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HBM4EU occupational e-waste study

Standard Operating Procedures (SOPs)

WP8 - Targeted field work surveys and alignment at EU level

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1 Introduction

Within the HBM4EU project, several priority chemicals were identified (https://www.hbm4eu.eu/the-substances/), which may be of concern for the European population. Several of those are also relevant at European workplaces, including companies taking part in the chain of e-waste processing.

E-waste is defined (Encyclopedia Britannica, 2016) as: 'various forms of electric and electronic equipment that have ceased to be of value to their users or no longer satisfy their original purpose (…) including both "white goods" such as refrigerators, washing machines, and microwaves and "brown goods" such as televisions, radios, computers, and cell phones.' A classification of e-waste items is in a report endorsed by ESCAP, ESCWA, ITU, OECD, UNCTAD, UNECA, EUROSTAT, UNEP/SBC and UNU, referring to two existing EU waste classification systems (Baldé et al., 2015). In the FP7 project CWIT (2012) it was estimated that only 35% (3.3 million tonnes of 9.5 million tonnes) of used (but still functioning) e-waste was processed within the EU. Annually approximately 400,000 tonnes of discarded electronics left the EU as part of 'undocumented mixed exports'. When taking into account the EU new circular economy policy (EEA, 2019) and the need to enhance the recycling of e-waste, the waste management/recycling sector is expected to grow. This e-waste stream is complex because it contains many composite materials such as circuit boards, cathode ray tubes, flat screen monitors, batteries, connectors and transformers, plastic casings and cables. The e-waste stream contains a broad range of hazardous ingredients, including toxic metals, polybrominated diphenyl ether (BPDE) and organophosphate ester (OPE) flame retardants, phthalates, polychlorinated biphenyls (PCBs), hexabromocyclododecanes (HBCDs), polychlorinated dibenzo-p-dioxins (PCDD), polybrominated dibenzo-p-dioxins (PBDD) and polychlorinated dibenzofurans (PCDF). Plastic materials may contain chemicals that were legal at the time they were manufactured but are now either restricted or banned, such as lead, PCBs, some phthalates and flame retardants (Grant et al., 2013). The recycling of these materials can result in exposure of workers involved in different steps in the chain of waste processing such as collection, sorting, dismantling, shredding and further pre-processing and purification of waste components for the market of recycled polymer plastics and metals.

HBM4EU could support the development of sustainable practices in e-waste management by providing suitable methods for exposure assessment to support the development of sound practices in professional processing of e-waste. This would prevent e-waste from being dumped in, and also outside European countries and would support a development towards more sustainable processing of this waste stream in line with the Basel Convention on the Control of Transboundary Movement of Hazardous Wastes and their Disposal (Basel Convention, 1989). A recent literature review by Arain and Neitzel (2019) shows that so far occupational exposures were only studied in few European countries and only one study used biomonitoring to assess the level of exposure (Julander et al., 2014). An HBM4EU study could focus on a range of substances on the 1st an 2nd list of priority substances (EEA, 2018), for which biomonitoring methods have been developed and tested in multiple laboratories as part of HBM4EU and may also include substances for which this process is still ongoing.

In the HMB4EU e-waste occupational exposure study (Santonen et al., 2020) exposed workers and controls from companies participating in the chain of e-waste processing will be recruited from nine countries, namely: Belgium, Finland, Germany, Latvia, Luxembourg, Poland, Portugal, The Netherlands and United Kingdom (UK). In order to achieve comparable data in a harmonised way, the enclosed Standard Operating Procedures (SOPs) have been prepared. Every participating country is obliged to, as far as is reasonable possible, follow these procedures. The SOPs for the selection of participants and recruitment, information to the participants, informed consent (annex

1), completion of questionnaires (annex 2) and instructions for blood, urine, settled dust, air, hair, dermal and buccal cells sampling (annexes 3-9), procedure for comparing occupational hygiene measurements with exposure estimates generated using REACH models (annex 10), and communication plan (annex 11), are annexed.

The overarching goal is to increase the share of processing e-waste that EU member states are producing, using EU's own processing capability, instead of exporting e-waste. In a partnership with the recycling sector in Europe (EEA, 2019), HBM4EU can help to ensure the sustainable processing of e-waste. For this study our focus will be on the occupational health and safety aspects. By conducting a HBM study we hope to contribute to awareness of potential hazards and stimulate good work practices that will lead to further improve protection of the worker's health from the risk of exposure to toxic components, including that of combined exposures.

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Annexes

Annex 1: Standard operation procedure for selection of participants and recruitment, information to the participants, informed consent (SOP 1)

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Annex 1

SOP 1:

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

Occupational E-waste study

WP 8

Task 8.5

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Summary

Most relevant aspects:

- ✓ Workers are from companies performing e-waste management processes.
- ✓ Workers from both genders and ages ranging from 18-70 years will be considered.
- ✓ Control subjects should match for age, gender and smoking status from companies in the same geographical area but working on activities not related with e-waste management processes.
- ✓ Additional criteria should be followed for workers and controls in the case of biomarkers of effect.
- ✓ SOP details the specific documents to be delivered to companies, workers and controls.

