2 – “Completion of company and worker questionnaires” is designed to support a targeted occupational study on chromate exposure performed under task 8.5.

This SOP has been created with the premise in mind that every participating country is obliged to try, as far as is reasonably practicable, to follow the HBM4EU documents to achieve comparable data in a (as much as possible) harmonised and consistent manner.

As it is stated in SOP1 (Selection of participants and recruitment, information to the participants, informed consent), the target population are workers from companies performing surface treatment with hexavalent chromium (Cr(VI))- chrome plating in baths, surface treatment by spraying or painting, and stainless steel welding. The exact welding and surface treatment techniques used in the companies and by workers will be specified during the collection of contextual information, which is guided on this SOP.

Some welding situations are associated with low levels of exposure to Cr(VI), which may be below the limit of detection for air sampling techniques. These low exposure levels are likely to be associated with TIG welding in large workshops with good ventilation controls. Higher concentrations are likely associated with flux-cored arc welding in small or medium-size companies where the standard of ventilation control is relatively poor. Poor control might be more likely when the work is done in-situ, e.g. maintenance work in confined spaces, rather than in a well-organized workshop.

Two questionnaires will be used to collect relevant contextual information for the study:

1. Self-completed company questionnaire
  - to be completed by the company representative, prior to the sampling campaign commencing
2. Interview led post-shift worker questionnaire
  - to be completed by the researcher while interviewing the worker, and
  - to be completed as close as possible to the end of work shift.

It should be noted that some questions will not be applicable to the control group (those participants not exposed to Cr(VI)) and these questions will be clearly identified as such.

## 2 Instructions to fill in the workplace questionnaire (self-administered by company representative)

This questionnaire is to be completed by the company representative before the sample collection campaign starts. The questionnaire should all be self-explanatory however, some further explanatory information is provided below should the company representative require further information. This explanatory information also acts as an aide memoir for the researcher.

Please ask the representative to fill in the questionnaire and return it directly to you (researcher) once completed.

Questionnaire explanatory text:

- **Company and occupational health care information** – This section is to be completed by all respondents
  - Information on sector and description of the workplace are needed to be able to present the study results in aggregated form. Sector of use (see Appendix 1) and nature of the business can be described in free text.
  - The researcher will fill the NACE Rev.2 code (see Appendix 1 for classification). Copy corresponding NACE code (with 4 digits) and label text from the link. The classification is available in several EU-languages:
   
[http://ec.europa.eu/eurostat/ramon/nomenclatures/index.cfm?TargetUrl=LST\\_NOM\\_DTL&StrNom=NACE\\_REV2&StrLanguageCode=EN&IntPcKey=&StrLayoutCode=HIERARCHIC](http://ec.europa.eu/eurostat/ramon/nomenclatures/index.cfm?TargetUrl=LST_NOM_DTL&StrNom=NACE_REV2&StrLanguageCode=EN&IntPcKey=&StrLayoutCode=HIERARCHIC)
  - Most of the sectors of use in this study belong to classes 25 to 45, and they are listed in Appendix 1.
- **Operational conditions** - This section is to be completed by all respondents
  - The work tasks performed in the company need to be ticked, which will then direct the company representative to the sections that they need to complete. Sections of the questionnaire relating to work tasks not relevant to the company are omitted.
  - The researcher should double check that the corresponding sections of the ticked work tasks are completed.
- **Section 1 Chrome plating in baths:** This section is to be completed only if the company undertakes this work activity.
  - Used quantities and frequency of plating metals are asked because they affect the level of exposure.
  - Nickel use is asked because companies performing chrome plating may also perform nickel plating. If nickel plating is performed in the company then there is an option to measure urinary nickel levels in the same time as chromium. In this case the researcher should make a note for nickel analysis to be undertaken.
  - The use of trivalent chromium is asked because the companies might also do plating with trivalent chromium. In this case the researcher should make a note for trivalent chromium analysis to be undertaken.
  - The use of cadmium is asked because the exposure to cadmium may affect genotoxicity/epigenetic effect markers, which might be measured as well. In this case the researcher should make a note for cadmium analysis to be undertaken.
  - Information on perfluoroalkylated substances (PFAS; like perfluorooctyl sulfonate, PFOS) via the use of mist suppressants may also occur. Since PFAS are priority compounds under HBM4EU, exposure to those will be assessed from the blood samples. If mist suppressants are used the researcher should make a note for PFAS

- analysis to be undertaken. Note. If blood samples are not being collected at the site this question can be removed from the questionnaire (or left blank).
- The number of employees working on plating activities is asked in order to obtain a total number of workers who might be exposed to chromium and other chemicals studied.
- **Section 2 - Surface treatment by spraying or painting:** This section is to be completed only if the company undertakes this work activity.
    - Content of hexavalent chromium in paint and used amount of paint as well as frequency of tasks are asked because they affect the level of exposure.
    - The number of employees working on surface treatment activities is asked in order to get a total number of workers who might be exposed to chromium.
  - **Section 3 - Welding:** This section is to be completed only if the company undertakes this work activity.
    - Frequency of welding tasks is asked to help to assess the total exposure time.
    - The number of employees working on welding activities is asked in order to obtain a total number of workers who might be exposed to chromium.
    - Used welding method might have an effect on the level of exposure to chromium.
  - **Previous measurements:** This section is to be completed by all respondents
    - Background information of the previous exposure measurements is very important when estimating the risks of the exposure. If data on previous measurements is available for the researchers, the exposure trends can be determined. The researcher should highlight the confidential nature of providing the previous measurement data.
    - If the company representative is unaware of the type of previous measurements that have been collected or the years in question, this should be recorded as free text on the questionnaire as 'Don't know'. A request should then be made for this information to be followed up and provided, where possible, by no later than the time of completion of the sampling campaign at the site.

Upon return of the questionnaire, the researcher should ensure that all questions are completed and that handwriting is legible. In the event that it is difficult to read the handwriting, the researcher should ask for clarification and rewrite the response in their own writing. The researcher is also required to complete the NACE Rev.2 (see Appendix 1 for classification). Finally for questions where a range of options relate to an answer e.g. grams or litres; daily, days/week or days/month, the researcher should double check with the respondent which option their response relates to.

### 3 Instructions for completion of the workers post-shift questionnaire (interviewed by researcher)

This is an interview-led questionnaire with the responses being entered by the researcher. The interview-led questionnaire is to be completed as close as possible to the end of work shift where possible. The researcher can ask the site if the workers can finish a little earlier to allow the completion of the questionnaire. If this is not possible, the questionnaire should be filled in the next possible moment with due consideration of worker and researcher availability. The administration of the questionnaire should take place (where possible) in a quiet area, free from distractions.

Questionnaire is divided into three parts

- Background information about worker

- to be completed for both exposed workers and controls
- Occupational history
  - to be completed for both exposed workers and controls. Please ask about all jobs lasting more than 12 months since leaving school or full-time education.
- Job description
  - to be completed for only the exposed workers (control group participants do not need to respond to these questions).
  - after selection of the job (chrome plating in baths, surface treatment by spraying or painting, welding) the respondent will be asked only the questions relevant to that given job. All other questions will be left blank.

The interviewer should pay attention especially to the following matters and it is recommended that capital letters are used to record free text answers to aid in reading at the time of data entry:

### 1. Background information about workers (to be completed for both exposed workers and control group)

- **Worker ID:** Country ID 'XX' is the country code, using the ISO Alpha-2 country codes for the participating countries<sup>1</sup>. Participant ID (XX), where XX stands for running participant number. For example, the first worker in Finland would be coded as FI-01

Country	ISO Alpha-2 country codes
Belgium	BE
Finland	FI
France	FR
Italy	IT
Poland	PL
Portugal	PT
The Netherlands	NL
United Kingdom	UK

- **Information related to the sample collection:** Fill in the information regarding the samples collected. Pay attention to inform the actual sampling time for each sample. Note that not all samples may be collected. In which case "N/A" (not applicable) should be recorded in the relevant box.

Sample codes have the following structure:

Country ID (XX) - Participant ID (XX) - Sample ID (BXX/UXX/EXX/AXX/WXX)

The Country and Participant ID components of the sample code have been described above. Sample ID 'UXX' is one letter (B/U/E/A/W) to identify the type of sample collected, followed by a two-digit identifier (XX) to identify the running number of each type of sample for that worker (e.g. 01 for the first sample, 02 for the second and so forth). The letter code applied for the sample types is as follows:

<sup>1</sup> [http://www.nationsonline.org/oneworld/country\\_code\\_list.htm](http://www.nationsonline.org/oneworld/country_code_list.htm)

Type of sample collected	Sample type code
Air	A
Blood	B
Exhaled breath	E
Urine	U
Wipe	W

The following scenario is provided to illustrate the application of this convention.

A worker is recruited in The Netherlands. He is the first worker recruited and is providing his first two wipe samples. The sample identification codes assigned are therefore:

NL-01-W01

NL-01-W02

- **Company name and department:** Company name will already be known but name of department will need to be requested
- **Worker name:** Name of the worker is needed in order to be able contact the worker in the future to tell him/her about his/her personal results or regarding the use of his/her stored sample(s) and personal data in the future studies. The name will be replaced with a code to protect worker's privacy.
- **Sex:** The background exposure to chemicals may differ in men and women. Circle response. If an individual advises, they do not wish to respond to this question, it should be left blank.
- **Date of birth:** This question is essential to identify potential differences in human exposures, as well as susceptibility associated with the age. Record as dd/mm/yyyy.

The front page of the worker questionnaire, which contain the personal information, should then be removed from the rest of the questionnaire. Before doing so please check that the remaining pages of the worker questionnaire have the relevant unique identification codes entered.

- **Height and current weight:** This information is used to calculate body mass index (BMI). Researcher to take care in ensuring correct units are assigned.
- **Free description of occupation:** Please describe as detailed as possible to help to choose the relevant ISCO-code (see the ISCO-08 classification in Appendix 2). Copy corresponding ISCO-08 code (with 4 digits) and text label from the link. The classification is available in English, German and French:  
[http://ec.europa.eu/eurostat/ramon/nomenclatures/index.cfm?TargetUrl=LST\\_NOM\\_DTL&StrNom=CL\\_ISCO08&StrLanguageCode=EN&IntPcKey=&StrLayoutCode=HIERARCHIC](http://ec.europa.eu/eurostat/ramon/nomenclatures/index.cfm?TargetUrl=LST_NOM_DTL&StrNom=CL_ISCO08&StrLanguageCode=EN&IntPcKey=&StrLayoutCode=HIERARCHIC)

Most of the occupations in this study belong to groups 72-74, 81-82 or 93, and they are listed in Appendix 2.

- **Outside or Inside work:** Exposure may vary depending whether the work is done outside or inside. Air flows or wind conditions may lower the exposure. Circle response
- **Duration of work shifts:** Please enter typical duration of a work shift with partial hours being recorded as follows: 30 mins is 0.5 hours; 15 mins is 0.25 hours. Therefore, a 7 and a half hour work shift would be recorded as 7.5 hours.
- **Type of work shifts:** Note 'back' shift typically refers to a shift starting in the afternoon and finishing in the evening, e.g. 14.00-22.00

- **Home address** is needed in order to be able contact the worker in the future to tell him/her about his/her personal results or regarding the use of his/her stored sample(s) and personal data in the future studies. Worker's contact details will be stored exclusively for this purpose and will not be disclosed to any third party.
- **Location and related characteristics:** These questions aim to characterize the environment where the participant lives, as differences could exist in human exposure associated with the area of residence. Urban areas are very developed, meaning there is a density of human structures such as houses, commercial buildings, roads, bridges, and railways. "Urban area" can refer to towns, cities, and suburbs. In general, a "Rural area" is a geographic area that is located outside towns and cities. In other words, whatever is not urban is considered rural. Circle response
- **Industrial plants, incinerators or landfill sites in the surroundings of house:** It is necessary to collect information on facilities considered as potential sources of exposure to pollutants, which might lead to differences in human exposure levels. Likewise, this question provides information on the general characteristics of the living environment (e.g. if the house is located in a heavily industrialized area there might be high background exposure). Circle response and in the event that a Yes response is given prompt for the distance in km. In the event that the respondent indicates that they do not know the answer to this question 'Don't know' should be recorded.
- **Vehicular traffic density:** Traffic density may have an impact on exposure to Cr. Circle response.
- **Smoking habits (including all tobacco and e-cigarette products):** Information on smoking habits and passive exposure to tobacco smoke have to be collected since these are well known sources of exposure to a wide variety of substances such as Cr and Cd. The researcher should circle whether the respondent is a current smoker and if not, a former smoker. To assist the respondent in estimating the number of cigarettes smoked per day, a standard cigarette pack contains 20 cigarettes.
- **Metal containing implants:** This question is used to identify persons with artificial joints etc. in their body. Their data may need to be treated separately in some data analysis.
- **Dental fillings:** Amalgam fillings could be a source of exposure to metals
- **Medical X-ray or Computerised Axial Tomography (CAT):** This question is asked because such treatment during the last 3-months may affect on the genotoxicity markers. If the workers responds 'yes' their blood sample number Tube 1 will not be analysed for these biomarkers and a note of this should be made on the corresponding blood sample information form (see blood sampling SOP).
- **Cancer:** If the worker has been treated for cancer, it may affect on the genotoxicity markers. If the workers responds 'yes' their blood sample number Tube 1 will not be analysed for these biomarkers and a note of this should be made on the corresponding blood sample information form (see blood sampling SOP).
- **Alcohol consumption:** Alcohol has been identified as an important confounder in many epidemiological studies. When asking how many drinks a participant usually has it may help to ask, how many pints of beer/ glasses of wine/ glasses of spirit etc so to help them provide an estimated number. The types of alcoholic beverage do not need to be noted. The researcher should highlight that the samples will not be analyzed for alcohol (nor for prescription or illegal drugs).

- **Consumption of other beverages:** This question assesses the possible exposure to chemicals via other beverages (like coffee, tea and energy drinks). Energy drinks will include, for example, sports drinks or gels, Red Bull or other caffeinated drinks. Circle which additional beverages are consumed and for those indicated how many times in a typical working day.
- **Dietary habits:** Certain foodstuffs can be the source of exposure to chemicals. Other dietary habits may include gluten free, lactose free diets and should be recorded as free text.
- **Use of food supplements:** Diet pills may contain chromium. Human metabolism of some xenobiotics could be affected/modulated by the concentration of vitamins.
- **Recreational activities or hobbies:** Some recreational activities (e.g. welding, paint spraying, metal works) may result in exposure to Cr, Cd, Mn, Ni and PFAS. If the respondent indicates that they do have recreational activities that may cause additional chromate exposure, details of what they are should be recorded.

## 2. Occupational history (to be completed for both exposed workers and control group)

The information on occupational history and the exposure years are used when assessing the total cumulative exposure. This is important especially with the accumulative chemicals as their effects may show up even after long lag period. Please list all the possible work periods in the activities mentioned.

Researcher should start by asking about the respondents' current job (mentioning that this will be discussed in more detail if they are an exposed worker) and then ask the respondent to work back from this job through the jobs they have had. As a prompt it may be helpful to ask what year the respondent left school to ensure that all work periods are covered. In the event that the respondents work did not involve any of the activities of interest the activity boxes should be left unticked. In the event that the respondent did not work during a particular time period, for example, due to a period of study, unemployment or maternity leave this should be recorded as 'not employed' for the time period in question with the activity boxes left unticked.

Start and finish years should be recorded as YYYY, e.g. 1990.

Job description – The researcher should record which work task was performed by the participant which will then guide the next set of questions to be asked.

In the event that the respondent indicates that they did not complete any of these activities (in other words they are part of the control group), they should be advised that the questionnaire is complete and thanked for their contribution to the project.

## 3. Job description on different activities (to be completed for only the exposed workers)

### Job description in chrome plating in baths (administered to those who completed this activity)

- Please select all type of work tasks the worker has been involved in today, not only the main task (which is 'application in baths').
- Ask the requested contextual information:

- duration (hours / mins) and frequency of the tasks (times per week)
  - Process type – record manual or automatic.
  - risk management by personal protection equipment (PPE). Show Flash Card 1 (see Appendix 3) to assist respondent in their response concerning PPE use and record the relevant numbers on the form. If 'other' PPE is used, record '8' and details of what was used.
  - If local exhaust ventilation (LEV) was in use or not as this is very important in assessing adequate risk management of exposure. Record 'yes' or 'no'
- It is important to know whether the respiratory protection equipment (RPE) (mask) has been fit tested. To assist the respondent fit testing can be explained as a method of checking that a tight-fitting facepiece matches the wearer's facial features and seals adequately to their face. If they respond yes, that they have been face fit tested the year of testing should be recorded as 4-digits, e.g. 2017.
  - Record as 'yes' or 'no' whether the worker has received information, instruction or training on the use of safe working practices for this activity
  - Hygiene facilities – tick all that apply. In the event that 'other' is indicated, further details should be recorded as free text.
  - If the work conditions were not normal, please specify all the possible problems during the working day (e.g. problems with mask or extraction not working) as this may reflect to the level of exposure.

**Job description in surface treatment by spraying or painting (administered to those who completed this activity):**

- Please select all type of work tasks the worker has been involved in today, not only the main task. Note that tasks 2-4 are all spraying tasks in different facilities and tasks 5-6 are other kind of surface treatments. Machining operations (grinding) are tasks 10 (parts containing chromium) or task 11 (parts covered with chromium).
- Ask the requested contextual information:
  - duration (hours / mins) and frequency of the tasks (times per week)
  - risk management by personal protection equipment (PPE). Show Flash Card 1 (see Appendix 3) to assist respondent in their response concerning PPE use and record the relevant numbers on the form. If 'other' PPE is used, record '8' and details of what was used.
  - If local exhaust ventilation (LEV) was in use or not as this is very important in assessing adequate risk management of exposure. Record 'yes' or 'no'
- It is important to know whether the respiratory protection equipment (RPE) (mask) has been fit tested. To assist the respondent fit testing can be explained as a method of checking that a tight-fitting facepiece matches the wearer's facial features and seals adequately to their face. If they respond yes, that they have been face fit tested the year of testing should be recorded as 4-digits, e.g. 2017.
- Record as 'yes' or 'no' whether the worker has received information, instruction or training on the use of safe working practices for this activity

- Hygiene facilities – tick all that apply. In the event that ‘other’ is indicated, further details should be recorded as free text.
- If the work conditions were not normal, please specify all the possible problems during the working day (e.g. problems with mask or extraction not working) as this may reflect to the level of exposure.

**Job description in welding (administered to those who completed this activity):**

- Please select all type of work tasks the worker has been involved in today, not only the main task.
- Ask the requested contextual information:
  - duration (hours / mins) and frequency of the tasks (times per week)
  - risk management by personal protection equipment (PPE). Show Flash Card 2 (see Appendix 4) to assist respondent in their response concerning PPE use and record the relevant numbers on the form. If ‘other gloves’ are used, record ‘8’ and details. If ‘other’ PPE is used, record ‘9’ and details.
  - If local exhaust ventilation (LEV) was in use as this is very important in assessing adequate risk management of exposure. Show Flash Card 3 (see Appendix 5) and record relevant number.
- It is important to know whether the respiratory protection equipment (RPE) (mask) has been fit tested. To assist the respondent fit testing can be explained as a method of checking that a tight-fitting facepiece matches the wearer’s facial features and seals adequately to their face. If they respond yes, that they have been face fit tested the year of testing should be recorded as 4-digits, e.g. 2017.
- Record as ‘yes’ or ‘no’ whether the worker has received information, instruction or training on the use of safe working practices for this activity
- Hygiene facilities – tick all that apply. In the event that ‘other’ is indicated, further details should be recorded as free text.

If the work conditions were not normal, please specify all the possible problems during the working day (e.g. problems with mask or extraction not working) as this may reflect to the level of exposure.

**Operational conditions in welding**

- Material welded – circle whether this is ‘stainless steel’ or ‘other’. If ‘other’ is indicated ask what material was welded and record free text response.
- Chromium and nickel content of the welded material – If the % is known this should be recorded numerically. If unknown, ‘don’t know’ should be circled.
- Welding method used – tick all that were used during the sampling period. If ‘other’ is indicated, record free text response.
- If gas welding, e.g. TIG, then what gas mixture was used? If known, this should be recorded numerically as a percentage. If unknown enter ‘don’t know’

- Was the base metal painted and was it a chromium containing paint (circle response: yes, no or don't know)?
- Record the welding voltage and current numerically where known, otherwise circle 'don't know'.
- Material and type of welding rod - record as free text the type(s) of welding rod used during the sampling period. If the respondent does not know enter 'don't know'.
- Welding flux – record as free text the type(s) of welding flux used during the sampling period. If the respondent does not know enter 'don't know'.
- Where do you weld? This question relates to the locations where the participant welded during the sampling period. Tick all that apply and where confined spaces are indicated; ask respondent for an estimate of the size of the space in m<sup>3</sup>.
- Record whether the welder needed to position his/her head in the fume plume (tick the box that applies)?

### **Completion of questionnaire**

Upon completion of the questionnaire, participants should be informed that the interview is now complete and thanked for their contribution to the project.

## **4 Storing of gathered information**

The gathered hard copy questionnaires should be placed in secure storage in the manner described below, which is accessible only to designated members of the project team.

The front page of the worker questionnaire, which contain the personal information, should be removed from the rest of the questionnaire. Before doing so check that the remaining pages of the worker questionnaire have the relevant unique identification codes entered. The first page of the worker questionnaire should be stored in a secure physical storage separate to the rest of the coded questionnaire so to ensure confidentiality of the collected information.

The company questionnaire should also be stored in a secure physical storage separate. The company questionnaire should remain intact, e.g. there is no need to remove the front page.

The hard copy questionnaire data should be entered into the central electronic template in a timely manner.

Both hard copy and electronic data should be archived and kept for 5 years, with storage of these files being in compliance with relevant national and European data protection legislation.

## Appendix 1: Statistical Classification of Economic Activities in the European Community, NACE Rev 2. (2008)

Copy corresponding NACE code (with 4 digits) and label text from the link. The classification is available in several EU-languages:

[http://ec.europa.eu/eurostat/ramon/nomenclatures/index.cfm?TargetUrl=LST\\_NOM\\_DTL&StrNom=NACE\\_REV2&StrLanguageCode=EN&IntPcKey=&StrLayoutCode=HIERARCHIC](http://ec.europa.eu/eurostat/ramon/nomenclatures/index.cfm?TargetUrl=LST_NOM_DTL&StrNom=NACE_REV2&StrLanguageCode=EN&IntPcKey=&StrLayoutCode=HIERARCHIC)

Most of the sectors of use in this study belong to classes 25 to 45, and they are listed here:

### SECTION C — MANUFACTURING

#### **25 Manufacture of fabricated metal products, except machinery and equipment**

- 25.1 Manufacture of structural metal products
  - 25.11 Manufacture of metal structures and parts of structures
  - 25.12 Manufacture of doors and windows of metal
- 25.2 Manufacture of tanks, reservoirs and containers of metal
  - 25.21 Manufacture of central heating radiators and boilers
  - 25.29 Manufacture of other tanks, reservoirs and containers of metal
- 25.3 Manufacture of steam generators, except central heating hot water boilers
  - 25.30 Manufacture of steam generators, except central heating hot water boilers
- 25.4 Manufacture of weapons and ammunition
  - 25.40 Manufacture of weapons and ammunition
- 25.5 Forging, pressing, stamping and roll-forming of metal; powder metallurgy
  - 25.50 Forging, pressing, stamping and roll-forming of metal; powder metallurgy
- 25.6 Treatment and coating of metals; machining
  - 25.61 Treatment and coating of metals
  - 25.62 Machining
- 25.7 Manufacture of cutlery, tools and general hardware
  - 25.71 Manufacture of cutlery
  - 25.72 Manufacture of locks and hinges
  - 25.73 Manufacture of tools
- 25.9 Manufacture of other fabricated metal products
  - 25.91 Manufacture of steel drums and similar containers
  - 25.92 Manufacture of light metal packaging
  - 25.93 Manufacture of wire products, chain and springs
  - 25.94 Manufacture of fasteners and screw machine products
  - 25.99 Manufacture of other fabricated metal products n.e.c.

#### **26 Manufacture of computer, electronic and optical products**

- 26.1 Manufacture of electronic components and boards
  - 26.11 Manufacture of electronic components
  - 26.12 Manufacture of loaded electronic boards
- 26.2 Manufacture of computers and peripheral equipment
  - 26.20 Manufacture of computers and peripheral equipment
- 26.3 Manufacture of communication equipment
  - 26.30 Manufacture of communication equipment
- 26.4 Manufacture of consumer electronics
  - 26.40 Manufacture of consumer electronics
- 26.5 Manufacture of instruments and appliances for measuring, testing and navigation; watches and clocks
  - 26.51 Manufacture of instruments and appliances for measuring, testing and navigation
  - 26.52 Manufacture of watches and clocks
- 26.6 Manufacture of irradiation, electromedical and electrotherapeutic equipment

- 26.60 Manufacture of irradiation, electromedical and electrotherapeutic equipment
- 26.7 Manufacture of optical instruments and photographic equipment
  - 26.70 Manufacture of optical instruments and photographic equipment
- 26.8 Manufacture of magnetic and optical media
  - 26.80 Manufacture of magnetic and optical media

## **27 Manufacture of electrical equipment**

- 27.1 Manufacture of electric motors, generators, transformers and electricity distribution and control apparatus
  - 27.11 Manufacture of electric motors, generators and transformers
  - 27.12 Manufacture of electricity distribution and control apparatus
- 27.2 Manufacture of batteries and accumulators
  - 27.20 Manufacture of batteries and accumulators
- 27.3 Manufacture of wiring and wiring devices
  - 27.31 Manufacture of fibre optic cables
  - 27.32 Manufacture of other electronic and electric wires and cables
  - 27.33 Manufacture of wiring devices
- 27.4 Manufacture of electric lighting equipment
  - 27.40 Manufacture of electric lighting equipment
- 27.5 Manufacture of domestic appliances
  - 27.51 Manufacture of electric domestic appliances
  - 27.52 Manufacture of non-electric domestic appliances
- 27.9 Manufacture of other electrical equipment
  - 27.90 Manufacture of other electrical equipment

## **28 Manufacture of machinery and equipment n.e.c.**

- 28.1 Manufacture of general — purpose machinery
  - 28.11 Manufacture of engines and turbines, except aircraft, vehicle and cycle engines
  - 28.12 Manufacture of fluid power equipment 2812
  - 28.13 Manufacture of other pumps and compressors 2813\*
  - 28.14 Manufacture of other taps and valves 2813\*
  - 28.15 Manufacture of bearings, gears, gearing and driving elements 2814
- 28.2 Manufacture of other general-purpose machinery
  - 28.21 Manufacture of ovens, furnaces and furnace burners 2815
  - 28.22 Manufacture of lifting and handling equipment 2816
  - 28.23 Manufacture of office machinery and equipment (except computers and peripheral equipment)
  - 28.24 Manufacture of power-driven hand tools 2818
  - 28.25 Manufacture of non-domestic cooling and ventilation equipment 2819\*
  - 28.29 Manufacture of other general-purpose machinery n.e.c. 2819\*
- 28.3 Manufacture of agricultural and forestry machinery
  - 28.30 Manufacture of agricultural and forestry machinery 2821
- 28.4 Manufacture of metal forming machinery and machine tools
  - 28.41 Manufacture of metal forming machinery
  - 28.49 Manufacture of other machine tools
- 28.9 Manufacture of other special-purpose machinery
  - 28.91 Manufacture of machinery for metallurgy
  - 28.92 Manufacture of machinery for mining, quarrying and construction
  - 28.93 Manufacture of machinery for food, beverage and tobacco processing
  - 28.94 Manufacture of machinery for textile, apparel and leather production
  - 28.95 Manufacture of machinery for paper and paperboard production
  - 28.96 Manufacture of plastic and rubber machinery
  - 28.99 Manufacture of other special-purpose machinery n.e.c.

## **29 Manufacture of motor vehicles, trailers and semi-trailers**

- 29.1 Manufacture of motor vehicles
  - 29.10 Manufacture of motor vehicles
- 29.2 Manufacture of bodies (coachwork) for motor vehicles; manufacture of trailers and semi-trailers
  - 29.20 Manufacture of bodies (coachwork) for motor vehicles; manufacture of trailers and semi-trailers
- 29.3 Manufacture of parts and accessories for motor vehicles
  - 29.31 Manufacture of electrical and electronic equipment for motor vehicles
  - 29.32 Manufacture of other parts and accessories for motor vehicles

### **30 Manufacture of other transport equipment**

- 30.1 Building of ships and boats
  - 30.11 Building of ships and floating structures
  - 30.12 Building of pleasure and sporting boats
- 30.2 Manufacture of railway locomotives and rolling stock
  - 30.20 Manufacture of railway locomotives and rolling stock
- 30.3 Manufacture of air and spacecraft and related machinery
  - 30.30 Manufacture of air and spacecraft and related machinery
- 30.4 Manufacture of military fighting vehicles
  - 30.40 Manufacture of military fighting vehicles
- 30.9 Manufacture of transport equipment n.e.c.
  - 30.91 Manufacture of motorcycles
  - 30.92 Manufacture of bicycles and invalid carriages
  - 30.99 Manufacture of other transport equipment n.e.c.

### **31 Manufacture of furniture**

- 31.0 Manufacture of furniture
  - 31.01 Manufacture of office and shop furniture
  - 31.02 Manufacture of kitchen furniture
  - 31.03 Manufacture of mattresses
  - 31.09 Manufacture of other furniture

### **32 Other manufacturing**

- 32.1 Manufacture of jewellery, bijouterie and related articles
  - 32.11 Striking of coins
  - 32.12 Manufacture of jewellery and related articles
  - 32.13 Manufacture of imitation jewellery and related articles
- 32.2 Manufacture of musical instruments
  - 32.20 Manufacture of musical instruments
- 32.3 Manufacture of sports goods
  - 32.30 Manufacture of sports goods
- 32.4 Manufacture of games and toys
  - 32.40 Manufacture of games and toys
- 32.5 Manufacture of medical and dental instruments and supplies
  - 32.50 Manufacture of medical and dental instruments and supplies
- 32.9 Manufacturing n.e.c.
  - 32.91 Manufacture of brooms and brushes
  - 32.99 Other manufacturing n.e.c.

### **33 Repair and installation of machinery and equipment**

- 33.1 Repair of fabricated metal products, machinery and equipment
  - 33.11 Repair of fabricated metal products
  - 33.12 Repair of machinery
  - 33.13 Repair of electronic and optical equipment
  - 33.14 Repair of electrical equipment
  - 33.15 Repair and maintenance of ships and boats
  - 33.16 Repair and maintenance of aircraft and spacecraft

- 33.17 Repair and maintenance of other transport equipment
- 33.19 Repair of other equipment
- 33.2 Installation of industrial machinery and equipment
  - 33.20 Installation of industrial machinery and equipment

## **SECTION D — ELECTRICITY, GAS, STEAM AND AIR CONDITIONING SUPPLY**

### **35 Electricity, gas, steam and air conditioning supply**

- 35.1 Electric power generation, transmission and distribution
  - 35.11 Production of electricity
  - 35.12 Transmission of electricity
  - 35.13 Distribution of electricity
  - 35.14 Trade of electricity
- 35.2 Manufacture of gas; distribution of gaseous fuels through mains
  - 35.21 Manufacture of gas
  - 35.22 Distribution of gaseous fuels through mains
  - 35.23 Trade of gas through mains
- 35.3 Steam and air conditioning supply
  - 35.30 Steam and air conditioning supply

## **SECTION E — WATER SUPPLY; SEWERAGE, WASTE MANAGEMENT AND REMEDIATION ACTIVITIES**

### **36 Water collection, treatment and supply**

- 36.0 Water collection, treatment and supply
  - 36.00 Water collection, treatment and supply

### **37 Sewerage**

- 37.0 Sewerage
  - 37.00 Sewerage

### **38 Waste collection, treatment and disposal activities; materials recovery**

- 38.1 Waste collection
  - 38.11 Collection of non-hazardous waste
  - 38.12 Collection of hazardous waste
- 38.2 Waste treatment and disposal
  - 38.21 Treatment and disposal of non-hazardous waste
  - 38.22 Treatment and disposal of hazardous waste
- 38.3 Materials recovery
  - 38.31 Dismantling of wrecks
  - 38.32 Recovery of sorted materials

### **39 Remediation activities and other waste management services**

- 39.0 Remediation activities and other waste management services
  - 39.00 Remediation activities and other waste management services

## **SECTION F — CONSTRUCTION**

### **41 Construction of buildings**

- 41.1 Development of building projects
  - 41.10 Development of building projects
- 41.2 Construction of residential and non-residential buildings
  - 41.20 Construction of residential and non-residential buildings

### **42 Civil engineering**

- 42.1 Construction of roads and railways
  - 42.11 Construction of roads and motorways
  - 42.12 Construction of railways and underground railways
  - 42.13 Construction of bridges and tunnels
- 42.2 Construction of utility projects
  - 42.21 Construction of utility projects for fluids
  - 42.22 Construction of utility projects for electricity and telecommunications

- 42.9 Construction of other civil engineering projects
  - 42.91 Construction of water projects
  - 42.99 Construction of other civil engineering projects n.e.c.

**43 Specialized construction activities**

- 43.1 Demolition and site preparation
  - 43.11 Demolition
  - 43.12 Site preparation
  - 43.13 Test drilling and boring
- 43.2 Electrical, plumbing and other construction installation activities
  - 43.21 Electrical installation
  - 43.22 Plumbing, heat and air conditioning installation
  - 43.29 Other construction installation

For more detailed descriptions and guidance see:

<http://ec.europa.eu/eurostat/documents/3859598/5902521/KS-RA-07-015-EN.PDF>

## Appendix 2: Structure of the international standard classification of occupations (ISCO-08)

Copy corresponding ISCO-08 code (with 4 digits) and text label from the link. The classification is available in English, German and French:

[http://ec.europa.eu/eurostat/ramon/nomenclatures/index.cfm?TargetUrl=LST\\_NOM\\_DTL&StrNom=CL\\_ISCO08&StrLanguageCode=EN&IntPcKey=&StrLayoutCode=HIERARCHIC](http://ec.europa.eu/eurostat/ramon/nomenclatures/index.cfm?TargetUrl=LST_NOM_DTL&StrNom=CL_ISCO08&StrLanguageCode=EN&IntPcKey=&StrLayoutCode=HIERARCHIC)

Most of the occupations in this study belong to groups 72-74, 81-82 or 93, and they are listed here:

### 7 Craft and Related Trades Workers

#### 71 Building and Related Trades Workers (excluding Electricians)

- 711 Building Frame and Related Trades Workers
  - 7111 House Builders
  - 7112 Bricklayers and Related Workers
  - 7113 Stonemasons, Stone Cutters, Splitters and Carvers
  - 7114 Concrete Placers, Concrete Finishers and Related Workers
  - 7115 Carpenters and Joiners
  - 7119 Building Frame and Related Trades Workers Not Elsewhere Classified
- 712 Building Finishers and Related Trades Workers
  - 7121 Roofers
  - 7122 Floor Layers and Tile Setters
  - 7123 Plasterers
  - 7124 Insulation Workers
  - 7125 Glaziers
  - 7126 Plumbers and Pipe Fitters
  - 7127 Air Conditioning and Refrigeration Mechanics
- 713 Painters, Building Structure Cleaners and Related Trades Workers
  - 7131 Painters and Related Workers
  - 7132 Spray Painters and Varnishers
  - 7133 Building Structure Cleaners

#### 72 Metal, Machinery and Related Trades Workers

- 721 Sheet and Structural Metal Workers, Moulders and Welders, and Related Workers
  - 7211 Metal Moulders and Coremakers
  - 7212 Welders and Flame Cutters
  - 7213 Sheet Metal Workers
  - 7214 Structural Metal Preparers and Erectors
  - 7215 Riggers and Cable Splicers
- 722 Blacksmiths, Toolmakers and Related Trades Workers
  - 7221 Blacksmiths, Hammersmiths and Forging Press Workers
  - 7222 Toolmakers and Related Workers
  - 7223 Metal Working Machine Tool Setters and Operators
  - 7224 Metal Polishers, Wheel Grinders and Tool Sharpeners
- 723 Machinery Mechanics and Repairers
  - 7231 Motor Vehicle Mechanics and Repairers
  - 7232 Aircraft Engine Mechanics and Repairers
  - 7233 Agricultural and Industrial Machinery Mechanics and Repairers
  - 7234 Bicycle and Related Repairers

#### 73 Handicraft and Printing Workers

- 731 Handicraft Workers
  - 7311 Precision-instrument Makers and Repairers

- 7312 Musical Instrument Makers and Tuners
- 7313 Jewellery and Precious Metal Workers
- 7314 Potters and Related Workers
- 7315 Glass Makers, Cutters, Grinders and Finishers
- 7316 Signwriters, Decorative Painters, Engravers and Etchers
- 7317 Handicraft Workers in Wood, Basketry and Related Materials
- 7318 Handicraft Workers in Textile, Leather and Related Materials
- 7319 Handicraft Workers Not Elsewhere Classified
- 732 Printing Trades Workers
  - 7321 Pre-press Technicians
  - 7322 Printers
  - 7323 Print Finishing and Binding Workers

#### **74 Electrical and Electronics Trades Workers**

- 741 Electrical Equipment Installers and Repairers
  - 7411 Building and Related Electricians
  - 7412 Electrical Mechanics and Fitters
  - 7413 Electrical Line Installers and Repairers
- 742 Electronics and Telecommunications Installers and Repairers
  - 7421 Electronics Mechanics and Servicers
  - 7422 Information and Communications Technology Installers and Servicers

### **8 Plant and Machine Operators and Assemblers**

#### **81 Stationary Plant and Machine Operators**

- 811 Mining and Mineral Processing Plant Operators
  - 8111 Miners and Quarriers
  - 8112 Mineral and Stone Processing Plant Operators
  - 8113 Well Drillers and Borers and Related Workers
  - 8114 Cement, Stone and Other Mineral Products Machine Operators
- 812 Metal Processing and Finishing Plant Operators
  - 8121 Metal Processing Plant Operators
  - 8122 Metal Finishing, Plating and Coating Machine Operators
- 813 Chemical and Photographic Products Plant and Machine Operators
  - 8131 Chemical Products Plant and Machine Operators
  - 8132 Photographic Products Machine Operators
- 814 Rubber, Plastic and Paper Products Machine Operators
  - 8141 Rubber Products Machine Operators
  - 8142 Plastic Products Machine Operators
  - 8143 Paper Products Machine Operators
- 815 Textile, Fur and Leather Products Machine Operators
  - 8151 Fibre Preparing, Spinning and Winding Machine Operators
  - 8152 Weaving and Knitting Machine Operators
  - 8153 Sewing Machine Operators
  - 8154 Bleaching, Dyeing and Fabric Cleaning Machine Operators
  - 8155 Fur and Leather Preparing Machine Operators
  - 8156 Shoemaking and Related Machine Operators
  - 8157 Laundry Machine Operators
  - 8159 Textile, Fur and Leather Products Machine Operators Not Elsewhere Classified
- 816 Food and Related Products Machine Operators
  - 8160 Food and Related Products Machine Operators
- 817 Wood Processing and Papermaking Plant Operators
  - 8171 Pulp and Papermaking Plant Operators
  - 8172 Wood Processing Plant Operators
- 818 Other Stationary Plant and Machine Operators
  - 8181 Glass and Ceramics Plant Operators
  - 8182 Steam Engine and Boiler Operators

- 8183 Packing, Bottling and Labelling Machine Operators
- 8189 Stationary Plant and Machine Operators Not Elsewhere Classified

## **82 Assemblers**

- 821 Assemblers
  - 8211 Mechanical Machinery Assemblers
  - 8212 Electrical and Electronic Equipment Assemblers
  - 8219 Assemblers Not Elsewhere Classified

## **9 Elementary Occupations**

### **93 Labourers in Mining, Construction, Manufacturing and Transport**

- 931 Mining and Construction Labourers
  - 9311 Mining and Quarrying Labourers
  - 9312 Civil Engineering Labourers
  - 9313 Building Construction Labourers
- 932 Manufacturing Labourers
  - 9321 Hand Packers
  - 9329 Manufacturing Labourers Not Elsewhere Classified
- 933 Transport and Storage Labourers
  - 9331 Hand and Pedal Vehicle Drivers
  - 9332 Drivers of Animal-drawn Vehicles and Machinery
  - 9333 Freight Handlers
  - 9334 Shelf Fillers

## **Appendix 3: Flash Card 1: PPE (personal protective equipment) used for chrome plating and spray painting**

1. Powered or air-fed, filtering respirator
2. Reusable half or full face mask respirator (without powered or air-fed respirator)
3. Disposable face mask
4. Other Respiratory Protection Equipment (please specify)
5. Coveralls
6. Reusable Gloves
7. Disposable gloves
8. Other (please specify)

## **Appendix 4: Flash Card 2: PPE (personal protective equipment) used for welding**

1. Welding helmet with powered or air-fed, filtering respirator
2. Welding helmet with half mask re-usable dust respirator
3. Welding helmet with disposable particulate respirator
4. Welding helmet without any respirator
5. Welding helmet with other respiratory protection equipment (please specify)
6. Fire/flame resistant clothing
7. Welding gloves
8. Other gloves
9. Other (please specify)

## **Appendix 5: Flash Card 3: LEV (local exhaust ventilation) used for welding**

1. Gun fixed extraction
2. Movable welding hood
3. Extracted work bench
4. Extracted welding booth
5. General ventilation
6. Other (please specify)



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## **SOP 3:**

**Standard operating procedure for blood sampling,  
including sample storage and transfer**

**WP8**

**Task 8.5**

## Table of Contents

Authors and Acknowledgements.....	3
1 Introduction.....	3
2 Precautions in the pre-analytical phase.....	4
3 Blood sampling .....	6
3.1 Blood collection schedule .....	6
3.2 Sampling material .....	6
3.3 Instructions for blood sampling .....	8
4 Conservation, transport and storage of the samples .....	9
4.1 Processing and storage of collected blood samples at the local laboratory .....	9
4.2 Transportation of the samples to the laboratory .....	11
4.3 Storage of the samples in the laboratory until analysis.....	12
References .....	12
Annex 1 – Blood sampling form .....	13
Annex 2 – Schemes for blood collection and transportation .....	14
Annex 3 - An illustration of best practices in phlebotomy (WHO, 2010).....	17

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The text has been partially adapted for the chromate occupational study based on the document SOP 3: Procedure for obtaining human samples, prepared under WP 7, Task 7.2, D 7.3.

The draft version was revised upon suggestions made by laboratories with expertise in chromium analysis.

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## 1 Introduction

Within HBM4EU project, several priority chemicals were identified, which may be of concern for the European population. Several of those are also relevant at European workplaces, such as Hexavalent chromium, Cr(VI), which is one of the most important occupational carcinogens, known to cause lung cancer in humans. It is currently an issue in EU since Cr(VI) compounds are authorized under Registration, Evaluation, Authorization and Restriction of Chemicals (REACH). Moreover, the European Commission has published a proposal to set a binding limit value for Cr(VI) under the Carcinogens and Mutagens Directive (CMD) i.e. EU Directive 2004/37/EC on the protection of workers from the risks related to exposure to carcinogens or mutagens at work.

The relationship between occupational exposure levels and chromium levels has been investigated in numerous studies, with results showing correlations between exposure levels and chromium levels in blood and urine (reviewed in Wilbur et al. 2012).

Cr(VI) that enters blood is taken up by red blood cells, reduced to Cr(III), and, in the process, forms adducts with red blood cell haemoglobin and other proteins. These complexes are sufficiently stable to remain in the red blood cells for a substantial fraction of the lifespan of the red blood cell (Wilbur et al. 2012). Therefore, following absorption of Cr(VI) into blood, the elimination half-time of chromium in blood will be substantially longer than that in plasma. Based on a half-time of 30 days in red blood cells, with cessation of exposure to and absorption of Cr(VI), levels of chromium in red blood cells will reach 5% of a previous steady state level within 130 days.

Therefore, Cr(VI) compounds can be analytically determined in red blood cells (RBC) or plasma. An increase in plasma levels of chromium may reflect both recent exposure and exposure that occurred during the past few months (e.g., chromium that is sequestered within erythrocytes for the lifespan of the cell). Also, distinct measurements of chromium in plasma and whole blood (reflecting intracellular distribution to erythrocytes) may also be useful in distinguishing exposures to Cr(VI) compounds *versus* Cr(III) compounds; increased plasma levels of chromium may indicate

exposure to both Cr(VI) and Cr(III), whereas increased chromium in erythrocytes indicates exposure to Cr(VI), since Cr(III) is not taken up by erythrocytes (Wilbur et al. 2012).

According to Wilbur et al. (2012), the IOM (2001) reported average plasma chromium concentrations of 2–3 nmol/L (equivalent to 0.10–0.16 µg/L) and endogenous chromium concentrations also have been reported as 0.01–0.17 µg/L (median 0.06 µg/L) in serum. However, normal chromium levels in human fluid and tissues should be interpreted with caution. The low sensitivity of the most commonly used detection methods and the ubiquitous presence of chromium in laboratories make detection of low levels of chromium in blood and urine difficult.

Several studies demonstrated that chromium forms protein-DNA crosslinks and DNA adducts, and that these endpoints may be potentially useful biological markers, indicating the possibility of genotoxic effects or cancer in humans exposed to chromium (Wilbur et al. 2012). Therefore, micronucleus frequency in human lymphocytes and reticulocytes is a biomarker for early biological effects that may be of interest in studies concerning Cr(VI) and can be performed using whole blood collected in sodium heparin-coated tubes. Other assays for genotoxic damage, such as comet or chromosomal aberrations assay, as well as epigenetic changes may be relevant biomarkers of early biological effects.

In chrome electroplating in baths, mist suppressants are used to prevent the formation of aerosols. These mist suppressants often contain PFAS compounds. Therefore, it is interesting to study the PFAS exposure of electroplaters. PFAS analyses can be done from serum or plasma. Several PFAS compounds can be analysed from the same samples. In this study, we collect plasma, which will be then used for PFAS analysis.

This guideline is intended to be used in the framework of the Human Biomonitoring Initiative (HBM4EU). The Standard Operating Procedure (SOP) for blood sampling provides the general procedure for the collection, storage and transfer of human blood samples to be analysed within the chromate occupational study.

## 2 Precautions in the pre-analytical phase

The pre-analytical phase comprises all actions and aspects that occur prior to the analytical phase and should be considered as part of the laboratory work. This phase involves the collection, handling, transport and conservation, distribution and storage of samples until analyses, which this SOP addresses.

Although quality control measures are often absent from the pre-analytical phase, it is essential to avoid, or at least minimise, samples misidentification and possible sources of contamination. In this regard, two main groups of factors should be considered:

### a) Influencing factors:

These factors are specific for each biomarker and are already present before the sampling procedures. The biological half-life of a chemical, alcohol consumption, medication intake or individual habits such as smoking or diet, are examples of influencing factors that can modify the levels of the chemical of interest or the biomarkers of effects. Therefore, the influencing factors for the target biomarker must be identified and a sampling strategy that takes them into account must be considered.

Cr(VI) in the environment is almost totally derived from human activities (ATSDR, 2011). Environmental sources of chromium include: airborne emissions from chemical plants and incineration facilities, cement dust, contaminated landfill, effluents from chemical plants,

asbestos lining erosion, road dust from catalytic converter erosion and asbestos brakes, tobacco smoke, and topsoil and rocks. The general population is exposed to chromium by the oral route (food or food supplements, drinking water) and by inhalation (chromium in air). Therefore, apart from the exposure in occupational settings, also the exposure of workers through the referred sources should be assessed by the questionnaire.

b) Interfering factors

These factors can modify the concentration of the biomarker after sampling due to different processes such as the external contamination, physical or chemical changes in the biomarker during transport or storage, or changes in the biological matrix (e.g. coagulation or sedimentation). It is essential to identify and avoid possible sources of external contamination at the sampling site, as well as sample contamination due to inappropriate skin/mucosa disinfection or to the use of non sterile equipment/materials. Likewise, alterations due to adsorption of the substances under analysis to the vials wall should be also avoided.

Particularly, to reduce interferences in chromium analysis the following recommendations must be followed:

- The skin should be disinfected with alcohol and not with povidone iodine.
- Powder-free gloves should be used;
- Appropriate tubes for trace element detection (e.g. Vacuette® Trace Elements 6 mL) should be used to collect blood in order to eliminate any possible Cr contamination;
- The plastic material used thereafter should be trace element quality or should be soaked in 20% HNO<sub>3</sub> for 24 h prior to use and rinsed three times with deionized distilled water (Devoy et al., 2016).
- For venous blood collection it is recommended to use needles coated inside with silicone, although this is not consensual.

In addition, good aseptic techniques should always be employed in the collection of blood samples.

## 3 Blood sampling

### 3.1 Blood collection schedule

Blood is an ideal matrix for most chemicals because it is in contact with all tissues and is in equilibrium with the organs and tissues where chemicals are deposited. The collection of blood samples requires a clean, quiet and confined space, the availability of sterile material for blood collection and staff trained in phlebotomy knowing the special precautions related to the handling of biological material, according to each country rules.

One blood sample will be collected from each (exposed or non-exposed) worker, following signed informed consent of the participant (see SOP1: Selection of participants and recruitment, information to the participants, informed consent).

It will be convenient (for both the participant and researcher) to schedule the blood collection and urine sample collection at the same time, preferentially at the middle or end of a working day. It was assumed that the optimum timing for the sampling would be on the 3<sup>rd</sup> - 5<sup>th</sup> day of a working week (assuming a 5-day working week), preferentially in the afternoon. In addition, because samples have to be shipped to the laboratories that will perform the biomarkers analyses, **blood collections should be done on Wednesday or Friday and shipped on Wednesday or Monday, respectively**, to avoid being in transit for a long time, especially on weekend (see Annex 2, fig. A.1). **Please inform by email the laboratories that are going to receive samples about the number, date of samples collection and date estimated for samples arrival.**

Basic information shall be collected through an individual questionnaire with the support of the researcher or technician to avoid interpretation errors [see SOP2: Gathering contextual information and filling of the (including storing of gathered information)].

In addition, at the sampling time, **the following information should be recorded in the Blood Sampling Form** (Annex 1):

- Unique sample code attributed to worker and used to label sample tubes, for unambiguous identification of the specimens and related documents (questionnaires, personal data, etc.)
- Date and time of blood collection
- Number of tubes collected and their destination (according to the type of analysis and Lab that will perform it).

### 3.2 Sampling material

The following materials and equipment will be necessary for blood sampling and fractionation:

- **Tubes with anticoagulant:**
  - 2 Tubes - **tubes 1 and 4** - with sodium heparin (volume: 6 and 3 mL, respectively) for effect biomarkers (micronuclei);
  - 1 Tube – **tube 2** – with K<sub>2</sub> EDTA (volume: 3 mL) for effect biomarkers (epigenetics, telomere length); appropriate for -80°C (e.g., cryotubes Bio-one);
  - 1 Tube - **tube 3** - with K<sub>2</sub> EDTA or heparin (volume: 6 mL) for analysis of Cr in plasma and red blood cells and analysis of PFAS in plasma. Tubes for trace elements should be used to minimise the background contamination, e.g. *Greiner Vacuette®* Trace Elements, 6 ml or *BD Vacutainer®* Trace Element tubes (royal blue stopper).

- Vials for plasma and red blood cells storage that must be trace elements quality too (e.g., ICP-MS autosampler tubes) or pre-treated with HNO<sub>3</sub> [see 2.b)]
- Regular phlebotomy syringe with a stainless-steel needle; the use of a silicone-coated needle or butterfly is recommended (e.g. Sarstedt 21G for metal analysis ref. 85.1162.600); the vacutainer system can be optionally used
- powder-free disposable gloves
- 70% alcohol swabs for skin disinfection
- Labels
- Garrottes/tourniquets
- Adhesive bandages or tapes
- Container for disposal of used needles after venipuncture
- Bench centrifuge, refrigerated
- Pipettes for collecting the plasma and buffy coat
- NaCl solution (0.9%)
- Refrigerator for samples storage at +4°C
- Dry ice
- Freezer samples for storage at -80°C
- Containers appropriate for blood samples shipment (at + 4°C and -80°C)

A standardised convention will be used to assign unique identification codes for all samples collected. The identification code convention is as follows:

Country ID (XX) - Participant ID (XX) - Sample ID (BXX/UXX/EXX/AXX/WXX)

Country ID 'XX' is the country code, using the ISO Alpha-2 country codes for the participating countries<sup>1</sup>.

Country	ISO Alpha-2 country codes
Belgium	BE
Finland	FI
France	FR
Italy	IT
Poland	PL
Portugal	PT
The Netherlands	NL
United Kingdom	UK

Participant ID 'XX' is a two-digit running number of participants in each country (e.g. 01 for the first participant recruited, 02 the second and so forth).

<sup>1</sup> [http://www.nationsonline.org/oneworld/country\\_code\\_list.htm](http://www.nationsonline.org/oneworld/country_code_list.htm)

Sample ID 'UXX' is one letter (B/U/E/A/W) to identify the type of sample collected, followed by a two-digit identifier (XX) to identify the running number of each type of sample for that worker (e.g. 01 for the first sample, 02 for the second and so forth). The letter code applied for the sample types is as follows:

Type of sample collected	Sample type code
Air	A
Blood	B
Exhaled breath	E
Urine	U
Wipe	W

The following scenario is provided to illustrate the application of this convention.

A worker is recruited in The Netherlands. They are the first worker recruited and they are providing their first urine sample. The sample identification code assigned is therefore:

NL-01-U01

In the event that an air sample is also collected from this same worker, the sample identification code to be assigned would be:

NL-01-A01

### 3.3 Instructions for blood sampling

Blood sampling must only be done by personnel trained in phlebotomy techniques. In general, the blood is collected by venous puncture and manipulated under sterile conditions. The trained personnel shall be in charge of the procedure and shall use adequate personal protection equipment (lab coat and gloves). WHO (2010) provides the best practices on drawing blood and these should be followed (Annex 3). In general:

1. Keep the blood handling area clean and free of dust;
2. Use only the supplies provided by the study responsible as detailed in Section 3.2; wear talc-free gloves;
3. Prepare the tubes and label them with the code number and other relevant information (date, place);
4. Record relevant details in the record form (Annex 1 and Annex 3, fig. A.2);
5. Prepare the volunteer for phlebotomy;
6. Place the garrotte in the forearm and disinfect the collection site with 70% alcohol;
7. Collect approximately 18 mL (see table I) of venous blood by phlebotomy, loosen the garrotte and press a cotton ball with 70% alcohol against the puncture site;
8. Immediately distribute the blood sample among the four labelled tubes, filling them to the mark to avoid the risk of haemolysis. **Tube 3 should be the 1<sup>st</sup> tube to be filled** to avoid contamination of phlebotomy needle when puncturing the rubber stopper of other tubes;
9. Invert each tube gently 8 times, in order to mix the sample with the anticoagulant. After mixing, keep tube 3 upright until further processing to avoid contact with stopper;
10. Check that the worker is okay and provide a plaster for puncture site as necessary.

**Table I:** Distribution of samples according to the analyses to be performed.

Use	Fraction	Tube 1	Tube 2	Tube 3*	Tube 4	Volume of Blood collected
		Na heparin	K <sub>2</sub> EDTA	K <sub>2</sub> EDTA /heparin	Na heparin	
		6 ml	3 ml	6 ml	3 ml	
Cr determination	Plasma	0	0	X	0	18 ml
Cr determination	RBC	0	0	X	0	
PFAS determination	plasma	0	0	X	0	
Micronucleus & Comet assays	Whole blood	X	0	0	0	
Micronucleus assay	Reticulocytes	0	0	0	X	
Oxidative Lesions	Whole blood	0	X	0	0	
Epigenetics	Whole blood	0	X	0	0	

*\*Important: Tube 3 must be appropriate for trace elements analysis and must be the 1<sup>st</sup> to be filled*

A scheme for blood distribution among tubes and for shipment is additionally provided in Annex 2 (fig. A.3).

#### Important notes:

- All tubes should be transferred from the site of collection to the local laboratory as soon as possible (preferentially less than 2h after sampling) for further processing;
- All tubes should be transported at +4°C and tubes 1 and 4 need to be protected from light (wrapped in aluminium foil);
- Tube 2 can be immediately frozen at - 80°C at the site of collection (and transported at that temperature) or can be transported at +4 °C and immediately frozen at - 80°C after arrival. Tubes must be compatible with -80°C storage (e.g., cryotubes Bio-one);
- To ensure samples transport at +4°C (max +10°C) frozen ice packs shall be used, placed at the bottom and along the sides of the styrofoam box, making sure, however, that the samples will not freeze (Annex 2, fig. A.4).

## 4 Conservation, transport and storage of the samples

### 4.1 Processing and storage of collected blood samples at the local laboratory

Upon arrival to the local laboratory in the country of origin of the samples the following procedure must be immediately followed:

- **Check the blood sampling form** to confirm the number and type of tubes received per individual and to proceed accordingly.

- **Tube 1** will be kept at room temperature protected from light (wrapped in aluminium foil) until shipment to INSA (Portugal).
- **Tube 2** will be kept frozen at -80°C for effect biomarkers analysis and sent to KU Leuven (Belgium) without further manipulation (alternatively, DNA can be extracted and sent).
- **Tube 3** will be used for plasma and red blood cells (RBC) separation preferably within 8 h hours of the specimen collection, maximum 24 h, to avoid haemolysis. Separation can be done as described by Devoy et al. (2016). Briefly:
  1. Record the volume  $V_i$  of the total blood in the tube (or mark the blood volume on the tube) and determine the haematocrit 1 (HT1)\*;
  2. Centrifuge the total blood sample for 10 min at 1 000–2 000 x g (or 5 min at 2700 x g)
  3. Collect the supernatant (Fraction 1) consisting of plasma and white blood cells, in a PP tube (ICP-MS quality or beforehand washed with HNO<sub>3</sub> 20% and thrice rinsed with purified water); careful should be taken to avoid collection of RBC;
  4. Dilute the RBC pellet (Fraction 2) with NaCl solution (0.9%) up to the initial volume  $V_i$ ;
  5. Gently agitate at room temperature for 10 min;
  6. Centrifuge for 10 min at 1 000–2 000 x g (or 5 min at 2700 x g);
  7. Discard the supernatant (washing phase);
  8. Perform 2 more washings. Before the last centrifugation, measure the HT2;
  9. After removing the last washing phase, fill the tube containing RBCs with 1% Triton X-100 in deionised water/0.2% HNO<sub>3</sub> up to the initial volume  $V^{**}$ .
  10. Storage: RBC - store at room temperature up to 3 days or at -20°C for longer periods. It is possible to keep the samples at -20°C for 3 months. Plasma - store and ship at +4 °C up to 7 days; store at -20°C for longer periods. This is used for chromium analysis and possibly for PFAS analyses\*\*\* (see table I). For PFAS analysis, store plasma fraction at -20°C after collection.
- **Tube 4** will be kept at +4–8 °C (do not freeze) protected from light and sent to FIOH (Finland).

---

**Notes** (information kindly provided by Ogier Hanser and Guillaume Antoine, INRS):

\* the HT2 measurement allows to convert the final Cr concentration from µg/L of the sample analysed into µg/L of RBC. The ratio HT2:HT1 allows the correction of the RBCs loss along the washing steps.

\*\* The % of HNO<sub>3</sub> must not exceed 0.2%, otherwise the sample coagulates. Prepare the solution (1L) with 10 mL of triton X-100 and 2 mL HNO<sub>3</sub> in deionised water. The solution must be sonicated 1 h in a bath to dissolve Triton completely. This final procedure is adequate if Cr is going to be measured by Atomic Absorption Spectrophotometry with Graphite Furnace. For an ICP-MS analysis the Triton/HNO<sub>3</sub> must be replaced by 1% Triton X-100/0.2% NH<sub>4</sub>OH

\*\*\* PFAS analyses are made from workers performing electrolytic surface treatment in baths.

## 4.2 Transportation of the samples to the laboratory

As a general rule, samples should be shipped to the laboratory as soon as possible. During transportation, the storage conditions precluded above should be maintained, therefore:

- **Tube 1** - Ship whole blood for micronucleus analysis protected from light at +4°C (max +10°C). Blood samples have to arrive to the genotoxicology laboratory (INSA) within 1–4 days after sampling;
- **Tube 2** – Whole blood - shipped in dry ice (-80°C) to the laboratory (KU Leuven) or isolated DNA shipped at +4°C or -20°C;
- **Tube 3** - Following plasma separation and RBC preparation – send both fractions refrigerated (up to 3 days) to the analytic laboratory. Plasma can be shipped refrigerated or frozen at -20°C (PFAS fraction must be frozen à -20°C);
- **Tube 4** - For successful isolation of young transferrin-positive reticulocytes, the whole blood sample must be delivered to the genotoxicology laboratory (FIOH) within one week (preferably 1-4 days) after the sampling. The correct sample temperature must be ascertained also during the transport. The samples should be transported protected from light at +4°C (max +10°C).

To **ensure samples transportation at +4°C** (max +10°C) ice packs shall be used, placed at the bottom and along the sides of the styrofoam box, making sure, however, that the samples will not freeze. To ensure transportation at -80°C, samples shall be immersed in dry ice using an adequate styrofoam box (see Annex 2, fig. A.4).

A shipping date should be arranged between the sample collectors and the laboratory. When arrangements have been finalized, the addressee should be informed of the time and means of transportation.

The deliverable report *D.7.2 “Strategy and SOPs for human sample exchange, including ethical demands”* includes all information related to the proper conservation and transport of the samples in human biomonitoring studies as well as the conditions of storage until the chemical analysis. The recommendations there referred and included in D7.2. should be followed, namely:

- Standard operating procedure for Sample Exchange on a pan-European level to be used in the HBM4EU initiative
- Shipping Category B Biological Substances
- Pro-Forma Invoice
- Sample Transfer Protocol (Manifest)
- Data Transfer Template.

### 4.3 Storage of the samples in the laboratory until analysis

Once in the laboratory that will perform the analysis, the storage conditions described in the section 4.1. should be maintained, unless other specific procedure exists in the analytical lab. In addition, the blood remaining after testing will be preserved at least up to the end of the project, unless otherwise stated by national rules. Further procedures are described in each methodology SOP.

## References

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# Annex 1 – Blood sampling form

Confidential Data – do not send with sample

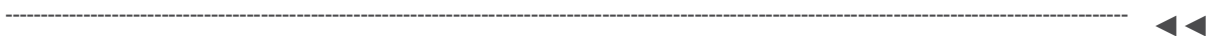
**Worker Identification:**

Country: \_\_\_\_\_  
\_\_\_\_\_

Company name and name of department: \_\_\_\_\_

Worker name: \_\_\_\_\_

Position: \_\_\_\_\_



Send with sample for analysis

**Questionnaire:**

Date: \_\_\_\_\_

Code Number: \_\_\_\_\_

**Blood Sample:**

Date: \_\_\_\_\_ Time of sampling: \_\_\_\_\_

**Blood Code Number:** \_\_\_\_\_

Number of Tubes collected: \_\_\_\_\_

Volume of Blood per tube: \_\_\_\_\_ ml

Destination of Tubes:

1 - \_\_\_\_\_

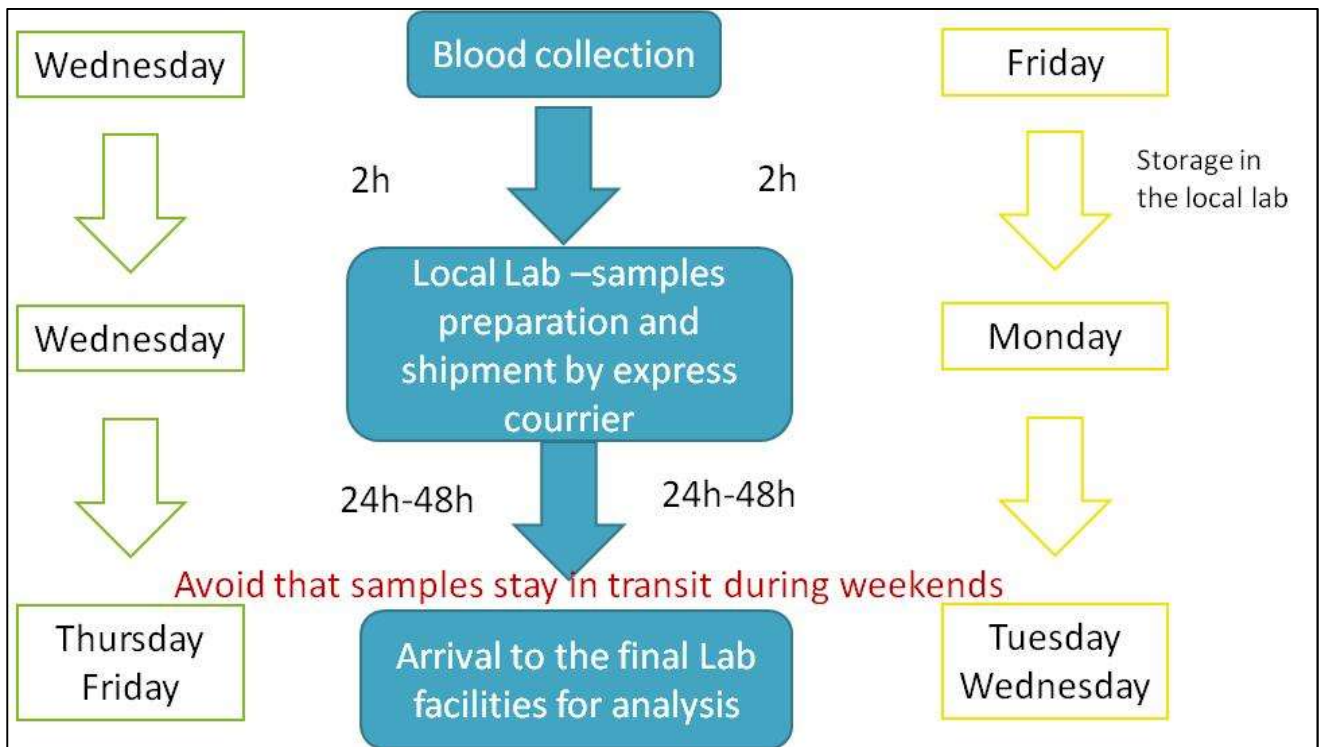
**Eppendorfs 1.2+1.3 -** \_\_\_\_\_

2- \_\_\_\_\_

3- \_\_\_\_\_

4 - \_\_\_\_\_

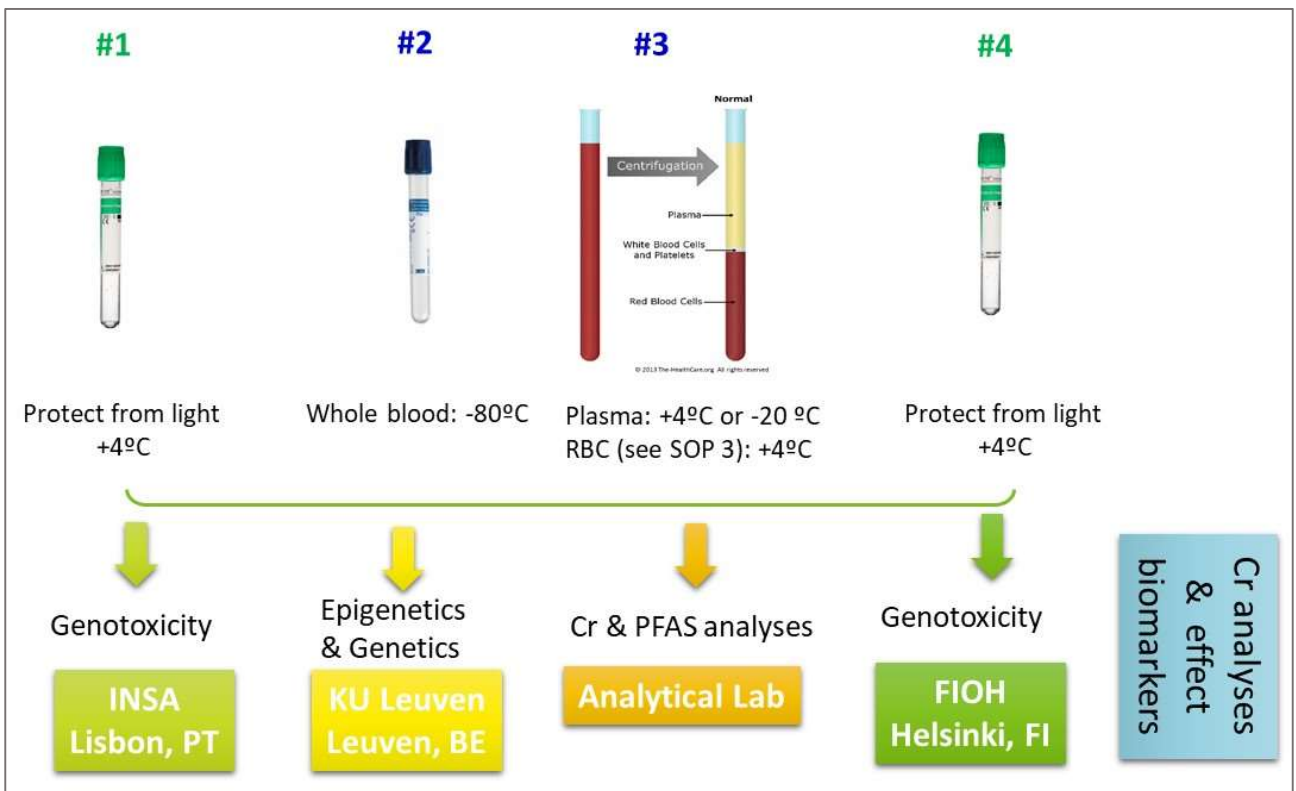
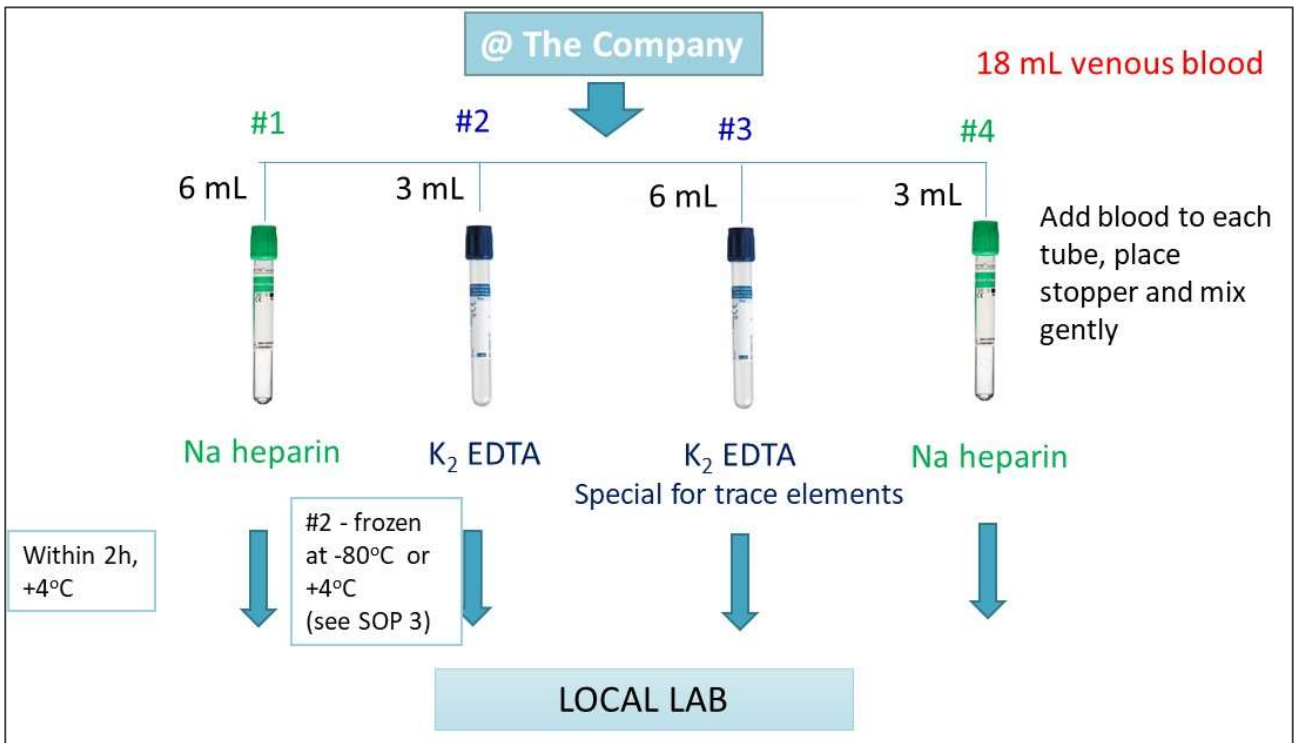
## Annex 2 – Schemes for blood collection and transportation



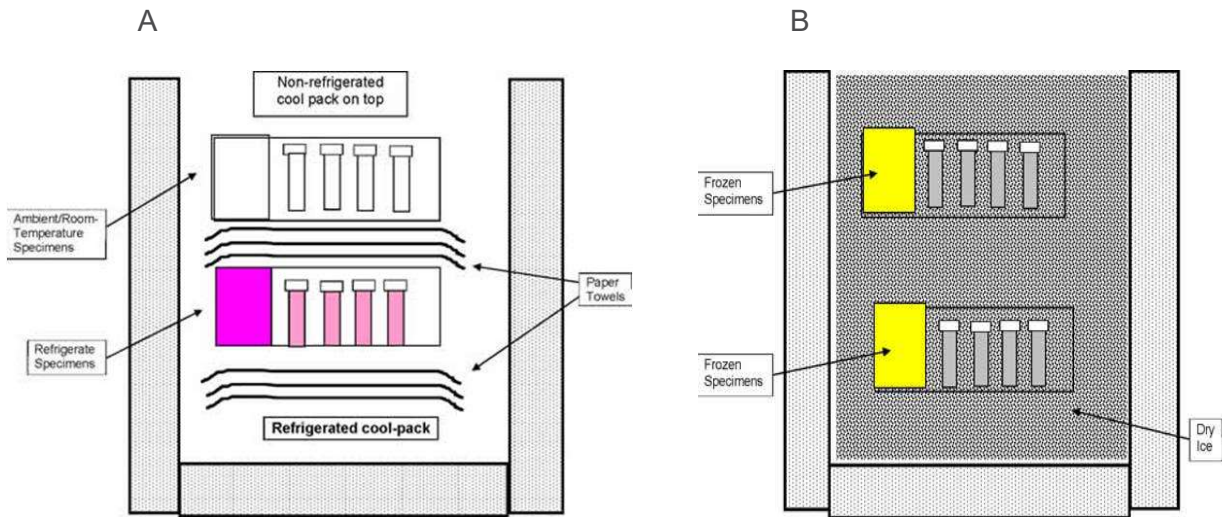
**Fig. A.1 -Timing for blood collection: Wednesday or Friday afternoon**

<input type="checkbox"/> Has the individual questionnaire been filled in? <input type="checkbox"/> Has a code been attributed to the worker? <input type="checkbox"/> Has the Informed consent been understood and signed by the worker? <input type="checkbox"/> Does the worker fulfill the inclusion criteria for the effect biomarkers study? <input type="checkbox"/> Did the partner agree to send blood samples for effect biomarkers study? - <b>YES – prepare to collect 18 mL of venous blood (Tubes 1 – 4)</b> - <b>NO - prepare to collect 3 mL of venous blood (Tube 3)</b> <input type="checkbox"/> Is the Blood Sampling form (Annex 1, SOP 3) filled in?
---

**Fig. A.2 - Check list for blood collection**

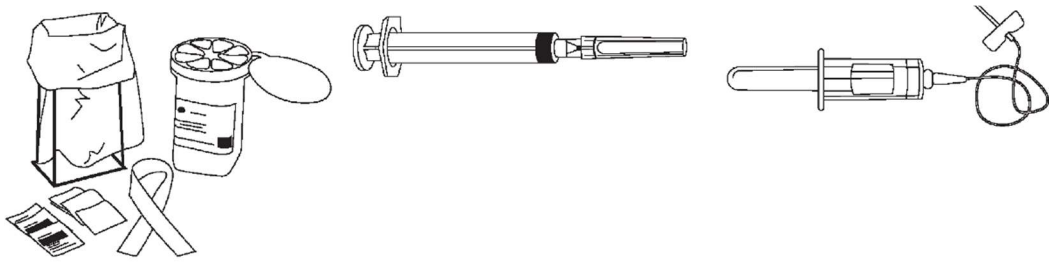


**Fig. A.3 - Blood sampling, processing and transportation**

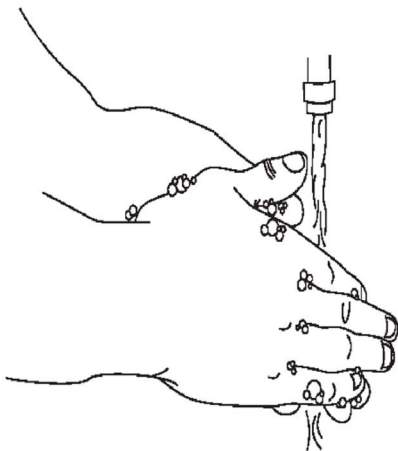


**Fig. A.4 – Specimens integrity during transportation - Containers for transportation of samples refrigerated and at room temperature (A) and for transportation of frozen samples (B). (Source: Mayo Clinics)**

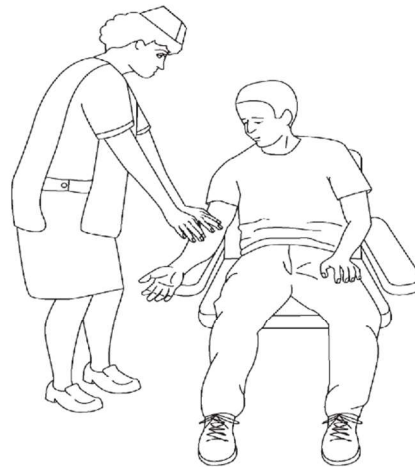
## Annex 3 - An illustration of best practices in phlebotomy (WHO, 2010)



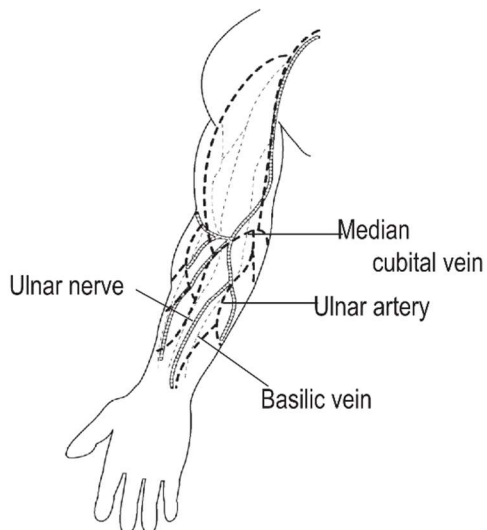
1. Assemble equipment and include needle and syringe or vacuum tube, depending on which is to be used.



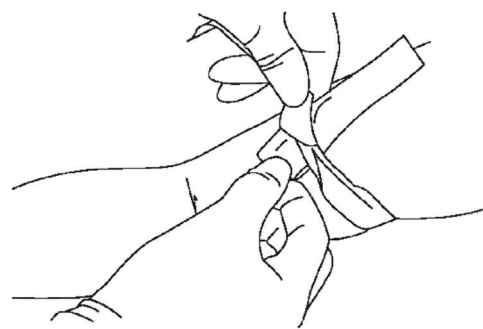
2. Perform hand hygiene (if using soap and water, dry hands with single-use towels).



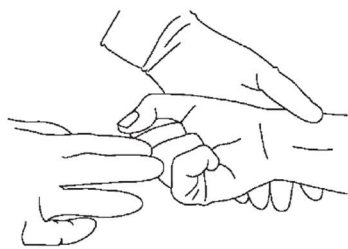
3. Identify and prepare the patient.



4. Select the site, preferably at the antecubital area (i.e. the bend of the elbow). Warming the arm with a hot pack, or hanging the hand down may make it easier to see the veins. Palpate the area to locate the anatomic landmarks. **DO NOT** touch the site once alcohol or other antiseptic has been applied.



5. Apply a tourniquet, about 4–5 finger widths above the selected venepuncture site.



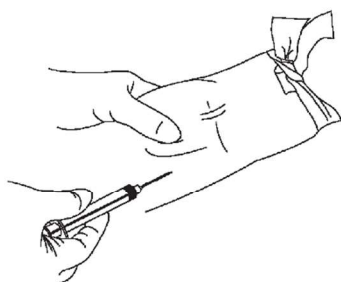
6. Ask the patient to form a fist so that the veins are more prominent.



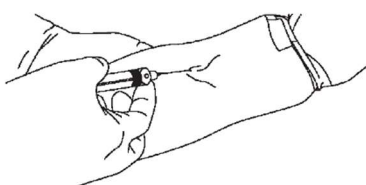
7. Put on well-fitting, non-sterile gloves.



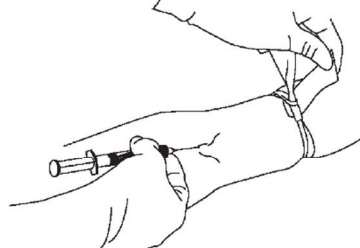
8. Disinfect the site using 70% isopropyl alcohol for 30 seconds and allow to dry completely (30 seconds).



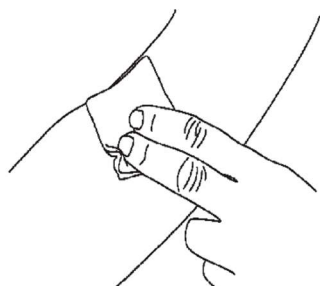
9. Anchor the vein by holding the patient's arm and placing a thumb **BELOW** the venepuncture site.



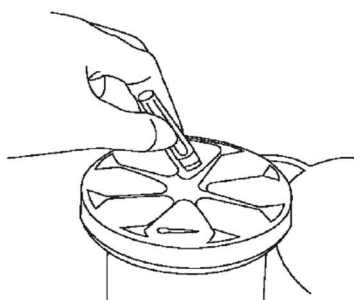
10. Enter the vein swiftly at a 30 degree angle.



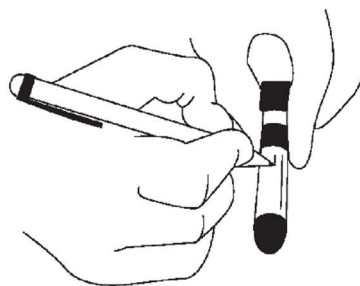
11. Once sufficient blood has been collected, release the tourniquet **BEFORE** withdrawing the needle.



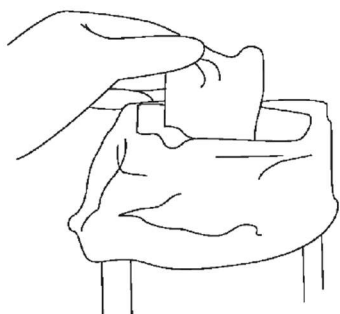
12. Withdraw the needle gently and then give the patient a clean gauze or dry cotton-wool ball to apply to the site with gentle pressure.



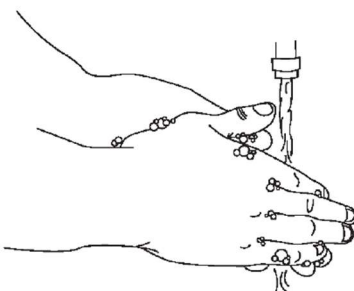
13. Discard the used needle and syringe or blood-sampling device into a puncture-resistant container.



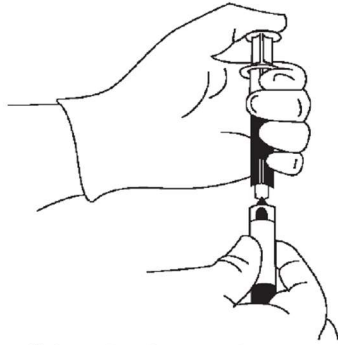
14. Check the label and forms for accuracy.



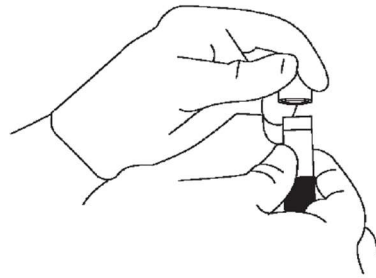
15. Discard sharps and broken glass into the sharps container. Place items that can drip blood or body fluids into the infectious waste.



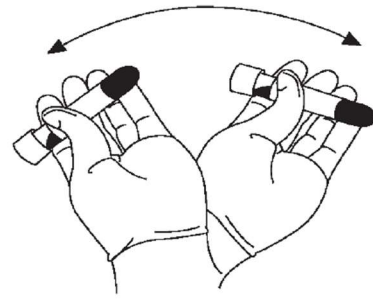
16. Remove gloves and place them in the general waste. Perform hand hygiene. If using soap and water, dry hands with single-use towels.



1. If the tube does not have a rubber stopper, press the plunger in slowly to reduce haemolysis (this is safer than removing the needle).



2. Place the stopper in the tube.



3. Following laboratory instructions, invert the sample gently to mix the additives with the blood before dispatch.

**SOP 4:**  
**Standard operating procedure for the collection of  
exhaled breath condensate samples**

**WP 8**  
**Task 8.5**

## Table of Contents

Authors and Acknowledgements.....	2
1 Introduction.....	2
2 Exhaled Breath Condensate (EBC).....	3
2.1 EBC collection devices .....	3
2.2 Complexation of EBC samples .....	4
2.3 Standardisation of EBC collection .....	4
2.4 Collection requirements .....	5
2.4.1 Equipment .....	5
2.4.2 Collection.....	5
2.4.3 Complexation with EDTA solution.....	7
2.5 Sample traceability .....	7
References .....	9

## Authors and Acknowledgements

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## 1 Introduction

According to IARC, hexavalent chromium (Cr(VI)) compounds are classified as carcinogenic to humans (Group I) whereas trivalent chromium (Cr(III)) is an essential element.

In established biomonitoring (urinary total chromium), Cr(VI) exposure cannot be specifically determined because Cr(VI) compounds are reduced to Cr(III) in the body before being eliminated in urine. As exposures reduce due to increased demands from regulators, urinary total chromium methods will become less useful as it will be difficult to separate harmful Cr(VI) exposure from normal dietary Cr(III) exposure. In light of this, more specific biomarkers are being investigated; namely, Cr(VI) in exhaled breath condensate and in red blood cells (where it is trapped and isolated from Cr(III) as only Cr(VI) can enter the red blood cells but in the end it is not Cr(VI) that is analyzed). This SOP covers the collection of exhaled breath condensate (EBC) samples for the analysis of Cr(VI); it is also possible to measure Cr(III) and possibly other metals in samples collected using this procedure.

## 2 Exhaled Breath Condensate (EBC)

EBC is a biological fluid which consists mainly of water vapour but also of small droplets of airway lining fluid from within the bronchial and alveoli regions of the lungs. These droplets of airway lining fluid contain an unknown fraction of both volatile and non-volatile substances but also environmental and occupational contaminants.

Inhalation is one of the primary routes of occupational exposure. It is thought that EBC might be a useful biological monitoring matrix when the current biological monitoring methods using traditional biological matrices such as urine or blood are not possible, or where the interpretation of elemental species in these matrices is difficult, for example hexavalent and trivalent chromium. Advancing the investigation of EBC may further the understanding of inhalation exposures and how elements behave and reside in the lungs.

The collection and analysis of EBC samples in this project is to further this understanding by gaining a more accurate picture of Cr(VI) exposure by correlating chromium species in EBC samples against industrial hygiene samples (for example personal air samples) in addition to urinary total chromium measurements.

The collection of EBC samples is a non-invasive technique and does not cause an inflammatory response itself. The collection of EBC results in low sample volumes and will take approximately 15 minutes to collect an adequate volume of sample (1-2 mL). Collection of EBC also involves the subsequent step of complexation with an EDTA solution to stabilise the hexavalent and trivalent chromium species immediately after collection.

The volume of EBC collected can vary from one individual to the next. EBC volume is directly correlated to tidal volume and minute/ventilation volume. Tidal volume refers to the volume of air displaced in the lungs between normal inhalation and exhalation. Minute/ventilation volume refers to the amount of gas inhaled or exhaled from an individual's lungs in one minute. Individuals with higher minute/ventilation volume and/or higher tidal volume will produce more EBC<sup>1-3</sup>. This variation in EBC sample volume means the concentration of Cr(VI) will also vary. As there is currently no proposed volume correction marker (such as creatinine for urine) it is therefore suggested that EBC results must be reported in µg/L per volume of EBC collected.

### 2.1 EBC collection devices

All EBC collection devices are based on a freezing cooling chamber, to cool and condense the exhaled breath. This must consist of an inert material for the surface of the condensing cooling chamber such as glass, aluminium or Teflon as recommended by the American Thoracic Society/European Respiratory Society Task Force<sup>1</sup>. The effectiveness of EBC collection depends on the volume of breath passing into the condenser, the condensing surface area and the temperature gradient of the exhaled air to the cooling chamber<sup>1</sup>.

A small number of commercial EBC devices are available to purchase for the collection of EBC. However, due to the differences in collection devices (for example the EBC collection temperature) only the TurboDECCS (Medivac SRL, Parma, Italy) system can be used to collect EBC samples as part of this project.

The TurboDECCS which stands for 'Transportable Unit for Research of Biomarkers Obtained from Disposable Exhaled Condensate Collections Systems', is a portable self-contained thermoelectric peltier cooling device. An external power source is required to operate the Turbo, whose function is to cool the EBC collection tube (which is inserted into the cooling unit of the Turbo). The collection tube connects to the disposable EBC sampling kit comprising of a mouthpiece connected to a one-way aspiration valve with a saliva trap (DECCS). (See Figure 1 in section 2.4.1) The temperature for

the cooling chamber is  $-5^{\circ}\text{C}$  (this is also the default temperature setting of the TurboDECCS so adjustment should not be necessary)..

A study by Goldoni et al<sup>4</sup> based on EBC collection volume, biomarker levels and collection variability determined the optimum EBC collection temperature of the TurboDECCS to be  $-5^{\circ}\text{C}$ . Published studies using the TurboDECCS which focus on elemental concentrations in EBC also maintained this collection temperature of approximately  $5^{\circ}\text{C}$ <sup>5-8</sup>.

It is not known how the differences in temperature and humidity of inspired air will affect the collection of EBC. A study by McCafferty et al<sup>9</sup> reported reduced EBC volume when individuals were inhaling cooler and drier air. For this project, EBC samples will be collected indoors in a standard office environment.

The volume of EBC collected can vary greatly however, 15 minutes of tidal breathing will collect on average between 1 – 2 mL of EBC. Several studies support the lack of correlation between EBC volume and gender, age, fitness, smoking status and lung status<sup>2,3,9</sup>.

## 2.2 Complexation of EBC samples

Maintaining the stability and integrity of both Cr(VI) and Cr(III) within any sample matrix can be challenging, as the stability of both species is pH dependent. Generally Cr(VI) is stable in alkaline conditions whilst Cr(III) is stable in acidic conditions and forms Cr(III) hydroxide compounds in weak acidic conditions.

EBC is slightly acidic, with samples checked in earlier work at HSL having a pH between pH 6 and pH 6.5. Stability studies at HSL have shown that EBC samples analysed within 24 hours of collection which contain Cr(III) will elute the Cr(III) at two different retention times when not complexed with EDTA. It is HSL's thought that that the two Cr(III) peaks could be Cr(III) and the product of its slow conversion to a Cr(III) hydroxide.

Secondly as EBC samples and the EDTA solution are both slightly acidic, any Cr(VI) in the EBC samples will begin to slowly convert to Cr(III) if the EDTA solution is not adjusted to pH 8.

Therefore, to maintain the integrity of both chromium species it is important that each EBC sample is complexed with an EDTA solution immediately after collection.

A solution of 0.5 mM EDTA is made in water (using ultrapure deionised water – 18.2 MΩm cm) and adjusted to pH 8 with 10% v/v ammonia solution. The EDTA complexes with Cr(III) and the adjustment of the pH to pH 8 stabilises Cr(VI).

To ensure that the same batch of EDTA solution prepared is used for both complexation of the EBC samples and for preparing standards and control material for the speciation analysis of those samples, a 2L EDTA solution is prepared. 1L can be taken to site to dilute with the EBC samples and the remaining 1L is used for the speciation analysis. The solution is kept at room temperature. Long term stability is unknown, as the solution was made fresh for each set of site visits/analysis in previous studies.

## 2.3 Standardisation of EBC collection

Although every volunteer will be asked to produce an EBC sample over 15 minutes, this will result in variable volumes of EBC. The variability is due to a result of the amount of air displaced in the lungs during normal inhalation and exhalation known as tidal volume and the amount of gas inhaled or exhaled from the lungs in one minute, known as minute of ventilation volume. In addition, the droplets of airway lining fluid will be considerably diluted by the condensed water vapour in each

EBC sample, and this dilution will also vary considerably between volunteers. Unfortunately, unlike a urine sample where, for example, creatinine content can be measured to correct for dilution, there has been no such dilution marker proposed for EBC. Until a suitable biomarker of dilution correction can be found it is advised that volume (or weight) of EBC produced by each volunteer is recorded and that results are reported per volume of EBC. Due to the high content of water vapour in an EBC sample, 1 mL of EBC sample will weigh 1 g.

To help standardise the collection of EBC samples further, it is advised the volunteer rinse their mouth with water prior to providing a sample (drinking a cup of water is acceptable). This helps to remove any accumulated food and/or saliva from the mouth, helping to avoid any contamination of the EBC samples.

In addition, for the pre working week EBC samples, it is important that the volunteer has performed no practices or duties where Cr(VI) may occur.

## 2.4 Collection requirements

### 2.4.1 Equipment

1. Only the EBC collection device known as a TurboDECCS with disposable EBC sampling kits made of an inert material can be used. The temperature of the TurboDECCS should be at  $-5^{\circ}\text{C}$  which is its default setting.
2. A suitable room away from the primary site of exposure/workshop floor (for example, office, meeting room, first aid/nurses room) with a operational plug socket (to power the EBC collection device) and a table and chair. For the comfort of the volunteer providing the EBC sample, a seated position with the collection device placed in front of them on a table is the most suitable.
3. Labels / or permanent marker pen
4. Nitrile disposable gloves or other suitable gloves
5. Secondary sample tubes with caps suitable for trace metal analysis. For example 15 mL - 50 mL polypropylene screw cap Sarstedt
6. Pipettes & tips (a 100 – 1000  $\mu\text{L}$ , 20 – 200  $\mu\text{L}$  and 500 – 5000  $\mu\text{L}$  pipettes are the most suitable).
7. 0.5 mM EDTA solution adjusted to pH 8 with 10% v/v ammonia solution.
8. Drinking water & cups.

### 2.4.2 Collection

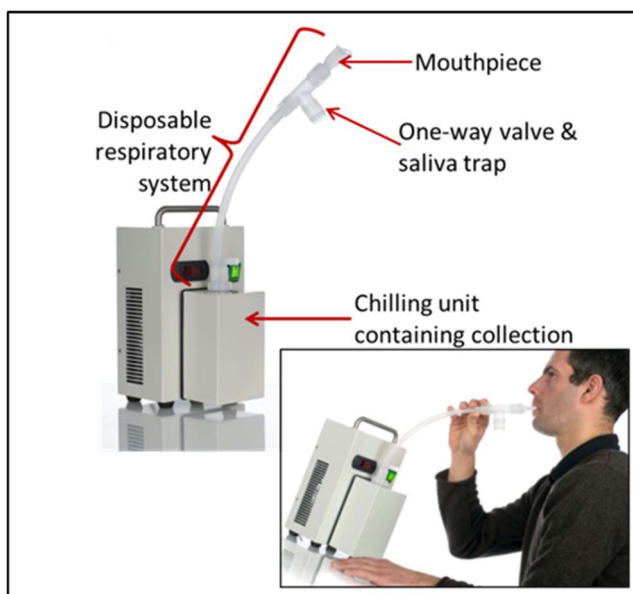
- A suitable room to collect EBC samples should already have been decided away from the primary site of exposure/workshop floor, for example office, meeting room or nurse/first aid room. To standardise the environmental conditions as much as possible ensure the room is within general office space conditions, for example a room temperature between  $18\text{-}25^{\circ}\text{C}$ .

Plug the Turbo into a suitable power source that enables it to stand on a table where the EBC collection will take place. From room temperature it can take up to 20 minutes for the cooling chamber to reach  $-5^{\circ}\text{C}$ .

- Wearing gloves unwrap and assemble a DECCS sampling kit and insert the EBC sample collection tube into the cooling chamber.
  - The DECCS sampling kits are individually wrapped and sealed and so will need opening and assembling according to the instructions available within each bag. For

hygiene and potential contamination reasons, each sampling kit is opened and assembled prior to each volunteer and not collectively beforehand.

- For each subsequent EBC sampling kit, ensure the collection tube is inserted in the cooling chamber for at least 5 minutes prior to a volunteer beginning to provide their breath sample to allow the collection tube to cool.
- Ask the volunteer to rinse/wash their mouth out with water (depending on the facilities in the room, the volunteer can rinse their mouth by drinking the water or rinsing and spitting out the water) and then begin providing their breath sample by regular tidal (normal) breathing into the mouth piece for 15 minutes. For the most part a complete seal around the mouthpiece with the mouth and lips must be maintained, however periodic removal will be required to allow any accumulated saliva to be swallowed.
  - It may be advantageous to liken breathing into the mouthpiece to that of breathing with a snorkel or scuba equipment, and to breath through their mouth and not their nose. Remind them to keep their breathing tidal, as heavy or deep breathing may cause them to feel light headed.
- After 15 minutes and the volunteer has ceased providing their sample, remove the entire sampling kit from the Turbo unit, unscrew, cap and label the sample collection tube. Dispose of the remaining sampling kit, for example, in a biohazard bag.
- All samples are to be kept refrigerated at approximately 2-8°C after collection and complexation, during transportation to the laboratory and once at the laboratory until analysis, DO NOT FREEZE.



Please note that the disposable respiratory system (sampling kit) has been redesigned by medivac to be much shorter. The position of the volunteer to the mouthpiece is now much closer to the chilling unit than depicted in this photograph.

Figure 1. The Turbo-DECCS exhaled breath condensate collection device



If the Turbo is left without a collection tube inserted into the condensing cooling chamber for too long, the surface of the chamber will begin to form ice, prohibiting another collection tube from being inserted.



If a tube containing an EBC sample is left in the condensing cooling chamber for too long after the volunteer has ceased breathing into the sampling kit, the sample will begin to freeze. A frozen sample will deteriorate the integrity of Cr(VI).

### 2.4.3 Complexation with EDTA solution

Each individual will produce a different amount of EBC sample. It is therefore necessary to make a judgement as to what volume of EBC to use in the complexation with the pH adjusted EDTA solution. This is done on-site immediately after collection. Wearing gloves:

- Label a secondary sample tube and aliquot a suitable amount of the EBC sample into this tube. Note how much of the EBC has been transferred to enable accurate calculation of the volume of EBC collected for the reporting of the results.
- Dilute the aliquoted EBC sample 10-fold with the pH adjusted EDTA solution and cap.

**An ideal scenario would be to aliquot 1 mL of EBC, so when diluted 10-fold with the EDTA solution it gives a final sample volume of 10 mL (ideally speciation analysis is performed in duplicate, and at least 1.5 mL is required per duplicate. This will leave the remaining complexed sample for any necessary repeats and if manganese & nickel analyses are required).**

- It is very possible a volunteer will produce less than 100 µl of EBC sample. This sample may not be suitable for analysis as a 10-fold dilution may produce an inadequate final volume for speciation analysis (determine minimum analytical volumes needed from your analysing laboratory).
- After complexation, place all EBC samples in a portable refrigeration unit/insulated box with ice pack until the samples arrive back at the analysing laboratory. DO NOT FREEZE.
- A short term storage study at HSL determined that these samples are stable for up to 6 weeks when stored refrigerated. It is not known how long the samples can be stored beyond this before Cr(VI) begins to deteriorate and convert.
- Upon returning to the laboratory, the remaining volume of EBC (uncomplexed) must be weighed and recorded. Each g of EBC correlates to 1 mL of EBC. To this weight, add (in g) the volume of EBC aliquoted for dilution with EDTA to give the original collected weight/volume of sample.

The collection pots are sealed within the sampling kits so cannot be weighed beforehand.

- Centrifuge all the collection pots (to remove EBC from the side walls)
- Weigh (g) and record an empty 30mL medicine beaker
- Transfer the EBC sample to the 30mL medicine beaker and weigh (g) and record again.
- Transfer back to the original container or another suitable container if retention of uncomplexed sample is required for other assays. Store appropriately for those assays.

## 2.5 Sample traceability

A standardised convention will be used to assign unique identification codes for all samples collected. The identification code convention is as follows:

Country ID (XX) - Participant ID (XX) - Sample ID (BXX/UXX/EXX/AXX/WXX)

Country ID 'XX' is the country code, using the ISO Alpha-2 country codes for the participating countries<sup>1</sup>.

Country	ISO Alpha-2 country codes
Belgium	BE
Finland	FI
France	FR
Italy	IT
Poland	PL
Portugal	PT
The Netherlands	NL
United Kingdom	UK

Participant ID 'XX' is a two-digit running number of participants in each country (e.g. 01 for the first participant recruited, 02 the second and so forth).

Sample ID 'UXX' is one letter (B/U/E/A/W) to identify the type of sample collected, followed by a two-digit identifier (XX) to identify the running number of each type of sample for that worker (e.g. 01 for the first sample, 02 for the second and so forth). The letter code applied for the sample types is as follows:

Type of sample collected	Sample type code
Air	A
Blood	B
Exhaled breath	E
Urine	U
Wipe	W

The following scenario is provided to illustrate the application of this convention.

A worker is recruited in The Netherlands. He is the first worker recruited and is providing his first EBC sample. The sample identification code assigned is therefore:

NL-01-E01

In the event that an air sample is also collected from this same worker, the sample identification code to be assigned would be:

NL-01-A01

<sup>1</sup> [http://www.nationsonline.org/oneworld/country\\_code\\_list.htm](http://www.nationsonline.org/oneworld/country_code_list.htm)

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**SOP 5:**  
**Standard operating procedure for urine sampling,  
including sample storage and transfer**

**WP 8**  
**Task 8.5**

## Table of Contents

Authors and Acknowledgements.....	2
1 Material needed for urine collection and storage.....	2
2 Pre-treatment of the urine collection containers .....	2
3 Urine sampling.....	3
3.1 Urine samples to be collected .....	3
3.2 Information to the workers .....	3
3.3 Sampling procedure.....	3
4 Urine processing.....	4
5 Conservation and storage of the samples .....	6
6 Transfer of the samples .....	6
Appendix 1: Poster with the sampling procedure .....	7
Appendix 2: Information record sheet.....	9

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## 1 Material needed for urine collection and storage

To undertake the collection of urine samples, researchers should ensure that they have sufficient quantities of the following:

- Urine collection containers (100 mL polypropylene vessel with a screw cap, pretreated as described in Section 2)
- Urine aliquot polyethylene tube of 1.5 mL
- Urine aliquot polypropylene tube of 5 mL
- Labels
- Nitrile or similar disposable gloves
- Biological and hermetic bags
- 65% nitric acid solution
- 10% nitric acid solution for washing
- 1% nitric acid solution for washing
- Pipettes and tips (1-3 mL and 30 µL)
- Modesty pads
- Aliquots boxes
- Freezers
- Posters with the hand washing and sampling procedures (Appendix 1)
- Trash cans
- Urine strip test (e.g. Combur-10 Test®, ref. 480770, <https://www.pharmacyonline.com.au/>)
- Pens
- Sample record sheets (Appendix 2)

## 2 Pre-treatment of the urine collection containers

Two types of vessel are used to collect urine samples: urine collection containers used by the worker to perform the sampling, and plastic tubes in which the collected urine samples are aliquoted.

Only the urine collection containers should be washed with 10% nitric acid solution in order to eliminate background contamination, according the following procedure:

1. Prepare a 10% nitric acid solution from nitric acid 65% extra pure and purified water.
2. Put the solution in a tank.
3. Open the containers and put them and the screw caps into the tank. Ensure that all of them are completely immersed.
4. Containers and screw caps should be immersed in this tank at least for 3 hours (preferably overnight).
5. Take out the containers and the screw caps from the acid tank, and rinse them three times with purified water.
6. Put containers and screw caps face down in a clean filter paper in order to dry them, preferably in an oven at 60°C.
7. After the drying is completed, screw the nitric acid pre-treated containers.

The washing of the urine collection containers can be avoided if the research team had already checked for contaminations on the supplies beforehand and that they are stored appropriately. In this case, the research team performed blank measurements with purified water in the containers to ensure that they obtain satisfying levels of metals (Cr, Ni, Mn).

### 3 Urine sampling

#### 3.1 Urine samples to be collected

Participants will collect two urine samples. (1) Urine sample before the work shift at the beginning of the working week (first-morning-void), and (2) after the work shift at the end of the working week (post-shift void). Non-occupationally exposed control persons will collect only sample number 1.

#### 3.2 Information to the workers

Participants should be orally informed of the sampling procedure. In order to remind about the guidelines, a poster with the sampling procedure can be displayed in the toilet where the sampling will be done (Appendix 1). Participants will be advised of the following points:

- The need to remove their work clothes (overalls) before the urine collection;
- The need to wash their hands thoroughly with soap and water;
- Information to write on the label of the collection container (name, date, hour);
- The need to return the collected sample to the research team as quickly as possible following collection.



#### 3.3 Sampling procedure

- For the sampling, decontaminated labelled containers are distributed to the participant, along with biological hermetic bags to place the container inside.
- The participant completes the label on the urine collection container with the required information (name, date, hour).
- Before the sampling, the participant is informed that he has to remove his work clothes (overalls) and wash his hands with soap and water in accordance with the provided instructions (Appendix 1). When it is done, the participant opens the urine collection

container, collects his urine, screws the cap firmly and places the container in the biological and hermetic bag provided to avoid any leak. Full instructions on the provision of the urine samples are provided in Appendix 1.

- The participant returns the urine collection container to the research team straight away. He can use a modesty pad, left at disposal by the research team, for the transport.
- The researcher checks that the required label details (see section 3.1) are recorded and that these are correct and legible.

## 4 Urine processing

If possible, the urine samples are directly conditioned on site by the HBM4EU research team in an area free from contamination, after hand washing and when wearing appropriate personal protective equipment, e.g. disposable gloves.

The research team complete a table with the listed information (Appendix 2):

- Sample ID number
- Date
- Hour
- Name of the participant
- Results of the Combur test (pH, presence of blood in the urine...)

The researcher washes thrice the urine and acid tips by pipetting 3 mL of nitric acid 1% and then prepares the aliquots for metals:

- 3 replicates of 3 mL of urine in a 5 mL aliquot tube, for metal (Cr, Ni, Mn) analyses;

The researcher uses a new tip which should not be washed to prepare the other aliquots:

- 3 replicates of 1 mL of urine in a 1.5 mL aliquot tube, for oxidative stress biomarkers (e.g. malondialdehyde, 8-isoprostane, 8-hydroxy-2-deoxyguanosine) and epigenetic changes (e.g. DNA methylation) analyses.
- 2 replicates of 3 mL of urine in a 5 mL aliquot tube for preservation of samples in each respective institute.

The aliquot tubes are properly labelled with the sample code determined for all of the HBM4EU study participants. A standardised convention will be used to assign unique identification codes for all samples collected. The identification code convention is as follows:

Country ID (XX) - Participant ID (XX) - Sample ID (BXX/UXX/EXX/AXX/WXX)

Country ID 'XX' is the country code, using the ISO Alpha-2 country codes for the participating countries<sup>1</sup>.

Country	ISO Alpha-2 country codes
Belgium	BE
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Participant ID 'XX' is a two-digit running number of participants in each country (e.g. 01 for the first participant recruited, 02 the second and so forth).

Sample ID 'UXX' is one letter (B/U/E/A/W) to identify the type of sample collected, followed by a two-digit identifier (XX) to identify the running number of each type of sample for that worker (e.g. 01 for the first sample, 02 for the second and so forth). The letter code applied for the sample types is as follows:

Type of sample collected	Sample type code
Air	A
Blood	B
Exhaled breath	E
Urine	U
Wipe	W

The following scenario is provided to illustrate the application of this convention.

A worker is recruited in The Netherlands. He is the first worker recruited and is providing his first two urine samples. The sample identification codes assigned are therefore:

NL-01-U01  
NL-01-U02

After the processing, a urine strip test (e.g. Combur10 test®) is performed.

Used tips, used washing solution and urine collection containers are thrown in the trash can. Ideally, the research team collect all the used trash cans to dispose of them on their own site to not clutter up the company.

<sup>1</sup> [http://www.nationsonline.org/oneworld/country\\_code\\_list.htm](http://www.nationsonline.org/oneworld/country_code_list.htm)

The researcher confirms collection of the required aliquots on the record form (Appendix 2).

Aliquots are then placed in a box and stored in freezers at  $-20^{\circ}\text{C}$ . If the transfer of the samples is scheduled for the same day, a cool box can be sufficient.

If immediate urine processing is not possible, urine samples can be transported in the HBM4EU research team laboratory to perform the processing. In this case, urine samples can be transferred in a cool box. The transfer of the samples should be scheduled for the same day.

Aliquots are then placed in a box and stored in freezers at  $-20^{\circ}\text{C}$ .

## **5 Conservation and storage of the samples**

The HBM4EU sampling teams conserve the aliquoted samples in their respective laboratories by maintaining the cold chain (in freezers). The samples are stored at  $-20^{\circ}\text{C}$  up to their transfer to the analysing laboratories.

## **6 Transfer of the samples**

Urine aliquoted samples will be sent to the laboratories which will perform the analyses by maintaining the cold chain, by using cool box or freezers.

## **Appendix 1: Poster with the sampling procedure**

## Urine sample and hand washing procedure

1. Take a urine collection container and biological bag from the research team
2. Complete the label with your name, date and time of collection
3. Carefully wash your hands following the instructions below



**NHS**

### Hand-washing technique with soap and water

1 Wet hands with water

2 Apply enough soap to cover all hand surfaces

3 Rub hands palm to palm

4 Rub back of each hand with palm of other hand with fingers interlaced

5 Rub palm to palm with fingers interlaced

6 Rub with back of fingers to opposing palms with fingers interlocked

7 Rub each thumb clasped in opposite hand using a rotational movement

8 Rub tips of fingers in opposite palm in a circular motion

9 Rub each wrist with opposite hand

10 Rinse hands with water

11 Use elbow to turn off tap

12 Dry thoroughly with a single-use towel

13 Hand washing should take 15–30 seconds

**clean your hands** campaign

**NHS**  
National Patient Safety Agency

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Adapted from World Health Organization Guidelines on Hand Hygiene in Health Care

4. Open the container and collect your urine
5. Screw on the cap and place container in the biological bag
6. Immediately return the sample to the research team





science and policy  
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Contract No. 733032 HBM4EU

**SOP 6:**  
**Standard operating procedure for air sampling of  
inhalable and respirable dust fraction  
and (hexavalent) chromium**

**WP 8**  
**Task 8.5**

## Table of Contents

Authors and Acknowledgements .....	2
1 Scope .....	2
2 Materials required for the collection of air samples.....	3
3 Recommendations and precautions for air sampling.....	4
4 Air sampling procedure .....	5
5 Sample traceability and contextual information .....	10
6 Storage of collected air samples .....	11
7 Quality control.....	11
8 Transportation of air samples to laboratory .....	12
References .....	13
Appendix 1: Sample record sheet .....	14

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## 1 Scope

This Standard Operating Procedure (SOP) is partially based on MDHS 14/4 "*General methods for sampling and gravimetric analysis of respirable, thoracic and inhalable aerosols*" and describes the air sampling of the inhalable (total) and respirable (alveolar) dust fraction and respective total chromium or chromium (VI) amount, in order to assess workplace exposure.

This SOP is also partially based on internal SOPs of the KU Leuven Laboratory for Occupational and Environmental Hygiene for the determination of the inhalable / respirable dust fractions. In accordance with these SOPs, sampling of these two fractions is performed using an IOM sampler and a Higgins-Dewell cyclone respectively. These samplers are selected as they are in best agreement with the inhalable / respirable convention.

Sampling of the inhalable dust fraction is performed at a flow rate of 2 L/min with an IOM-sampler containing an IOM-cassette fitted with a pre-weighed 25 mm PVC-filter (GLA-5000) or a 25 mm MCE filter (SKC 225-1930). For welders, alternatively the SKC Mini-sampler could be used with a pre-weighed 13 mm MCE filter (SKC 225-8050) at a flow rate of 0.75 L/min. Filter sampling of the respirable dust fraction is performed at a flow rate of 2.2 L/min with a Higgins-Dewell type cyclone (excepting for SKC 225-69 cyclone where a 3.0 L/min flow rate should be used) containing a cyclone cassette fitted with a pre-weighed 25 mm PVC-filter (GLA-5000) or a 25 mm MCE filter (SKC 225-1930). Even if the current SOP mainly focus on the utilisation of Higgins-Dewell type cyclones, other cyclones types might be used, if the international standards are followed mainly concerning the most appropriate flow-rate. For instance, SKC GS-3 respirable dust cyclone (225-103) with 25 mm three-piece filter cassette (225-3-25LF) and a flow rate of 2.75 L/min, conform to the ISO 7708 standard, could alternatively be used. Nevertheless, the other terms of utilisation for cyclones should be in agreement with the current SOP.

After drawing air over the pre-weighed filter, the filter is re-weighed and the concentration of particulate matter is calculated from the mass difference and the sampled air volume. The total chromium or chromium (VI) determination can be performed on this same filter, depending on the agreed analytical laboratory. Certain types of filters (other than GLA-5000

or MCE) have been reported to cause chromium (VI) reduction, so this should be investigated prior to their use.

The air samples are to be analyzed firstly gravimetrically for determination of the inhalable / respirable dust fraction. Depending on the agreements made with the project team and the analytical laboratories assigned to the air sample analysis, the following methods will be used for further analysis:

- OSHA Method ID-125G 'Metal and metalloid particulates in workplace atmospheres (ICP analysis)' for total chromium (OSHA, 2002)
- ISO 16740 Method, 'Workplace air – Determination of hexavalent chromium in airborne particulate matter – Method by ion chromatography and spectrophotometric measurement using diphenyl carbazide' for hexavalent chromium (ISO, 2005)

All air samples will be analyzed for both total chromium and chromium (VI).

## 2 Materials required for the collection of air samples

Below is a list with the different materials needed for performing air measurements. Samplers, as well as assembled IOM cassettes and cyclone cassettes (preloaded with pre-weighed PVC filters) should be provided by the agreed analytical laboratory.

- IOM inhalable sampler, in conductive plastic (SKC 225-70A) or stainless steel (SKC 225-76A)
- IOM-cassettes (25 mm), in conductive plastic (SKC 225-71A) or stainless steel (SKC 225-75A), with transport clip and cover.
- Higgings-Dewell cyclone, in conductive plastic (JS Holdings, FH022) or SKC cyclone, in conductive plastic (SKC 225-69)
- Cyclone cassettes (25 mm), in conductive plastic, with metal support grid and transport clip (SKC 225-62)
- Spare o-rings for sampling heads
- PVC membrane filter, GLA-5000, Pall 66466, diameter 25 mm, pore size 0.8 µm, 100/package (VWR 514-0466). These filters have been selected as they were evaluated for extraction and storage of hexavalent chromium, and found acceptable (OSHA ID-215). Moreover, these GLA-5000 filters also assure gravimetric stability with low moisture pick-up and low tare weight. Alternatively, MCE (mixed cellulose ester) filter, diameter 25 mm, pore size 0.8 µm (SKC-filter SKC 225-1930) could also be used.
- High flow air pump, capable of operating for up to 8 hours at a flow rate of 2 L/min (inhalable dust fraction with IOM sampler) or 2.2 L/min (respirable dust fraction with Higgings-Dewell cyclone) or 3 L/min (respirable dust fraction with SKC cyclone), with battery charger, e.g. Gilian GilAir-5 pump (Sensidyne), AirCheck 52 pump (SKC 224-52), Sidekick pump (SKC 224-52MTX)
- Flow rate calibrator, e.g. Defender 510 (BIOS DryCal), calibrated against a primary standard, capable of measuring the required flow rates
- IOM Calibration adaptor (SKC 391-01)
- Protective pump pouches (e.g. SKC 224-88 for Sidekick pump) and belts / harnesses to allow sampling equipment to be attached to wearer
- Supply of clips to attach sampling heads to participants (if not already on sampling head)
- Sufficient lengths of flexible tubing of suitable diameter for making a leak proof connection from the sampling head to the pump

- Calibrated timepiece, to chronometrate exact sampling time
- Sample record sheets (Appendix 1)

Below is an additional material list if measurements are to be performed inside welding masks and helmets using a Mini-sampler.

- Face Level Sampling Head Set supplied with 2 flexible arms, 4 hose connectors, 2 Luer plugs and 2 Luer connectors (SKC 225-6200)
- Mini-sampler for welding aerosol, from conductive aluminium alloy (SKC 225-6201)
- MCE (mixed cellulose ester) filter, diameter 13 mm, pore size 5.0  $\mu\text{m}$  (SKC 225-8050)
- Calibration adaptor for Mini-sampler (SKC 225-6202)
- Low flow sampling pumps, capable of operating for up to 8 hours at a flow rate of 0.75 L/min

### 3 Recommendations and precautions for air sampling

- Users of this SOP should first carry out a suitable, specific risk assessment, prior to performing air measurements. Appropriate health and safety practices should be established in order to ensure compliance with regulatory requirements.
- Sampling is preferably carried out by a person, familiar with collecting personal inhalation measurements, according to good occupational hygiene practices
- The Mini-Sampler is designed for sampling of manganese in welding aerosol, to the requirements of the standard ISO 10882-1 "Health and safety in welding and allied processes - Sampling of airborne particles and gases in the operator's breathing zone - Part 1: Sampling of airborne particles". The Mini-Sampler can also be used for analysis of other chemicals in welding aerosol and gravimetric analysis of welding aerosol, but with reduced sampling efficiency for particle sizes above 20 $\mu\text{m}$ .
- Before air sampling is performed, the type of PVC filters or MCE filters to be used, is defined by the analysing laboratory, according to the laboratory's SOP for analysis. Assembled, pre-loaded cassettes should be provided by the same lab.
- If the GLA-5000 PVC filter type is not used, it will be necessary to check the alternative PVC filter type, as it has been reported that there are interferences on some types of PVC filters which greatly reduce the hexavalent chromium to trivalent chromium (OSHA, 1998). At least the recovery upon spiking of a known amount of a chromium (VI) standard has to be evaluated then. Nevertheless we highly recommend to use the GLA-5000 filter type.
- The plastic IOM cassettes and cyclone cassettes should be treated as disposables (single use only), in order to avoid any possible contamination.
- IOM sampler, cyclone sampler and Mini-sampler should be maintained on a regular basis, and the o-rings checked each time before sampling. Samplers should be well pre-cleaned, checked for any defects and operated according to instructions of the manufacturer (see also SKC Operating Instructions: 'IOM Personal Samplers and IOM Samplers with Multidust-Operating Instructions', 'Cyclone Samplers for Respirable Dust-Operating Instructions', 'Mini-sampler for Welding Aerosol-Operating Instructions', 'Face Level Sampling Head Set-Operating Instructions')
- When collecting air samples, researchers must always wear a new pair of powder-free disposable gloves. Care should be taken to avoid cross contamination of samples.
- Air sampling should be performed during a time period, representative for the actual working period of the exposed person, but taking into account that this should be as long as is reasonably practical.

- Air sampling should be interrupted during lunch breaks and pumps should be switched off then and removed from the wearer.
- Spare pumps, sampling heads and cassettes must always be provided so that planned samplings are not compromised.
- Any possible contact with reducing components (e.g. organic material, elemental iron, divalent iron) should be avoided as they can lead to low results by reduction of Chromium (VI) collected on the filter.
- Water should be avoided as this will allow any metal interference to interact with Chromium (VI), thereby affecting results.

## 4 Air sampling procedure

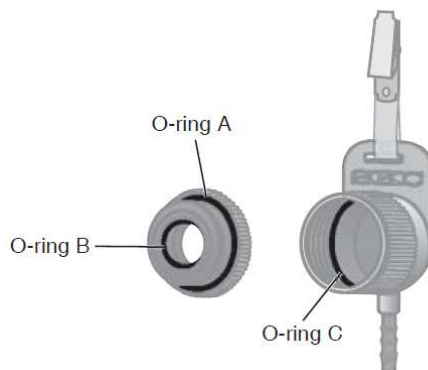
Sampling for both inhalable and respirable fraction can be performed simultaneously. Therefore the worker will need to wear two different pumps, preferably on two different sides (right and left side), preferably by alternating the sides of inhalable and respirable fraction collection between workers. For welding fumes only the inhalable fraction will be collected under the welding helmet (using either the IOM or Mini-sampler), thus welders will need to wear only one pump. This should be placed on the dominant side of the worker.

### Setting up sampling equipment

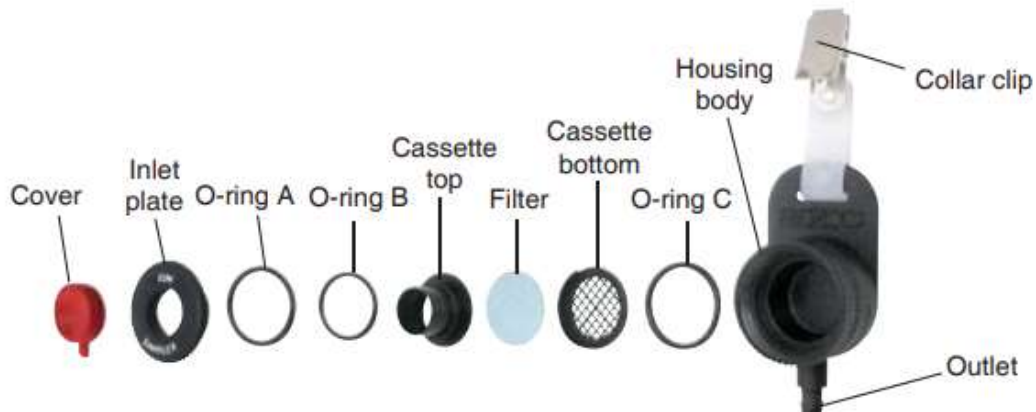
- Ensure that IOM, cyclone and Mini-sampler components are cleaned of any contamination using a detergent solution. Allow the components to dry fully before use.
- Charge the pump overnight with appropriate battery charger
- Set up the pump, sampler, cassette and flow calibrator in a clean area
- Use powder-free gloves and flat-tipped tweezers

#### IOM-sampler

- Remove IOM cassette (pre-loaded with pre-weighed filter) from its transport clip and remove protective cap.
- Unscrew top plate from IOM sampling head housing body. Ensure the O-rings are positioned correctly (Figure 1).
- Insert the IOM cassette into the IOM housing body. Screw the top plate into the housing body. Tighten securely to achieve a good seal. (Figure 2 for exploded view of an IOM sampler).



**Figure 1.** O-ring placement for plastic IOM sampler



**Figure 2.** Configuration for plastic IOM sampler and cassette

### Cyclone sampler

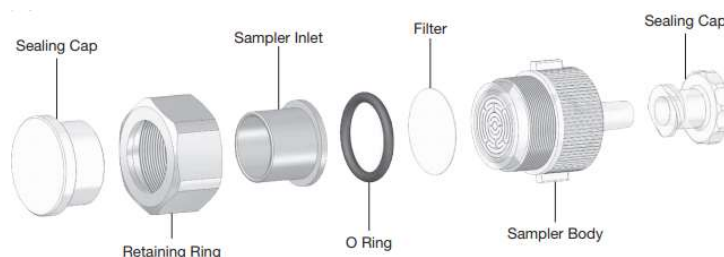
- Remove cyclone cassette (which contains the filter) from its sealing clip
- Unscrew cyclone sampler top from the sampler body. Ensure the O-rings are positioned correctly (see Figure 3).
- Fit the cyclone cassette into the cyclone sampler body with the cassette top upwards. Screw the sampler top into the sampler body. Tighten securely to achieve a good seal. Ensure that the clean and empty grit pot is securely fitted over the ridge around the bottom of the sampler body (see Figure 3).



**Figure 3.** Configuration for plastic cyclone sampler and cassette

### Mini-sampler

- Fit the sampler inlet into the retaining ring and fit the O ring into the retaining ring behind the sampler inlet (see Figure 4).
- Place the filter into the retaining ring on top of the O ring.
- Screw the retaining ring, sampler inlet, O ring, filter onto the sampler body, taking care not to dislodge the filter.
- Tighten carefully taking care not to damage the filter by overtightening (see Figure 4).
- Securely fit female Luer-Lok adapter fittings to both ends of one of the positionable gooseneck tubes.
- Press the male Luer-Slip fitting of the sampler body firmly into one of the female Luer-Lok fittings, attached to the gooseneck tube (see Figure 5).
- Fit the male Luer-Lok fitting of the tubing assembly (supplied with the Mini-sampler) to the remaining female Luer-Lok fitting on the gooseneck tube.



**Figure 4.** Configuration for Mini-sampler inlet components



**Figure 5.** Connection between Mini-sampler and gooseneck tube

- Connect IOM sampler / cyclone to the pump using flexible tubing of suitable diameter for making a leak proof connection from the sampling head to the pump.
- For Mini-sampler : connect the free end of the tubing assembly to the inlet port of the sample pump.
- This tubing should be of sufficient length allowing unimpeded movements of the worker.
- Switch on the pump and allow the flow to stabilise for a few minutes
- Attach flow meter to the inlet of the sampler (using a calibration adaptor for IOM sampler or calibration adaptor for Mini-sampler)

- Set the flow rate within  $\pm 0.1$  L / min of the prescribed flow rate, using a calibrated flow meter (e.g. Defender 510):
  - 2.0 L/min for IOM sampler
  - 2.2 L/min for Higgins-Dewell cyclone sampler
  - 3.0 L/min for SKC cyclone sampler
  - 0.75 L/min for Mini-sampler
- Measure and record the flow rate several times (e.g. 6 replicate readings from the calibrated flow meter)
- Disconnect the flow meter
- Perform a leak test by covering the sampler's inlet or kinking the tubing
- If the pump does not stall, this could indicate a leak and should be rectified and procedure above repeated.
- Switch off the pump
- Recap IOM sampler / Mini-sampler to prevent contamination of the filter

### **Placement of samplers on participants**

- Attach pump(s) to the worker's belt or harnesses so that they cause minimum inconvenience to the worker and safely secure the pump tubings
- Attach IOM and cyclone sampler to the worker's upper chest or lapel using the collar clips, preferably on two different sides (right and left side). Samplers should be placed in the breathing zone, not more than 30 cm away from the nose-mouth region (see Figure 6 and 7).
- For welders, whenever the IOM sampler is used this should be attached inside the welding helmet as close to the operator's mouth and nose.
- When using the min-sampler for welders, fit the gooseneck tube to the black plastic mounting clip, located on either side of the head set frame. Mount the sampling head set frame onto the worker. Carefully adjust the width of the head set frame to suit the size of the worker's head. Position the inlet of the Mini-sampler as close to the operator's mouth and nose as possible (see Figure 8).
- Make sure the opening of the IOM cassette is not directed upwards
- Cyclone sampler should be attached with the grit pot pointing downwards
- The inlet of the Mini-sampler should be facing forwards.
- When ready to begin sampling, remove protective cap from the IOM sampler / Mini-sampler (cyclone does not have a protective cap).
- Switch on the pump and record the time, using a calibrated timepiece
- Check the sampler and pump periodically during sampling to ensure that the equipment is still working.
- Air sampling should be interrupted during lunch breaks and pumps should be switched off and removed from the worker during this time. If possible, flow rates should be checked during lunch breaks for IOM and cyclone samplers, and at least at 2 hourly intervals for Mini-sampler, being adjusted as necessary.



**Figure 6.** IOM sampler and pump on worker (other than welders)  
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**Figure 7.** Cyclone sampler and pump on worker (© Copyright 2018 SKC Inc.)



**Figure 8.** Mini-sampler and headset on worker (© Copyright 2018 SKC Inc.)

### At end of sampling period

- Measure and record the flow rate several times (e.g. 6 replicate readings from the calibrated flow meter) before switching off the pump.
- Switch off the pumps and record the time, using a calibrated timepiece.
- Carefully disconnect the samplers from the tubing, without subjecting it to mechanical shocks.
- Cyclones must be always retained upright to avoid contents of the grit pot falling onto the filter
- Remove the IOM cassette from the IOM sampler and attach the protective cap on the IOM cassette and fasten with transport clip. Alternatively, it may be practical to cap the IOM sampler and return to the laboratory for disassembly
- Remove cyclone cassette and fit the sealing clip over the cassette.
- Remove Mini-sampler and fit the sealing cap to the sampler inlet.
- Calculate the average flow rate at the beginning and at the end of the measurement as well as the corresponding relative standard deviation (RSD). If the two flow rates differ by more than 5% or if a RSD value is higher than 2.5%, consider the air sample as invalid.
- Calculate the sampled air volume by multiplying the average flow rate with the sampling duration.
- For each air sample, a sample record sheet should be completed (Appendix 1). Following items are recorded : a unique identification code (including country ID, participant ID and sample ID, as explained below), sampling date, pump ID, start and end time, sampling duration (min), flow rate (L/min) before and after air sampling, sampled air volume (L) and other relevant sampling information (location, activities).

## 5 Sample traceability and contextual information

A standardised convention will be used to assign unique identification codes for all samples collected. The identification code convention is as follows:

Country ID (XX) - Participant ID (XX) - Sample ID (AXX/BXX/EXX/UXX/WXX)

Country ID 'XX' is the country code, using the ISO Alpha-2 country codes for the participating countries ([http://www.nationsonline.org/oneworld/country\\_code\\_list.htm](http://www.nationsonline.org/oneworld/country_code_list.htm))

Country	ISO Alpha-2 country codes
Belgium	BE
Finland	FI
France	FR
Italy	IT
Poland	PL
Portugal	PT
The Netherlands	NL
United Kingdom	UK

Participant ID 'XX' is a two-digit running number of participants in each country (e.g. 01 for the first participant recruited, 02 the second and so forth).

Sample ID '...XX' is one letter (A/B/E/U/W) to identify the type of sample collected, followed by a two-digit identifier (XX) to identify the running number of each type of sample for that worker (e.g. 01 for the first sample, 02 for the second and so forth). The letter code applied for the sample types is as follows:

Type of sample collected	Sample type code
Air	A
Blood	B
Exhaled breathe	E
Urine	U
Wipe	W

For stationary air samples, an extra letter 'S' is denoted in the sample ID. The following scenario is provided to illustrate the application of this convention. A worker is recruited in Belgium. It is the first worker being recruited and providing a first air sample. The sample identification code assigned is therefore BE - 01 - A01. In case of the first stationary air sample, the code assigned would be BE - 01 - SA01.

## 6 Storage of collected air samples

After sampling, air samples should be stored in the original cassettes (with transport or sealing clip). No cooled storage is required. Details of the time period for which the samples are stored before transportation to the lab, should be held.

## 7 Quality control

To check for possible contamination during the sampling procedure, transportation and storage, field blanks should be collected for each sampling survey. Submit at least one blank (filter, as well as cassette and cap) for every daily series of inhalable and respirable fraction samples respectively or alternatively for every set of more than 10 inhalable and respirable fraction samples. This blank should be handled in exactly the same way as the sampled filter containing cassette, but with no air drawn. The average mass found in the field blank should be subtracted from the corresponding mass found in the samples.

There are various factors that may affect the validity of the collected aerosol sample, such as: presence of projectile particles entering the sampler, large particles entering the sampler that are outside the inhalable definition, transportation losses (e.g. particles falling off the filter) and sample losses (e.g. wall losses onto the internal walls of the IOM cassette).

In some instances where the aerosol concentrations are unusually variable or there are significant projectile particles present, it is reasonable to assume that the sampler may be

unrepresentative of the personal exposure. This should be noted during the sampling and either disregard the result, or treat it as a 'worst-case' estimate of personal exposure. If projectile particles are present, then an unpumped sampler positioned next to the pumped sampler may be used to correct for unaspirated particles.

When sampling for welding aerosol it is important to sample as close as possible to the worker's nose and mouth because of the steep concentration gradients that occur in the immediate vicinity of the welding fume plume.

Possible losses on the internal walls of an IOM cassette should be also taken into account. The analyzing laboratory should perform wiping of the inside of the IOM cassette. Moreover, because wall deposition may not only result from air sampling, but may also occur upon shipment of sampled filters. The inside of the IOM cassette should be swabbed with a fresh filter of the same type as used for the air sampling.

## 8 Transportation of air samples to laboratory

After sampling, the labelled filter cassettes, accompanied by the sample record sheets, should be transported to the agreed analytical laboratory, who provided the assembled IOM cassettes and cyclone cassettes. The cassettes should be shipped with the top part directed upwards all the time.

For the Mini-sampler, the sampled filter should be re-assembled into a new, clean Mini-sampler with both sealing caps fitted to the sampler inlet and outlet, to prevent unwanted ingress or loss during transport.

Sample shipment should be ideally the same day as the sampling, however if this is not possible no later than the next day. Details of the numbers of samples being sent, sample identification codes, requested analysis and contact details of the responsible researcher should accompany the samples.

It is recommended that a hard copy of this information be included with the samples and that an electronic version is issued to the receiving laboratory at the time of sending the samples. This will allow sample numbers and identification codes to be checked upon receipt at the laboratory.

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Available from [https://www.skcltd.com/images/pdfs/007-09-016\\_Issue\\_A\\_Face\\_Level\\_Sampling\\_HeadSet\\_Instructions.pdf](https://www.skcltd.com/images/pdfs/007-09-016_Issue_A_Face_Level_Sampling_HeadSet_Instructions.pdf)

## Appendix 1: Sample record sheet

Identification code:		IOM or Cyclone type:	
Sampling date:		Company Name:	
Shipping date :		Company ID:	
Researcher:		Participant Name:	
Organisation:		Participant ID:	

Pump ID	Start time (00:00)	End time (00:00)	Sampling duration (min)	Flow rate (L/min) <i>before</i>	Flow rate (L/min) <i>intermediate</i>	Flow rate (L/min) <i>after</i>	Average flow rate (L/min)	Air volume (L)	Location	Activity
							Deviation (%)			
				<b>Average:</b>	<b>Average:</b>	<b>Average:</b>				
				<b>RSD (%):</b>	<b>RSD (%):</b>	<b>RSD (%):</b>				



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Contract No. 733032 HBM4EU

**SOP 7:**  
**Standard operating procedure for obtaining dermal  
wipe samples**

**WP 8**  
**Task 8.5**

## Table of Contents

Authors and Acknowledgements.....	2
1 Introduction.....	2
2 Materials required for the collection of wipe samples .....	3
3 Participant instructions.....	3
4 Researcher precautions.....	4
5 Wipe sampling procedures.....	4
6 Frequency of sample collection.....	5
7 Sample traceability and contextual information .....	5
8 Storage of collected wipe samples.....	6
9 Quality control.....	7
10 Transportation of wipe samples to laboratory .....	7
References .....	7
Appendix 1: Sample record sheet .....	8

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## 1 Introduction

This Standard Operating Procedure (SOP) is focussed on the collection of wipe samples to recover the mass of total chromium from the hands of volunteers participating in the workplace biomonitoring studies. The wiping technique is based on the removal of substances from the skin contamination layer by the application of a mechanical, fluid dynamic and/or chemical force to a moist medium that equals or exceeds the force of adhesion.

The collection and assessment of these occupational hygiene samples will provide information on the level of contamination experienced on the hands and can be used to evaluate protective glove effectiveness and workplace sanitation procedures. Average hand areas will be used in subsequent calculations, these being:

- 535 cm<sup>2</sup> per male hand (total 1070 cm<sup>2</sup> for both hands)
- 445 cm<sup>2</sup> per female hand (total 890 cm<sup>2</sup> for both hands) (US EPA, 2011)..

The SOP is based on internal SOPs employed by IOM to assess dermal exposure to various metals such as zinc and nickel as well as crude oil / base oils using wipe sampling methods. It involves the use of SKC Ghost sampling wipes, which can also be used to collect samples of several metals including chromium, as specified in OSHA Method ID-125G, Addendum B<sup>1</sup>. (It should however be noted that recovery, sampling and storage efficiency of the wipe sampling method has not been validated within the HBM4EU project). Alternatively lead wipes can be used. The goal is to provide a uniform methodology to collect representative samples of the hand contamination to total chromium in a standardized manner.

The Ghost wipes are to be analysed for total chromium using OSHA Method ID125G 'Metal and metalloid particulates in workplace atmospheres (ICP analysis)' by laboratories experienced in this analysis.

It should be highlighted that the Ghost wipe is made up of cellulose type material, and the digestion of the wipes results in a very exothermic reaction in which the wipes are digested within a few minutes and a lot of gas vapour and heat is released.

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<sup>1</sup> <http://www.skcltd.com/products2/9-uncategorised/310-surface-and-skin-1>

It is therefore essential that a thorough risk assessment is undertaken of the digestion and analyse activities prior to commencing the work. It is again highlighted that we advocate that laboratories experienced in the analysis of Ghost wipes undertake the analysis.

The Lead wipes are to be analysed using NIOSH method 9102 'Elements on wipes' (NIOSH, 2003).

## 2 Materials required for the collection of wipe samples

To undertake the collection of wipe samples, researchers should ensure that they have sufficient quantities of the following:

- Ghost wipes, moistened with deionised water (individually packed). Part No: 225-2414 (200 wipes); part No: 225-2413 (1,000 wipes) or:
- Lead wipes; moistened with deionized water, polyorbate 20, methylparaben, and propylparaben (individually packed). Part No: AR-LWIPE100 (100 wipes); part No: AR-LWIPE1000 (1,000 wipes). Lynx Products, New Jersey. To be used when samples are prepared as described in NIOSH method 9102, 2003.
- Self-seal sample bags of sufficient size to hold two wipes and have label secured.
- Sample labels
- Supply of powderless disposal nitrile gloves
- Sample record sheets (Appendix 1)
- Supply of disposable paper towels
- Pens
- Receptacle for storage of collected samples back to laboratory

## 3 Participant instructions

Participants should be fully informed of the sampling procedure. They will be advised of the following points before commencing their work shift:

- Rings should be removed (wherever possible) and securely stored.
- Participants will be asked to thoroughly wash and dry their hands with soap and water before commencing their work shift. Hands will be dried using fresh disposable paper towels (which can be provided as necessary) or hand dryers. Reusable fabric towels must not be used.
- A wipe sample will then be collected from each hand, prior to the participant commencing their work activity using the standardised wiping procedure.
- Further wipe samples will be collected from the participants prior to their refreshment/comfort and lunch and before they finish their work shift. Participants must not wash their hands before wipe samples are collected.
- The participant should also be informed that if for any reasons they do wash their hands between any two wipe sampling periods that they should inform the researcher that this is the case.

## 4 Researcher precautions

When collecting each wipe sample from the participants, researchers must always wear a new pair of unpowdered disposable nitrile gloves.

Care should also be taken to avoid cross contamination of samples. It is recommended that where possible the wipes are collected in an area considered to be free of potential contamination e.g. office space, medical room etc. Wipes should not be collected in the physical work area where, for example, welding or surface treatment activities are taking place due to the risk of cross contamination. Appropriate quality assurance procedures must be applied to check this is the case (see Section 9).

Researchers collecting the wipe samples should try to ensure that a consistent amount of pressure is applied when wiping the participants hands. It is recommended that the number of researchers collecting the wipe samples is minimised to one or two per country where possible.

Researchers should ensure that a site specific risk assessment of their work practices is undertaken prior to commencing the measurement campaign and that all necessary health and safety precautions are adopted and followed.

## 5 Wipe sampling procedures

The following procedures should be used to collect the wipe samples.

- Prepare a sufficient number of self-seal bags each labelled with a unique number (see Section 7).
- Wear a new pair of clean disposable gloves for each sample. DO NOT use powdered gloves.
- Record the sample number and record participant details on the sample record form (Appendix 1).
- Remove the wipe from its wrapper using gloved hands. Do not use metal tweezers to handle the wipe, as they could contaminate the sample.
- Note on the record sheet if the participant's hands are observed to be wet with water or if participant has washed their hands prior to sampling.
- A standardised wiping technique will be used which involves wiping the whole palmar and dorsal area and fingers:
  - Starting with the right hand, wipe five times the palm of the hand from the top of the hand to the start of the fingers and five times across
  - Repeat the procedure to sample the back of the hand.
  - Fold the wipe in half (with the contaminant side inward) and sample the fingers trying to wipe well in between the fingers (Figure 1).
  - Wipe twice the palm of each finger, from the top to the fingertip. Repeat, to sample the back of the fingers



**Figure 1:** Dermal sample collected from the fingers

- After wiping, fold the wipe again with the contaminant side inward. Place the wipe immediately in the labelled seal-seal bag.
- Repeat described wipe sampling procedure for the left hand, placing the wipe in the same labelled seal-seal bag.
- It is recommended that one blank sample is collected per participant, treated in the same fashion, but without wiping (See Section 9).

## 6 Frequency of sample collection

The number of wipe samples collected will be dependent on the shift duration and number of rest breaks the participant has and researchers should use their judgment to decide on the numbers of samples to be collected. As way of example, assuming that the participant works a full shift on the Cr(VI) activities and has one rest break and one lunch break, there will be four samples collection periods, these bring pre-shift, first break period, lunch and post-shift, resulting in four samples being collected per participant (excluding blanks).

## 7 Sample traceability and contextual information

A standardised convention will be used to assign unique identification codes for all samples collected. The identification code convention is as follows:

Country ID (XX) - Participant ID (XX) - Sample ID (BXX/UXX/EXX/AXX/WXX)

Country ID 'XX' is the country code, using the ISO Alpha-2 country codes for the participating countries<sup>2</sup>.

Country	ISO Alpha-2 country codes
Belgium	BE
Finland	FI
France	FR
Italy	IT
Poland	PL
Portugal	PT
The Netherlands	NL
United Kingdom	UK

Participant ID 'XX' is a two-digit running number of participants in each country (e.g. 01 for the first participant recruited, 02 the second and so forth).

Sample ID 'UXX' is one letter (B/U/E/A/W) to identify the type of sample collected, followed by a two-digit identifier (XX) to identify the running number of each type of sample for that worker (e.g. 01 for the first sample, 02 for the second and so forth). The letter code applied for the sample types is as follows:

Type of sample collected	Sample type code
Air	A
Blood	B
Exhaled breath	E
Urine	U
Wipe	W

The following scenario is provided to illustrate the application of this convention.

A worker is recruited in The Netherlands. He is the first worker recruited and has provided four wipe samples over the course of his work shift. The sample identification codes assigned are therefore:

NL-01-W01 (pre-shift collection)

NL-01-W02 (first break)

NL-01-W03 (lunch)

NL-01-W04 (post-shift)

The unique sample identification code will be clearly stated on the labelled self-seal bag. A sample record sheet should be completed when collecting the wipe samples (Appendix 1).

## 8 Storage of collected wipe samples

The collected wipe samples can be stored at room temperature in a clean box before transportation to the laboratory.

<sup>2</sup> [http://www.nationsonline.org/oneworld/country\\_code\\_list.htm](http://www.nationsonline.org/oneworld/country_code_list.htm)

## 9 Quality control

To check for contamination during the sampling procedure, transportation and storage, field blanks should be collected for each sampling survey.

These samples should be treated in the same way as the exposure samples, using the same procedures as previously described, but omitting the skin wiping. The average mass found in the field blank should be subtracted from the corresponding mass found in the samples. In the event of elevated concentrations being observed in the blank samples these will be investigated and used to flag any suspect participant wipe samples.

The number of field blanks should be no less than 10% of the number of dermal samples however it is recommended that one field blank is collected per participant.

## 10 Transportation of wipe samples to laboratory

Local arrangements will need to be put in place with respect to the transportation of the samples to the laboratory. For example, in some instances the samples may be driven by the researcher to the laboratory whereas in others, courier delivery may be necessary.

Samples should be transported to the agreed analytical laboratory ideally the same day however if this is not possible no later than the next day. Details of the numbers of samples being sent, sample identification codes, requested analysis and contact details of the responsible researcher should accompany the samples.

It is recommended that a hard copy of this information be included with the samples and that an electronic version is issued to the receiving laboratory at the time of sending the samples. This will allow sample numbers and identification codes to be checked upon receipt at the laboratory.

Whilst no storage stability tests have been established within the HBM4EU project it is recommended that all collected wipe samples are analysed as soon as possible by the receiving laboratory and certainly within no more than 14 days of collection.

## References

NIOSH Method 9102 'Metals on wipes'. URL: <https://www.cdc.gov/niosh/docs/2003-154/pdfs/9102.pdf>

OSHA Method ID125G 'Metal and metalloid particulates in workplace atmospheres (ICP analysis)'. URL: <https://www.osha.gov/dts/sltc/methods/inorganic/id125g/id125g.pdf>

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## Appendix 1: Sample record sheet

<b>Company Name:</b>	
<b>Company ID:</b>	
<b>Worker ID:</b>	
<b>Name (family name, initial):</b>	
<b>Job title:</b>	

<b>Researcher(s):</b>	
<b>Organisation:</b>	
<b>Date sampling:</b>	

<b>Collection round</b>	<b>Sample ID</b>	<b>Time collected (00:00)</b>	<b>Gloves worn prior to collection (Y/N)</b>	<b>Type gloves worn</b>	<b>Hands washed prior to collection (Y/N)</b>	<b>Hands observed to be wet (Y/N)</b>
Pre-shift						
Break 1						
Lunch						
End shift						
Blank						



science and policy  
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HORIZON2020 Programme  
Contract No. 733032 HBM4EU

**SOP 8:**

**Standard operating procedure for comparing  
occupational hygiene measurements with exposure  
estimates generated using REACH models**

**WP 8**  
**Task 8.5**

## Table of contents

Authors and Acknowledgements.....	2
1 Scope .....	2
2 Contextual information to be collected during site visits .....	3
3 Generation of exposure scenarios for use in modelling exercise.....	3
4 Models to be used.....	3
5 Who should complete the modelling exercise? .....	4
6 The modelling exercise .....	4
6.1 Overview.....	4
6.2 Background questionnaire .....	4
6.3 Introductory pack .....	4
6.4 Exposure situations .....	4
7 Data preparation .....	5
References .....	5
Appendix 1: Contextual information proforma .....	6
Appendix 2: Example of exposure scenarios to be generated.....	11

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## 1 Scope

Many different approaches can be used for estimating occupational exposure to chemical substances. More recently, and probably primarily due to regulatory influence following the introduction of the REACH regulations, the use of predictive exposure models is becoming more frequent as it is not possible for the occupational hygiene community to collect a sufficient number of exposure measurements to generate estimates for all relevant exposure scenarios (Fransman, 2017; Landberg et al., 2018).

When applying exposure assessment modelling tools, users are required to select options from a number of possible input parameters. Hence, results obtained with the tools could be affected by factors such as the professional experience and judgment of the tool user and access to an appropriate level of information.

Several Tier 1 screening models such as ECETOC-TRA, MEASE and others are recommended for use under REACH (ECHA, 2016) and were evaluated under the E-TEAM project of which the results have been reported in several papers (Lamb et al, 2015, 2017, Tischer et al, 2017, van Tongeren et al, 2017). Lamb et al (2017) reported a between-user reliability exercise where exposure estimates ranged over several orders of magnitude for the same exposure situation by different users. It was also noted that the amount of contextual information provided in the situations could have potentially affected the level of variation between users. To explore this further a standardised proforma will be used in the HBM4EU Hexavalent Chromium study to collect contextual information about the work activities observed during the measurement campaign. For each visit, an exposure scenario will be generated.

At a later stage (and without knowledge of the results of the measurement campaigns), participants with differing knowledge about the workplace environments and activities will be given the generated workplace exposure scenarios and asked to use selected REACH models. Those selected are: ECETOC TRA v 3, MEASE-2 and ART 1.5, with participants being asked to estimate both inhalation and dermal exposure (where applicable). Comparisons of the exposure estimates generated between the different types of users will be made, with these estimates also being compared with the actual exposure measurement results.

This Standard Operating Procedure (SOP) is partially based on earlier work undertaken by the IOM which has focused on the evaluation of exposure models (e.g. Lamb et al, 2015;2017; van

Tongeren et al, 2017). This SOP is focused on: a. the collection of contextual information to inform the development of exposure scenarios to be used in the modelling exercises and b. the administration of the modelling exercise to participants. Details of how the collected data will be analysed is not provided in this SOP.

## 2 Contextual information to be collected during site visits

A standardised proforma will be used to gather relevant contextual information during the field survey measurement campaigns (Appendix 1). Information to be gathered will be from researchers first hand observations of the workplaces and activities taking place there and will include, for example, details of the risk management measures in place and used, operational conditions, materials generated, used etc. This proforma is to be completed on the same day that the air and wipe samples are collected.

## 3 Generation of exposure scenarios for use in modelling exercise

The completed proformas will be returned to KG ([karen.galea@iom-world.org](mailto:karen.galea@iom-world.org)) and SV ([susana.viegas@estesl.ipl.pt](mailto:susana.viegas@estesl.ipl.pt)), who will use these to generate exposure scenarios to be used in the modelling exercise.

A standardised single A4 page format will be used for the situations to minimise participant uncertainty from differences in layout of the descriptive information. An example of what these may look like is provided in Appendix 2 (this example being from the IOM E-TEAM project).

The situation descriptions will not be tailored to a specific inhalable or dermal exposure route, however their content will be such that either or both of the routes are applicable. Similarly, situations will not be tailored to the applicability range of specific tools in terms of work activity or substance type. It is recognised that this will have led to situations being assessed by tools that were not applicable in terms of scope and/or exposure route.

## 4 Models to be used

Participants will be asked to generate exposure estimates for the various scenarios using the following selected models which are typically used under REACH:

- ECETOC TRA v3 – inhalation and dermal exposure estimate
- MEASE v2 - inhalation and dermal exposure estimate
- ART 1.5 - inhalation exposure estimate

ECETOC TRA and MEASE are referred to as Tier 1 models, with ART being considered as a higher tier model.

## 5 Who should complete the modelling exercise?

Each participating country where air and wipe samples will be collected as part of the Cr(VI) biomonitoring campaign, were invited to participate. In the end, six countries expressed an interest in participating in this parallel study, namely: Belgium, Finland, France, Portugal, The Netherlands and United Kingdom. In each country the following participants will be directly involved:

- The occupational hygienist / researcher who completed the contextual information template and collected the air and wipe sample measurements
- A member of the chromate project team who did not visit the sites
- An individual experienced in exposure assessment but who has no direct experience of the project or sites where Cr(VI) exposure may be present.

## 6 The modelling exercise

### 6.1 Overview

Participants will be asked to complete a short background questionnaire. They will then be provided electronically with an introductory pack containing simple instructions for completing the exercise, which will be instructions on how to use the models. Participants will then be forwarded the exposure scenarios and supporting worksheets to complete, along with details on how these are to be returned.

### 6.2 Background questionnaire

A short background questionnaire will be administered to collect key information on participants experience in relation to the Cr(VI) measurement campaign and also their use of the modelling tools.

The participants will be requested to provide the following information:

- Organisation they work for.
- Years of experience in exposure assessment.
- The nature of their involvement in the Cr(VI) measurement campaign, e.g. if they personally collected the contextual information / air and wipe samples at the sites; if they did not attend any site visits; had no involvement with the Cr(VI) study.
- Previous experience with the use of the various models.

### 6.3 Introductory pack

The introductory pack will include simple guides to installing and operating the models for the purposes of the exercise. Guidance and screenshots detailing the required tool outputs will also be included.

### 6.4 Exposure situations

Depending on the number of exposure situations which are eventually generated, participants may receive these in batches or all at one time.

For each exposure situation, participants will be instructed to undertake both an inhalation and dermal exposure assessment (were applicable) using a specified tool, even where the situation may be outwith the scope for that tool. For each exposure situation they will be asked to complete a worksheet to record their results for each fo the models used.

For each exposure situation/tool combination issued, participants will be required to document systematically the following contextual information on the worksheet:

- Previous experience of the given exposure situation.
- Instances where they found choice or description of parameter types difficult, i.e. the level of uncertainty in their choice for example when selecting substance characteristics or risk management measures.
- The outputs derived by the tool.
- Their perception of the level of over/ under-estimation of the exposure estimate generated by the tool(s).

Participants will be asked to complete the given exposure situation/tool combination and return the completed worksheet and tool files within a specified period of time. A reminder will be issued in the event of non-receipt.

## 7 Data preparation

The exposure assessment outputs will be harvested from the returned worksheets and questionnaires and tabulated for analysis in Microsoft Excel spreadsheets.

## References

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## Appendix 1: Contextual information proforma

Worker Information:	
Date:	
Participant ID:	
Job Title:	
Shift Length (hours)	

### General working environment:

- Indoor / outdoor
- Room temperature: .....°C
- Approximate size of room where the participant works: ..... m<sup>3</sup>
- Natural ventilation / Mechanical general room ventilation / Both
- Air change rate: ..... air changes per hour
- Description of how well room is generally ventilated  
.....
- General impression of cleanliness and tidiness of workplace      Poor / Good / Excellent
- Frequency of cleaning per day and how  
.....
- General hygiene /welfare provision
  - Hand-washing facilities in immediate work location:      Yes / No
  - Do workers shower at end of the shift:      Yes / No
  - Separate eating/ drinking area:      Yes/ No
  - Others: .....
- Workers received previous training on the health risks associated with Cr(VI)?
  - Yes/ No
  - If yes, please specify.....

**Activities and tasks within these**

- Description of activities taking place in this environment and numbers of workers involved (for each activity and in total)

.....  
.....

- Description of tasks undertaken by workers and how they may be exposed:

- plating / surface treatment:

.....

- welding activities:

.....

- others:

.....

- Process classification (continuous/batch) across operations and tasks

.....  
.....

- Level of automation, mechanisation and manual interventions in process

.....  
.....

- Any tools being used / manipulated and how this is done?

- Description of tools used :

.....  
.....

- Please shortly describe the tools utilization for the different tasks

.....  
.....

- Time spent on each task / activity during the day

.....  
.....

- Approximate working distance of worker from the exposure source: .....meters

- If different distances for different workers please specify:

.....

- Any observed differences in working behavior between workers involved in same activity.

.....

.....

- Were the tasks /activities observed on the day typical of usual work activities:

- Yes / No : .....

- If no, were samples/information collected during periods of lower / higher work rates?

.....

- How do these differ over the course of a week/year?

.....

**Nature and sources of hazardous substance**

- Physical state of substance:

- solid..... Dustiness: .....

- liquid..... Vapour pressures (Pa) at 20°C: .....

- Concentration of Cr(Vi) in preparation/product?

<1%       1-5 %       5-25%       >25%       100%

- Sources of emission and subjective assessment of where emissions may be high.

.....

.....

**Operational Conditions**

- Frequency and duration of exposure of workers conducting the tasks that can imply exposure (e.g. Task X: 120 min. twice a shift)

.....

.....

- Amount of substance handled: .....Kg per shift
- Use rate (include units):  
.....
- Process conditions that can be relevant (e.g. heated bath, high current applied)

.....

- Process temperature: .....°C
- Level of automation (e.g. manual)

.....

**Risk management measures (description and comment on each of these)**

- Segregation – description

.....

.....

- Enclosure

.....

.....

- Local exhaust ventilation controls, description and comment on position, use, effectiveness

.....

.....

- Suppression techniques

.....

.....

- Control rooms description – time spent (minutes or hours)

.....

- Others

.....

**PPE**

Respiratory Protection Equipment usage by worker during activities cleaning / storage regime:

- Type of RPE used: .....Supplier APF: .....
- % of time/tasks being used:  
.....

• Protective gloves

- Type: .....
- % of time/tasks being used:  
.....
- donning and doffing procedure:  
.....
- frequency of replacing disposable gloves:.....;

**Dermal and ingestion exposure**

• What is the frequency of skin contact with the contaminant?

- hands: .....
- other body regions: .....

• What kind of skin contact with the contaminant occurs:

- direct contact: Yes/No
- touching contaminated surfaces: Yes/No
- other:.....

• Are significant amounts of aerosols or splashes generated in the tasks?

.....

• Observed Hand/object-to-mouth contact: Yes /No

- If yes please specify frequency (for hand-mouth route) during the activities:

.....  
.....

• Hand washing behaviors during work shift, (particularly prior to refreshment breaks).

.....  
.....

## Appendix 2: Example of exposure scenarios to be generated

Example exposure situation from E-TEAM project (Lamb et al, 2017).

### Situation 15: Packing of Nickel Metal Powder

Please assess inhalation and dermal exposure to **nickel** in the situation described below.

When entering data into the tools during the exercise, please use the CAS number, molecular weight and vapour pressure value given in the table below.

#### 1. General Description of Exposure Situation

This situation describes the packing of nickel powder in drums.

The operator removes excess powder (Product R) from a pre-weighed drum using a hand scoop and places the surplus material into a storage bin located at the packing station (Work Area R). If the containers are below the required weight, the operator uses the scoop to transfer powder back from the storage bin into the drum.

The operator then fixes a sealing cap onto an open aperture on the top of the drum.

The packing station is provided with local exhaust ventilation at the filling point. An air assisted filtering visor fitted with P3 filters is worn. All packing operators wear cotton overalls and safety boots. Gloves are not worn during scooping of powders.

The activity takes place at room temperature (20°C) in a small room with general ventilation.

The activity takes place for approximately 3 hours per 8 hour shift.

#### 2. Product/ Substance Information

Product	Supplier	Substance Name	CAS Number	Molecular Weight/ g mol <sup>-1</sup>	Vapour pressure at 20°C/ Pa	Concentration of Nickel in Product R (%)
Product R	Supplier R	Nickel	7440-02-0	59	1 (Negligible)	100