1 Introduction

Standard Operation Procedure 1 (SOP 1) is focussed on the selection of participants and recruitment, information to the participants and informed consent. This was developed with due consideration of the contents of the "SOP1: Standard operating procedure for selection of participants and recruitment, information to the participants, informed consent (SOP 1)" (lead authors Carina Ladeira, Edna Ribeiro, Susana Viegas, ESTeSL) developed for the HBM4EU occupational biomonitoring study on hexavalent chromium and other harmful chemicals (Porras et al., 2019)

2 Study design and participants

The study is focussed on companies performing e-waste management processes located in several countries from Europe, namely: Belgium, Finland, Germany, Hungary, Latvia, Luxembourg, The Netherlands, Poland, Portugal and United Kingdom.

The target population will be workers from companies performing e-waste management processes such as:

1) Sorting e-waste from household and industrial waste streams (by hand or semi-automatic)
2) Dismantling to split casings from electronic components such as circuit boards and batteries (often by hand)
3) Shredding and pre-processing (e.g. on a belt by electrostatic, density, magnetism, colour separation)
4) Further purification of e-waste materials for re-use and re-sell (melting metals to a product that can be re-used and polymer processing to a granulated product that can be re-used).

2.1 Target population

The target population will be workers involved in the above described e-waste processes. In addition, a control population of workers will also be recruited. These workers will be from the same geographical region but working in offices/administrative tasks.

Both genders will be eligible, with ages ranging from 18-70 years. For some analyses, e.g., effect biomarkers characterization, specific inclusion/exclusion criteria are 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 25 - 50 workers *per* country. The sample size is indicative and may need further justification due to expected population variability of the biomarkers. Additionally, 25 workers (controls) *per* country will be also engaged in the study. Overall, this would result in 500 exposed workers and 250 control workers.

3 Selection of sampling locations

Sampling will be conducted in previously identified companies located in the ten countries participating in the study, namely: Belgium, Finland, Germany, Hungary, Latvia, Luxembourg, The Netherlands, Poland, Portugal and United Kingdom. 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 the companies workers. It should be noted that in some instances a site visit prior to the day of the actual sample collection may be necessary to obtain the necessary written consents, whereas in other instances the written consent may be obtained on the same day, immediately before commencement of the sample collections.

Company recruitment

Establishment of phone contact or e-mail with the Company responsible. Upon company expressing interest in participating in the study, the information leaflet, "INFORMATION FOR PARTICIPATING COMPANIES" will be issued. Where country specific rule requires its use (e.g., Belgium) upon a company agreeing to participate in the study an authorised representative of the Company must complete the ***"EMPLOYER CERTIFICATE OF INFORMED CONSENT"***. Where country specific rules do not require it's use completion of this certificate is not necessary. Then, request to the company responsible of a list of names of those involved in e-waste management processes so that we can approach them to explain the project and invite them to participate.

Worker recruitment

In most countries recruitment cannot start before ethics approval has been obtained.

1. Establishment of a direct contact between the researcher and 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 reasonable period of time 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.

Therefore, and following workers' acceptance to participate in the study, we will recruit workers directly involved in the processes already described in Section 2.

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 but involved in activities not related with e-waste management processes. The same approach used for workers will be followed to recruit controls.

All the 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) have not been diagnosed for cancer.

Results communication

Results communication should follow SOP 9 (Communication Plan). Moreover, the definition of which results, to whom and how the HBM results will be communicated should be defined in the beginning of the study following the General Data Protection Regulation requirements.

5 Informed Consent form

Prior to the workers contact, and where country specific rule requires its use (e.g., Belgium), the company should analyse and sign the Informed Consent Form ***("EMPLOYER CERTIFICATE OF INFORMED CONSENT"***). 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 only 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 of the consent form by the participants (company representative, workers and controls). The workers should have the time and opportunity to ask questions to the researcher and not be under pressure to decide.

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

6 Assignment of participant and sample codes

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

E-waste (E) - Country ID (XX) - Company ID (XX) - Participant ID (XXX) - Sample ID (AX/BX/LCX/RCX/HX/SDX/UX/WX/WBX)

'E' is to denote that the samples and data relate to the e-waste occupational study.

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).

Country	ISO Alpha-2 country codes
Belgium	BE
Finland	FI
Germany	DE
Latvia	LV
Luxembourg	LU
Poland	PL
Portugal	PT
The Netherlands	NL
United Kingdom	UK

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

Participant ID 'XXX' is a three-digit running number of participants in each country (e.g. 001 for the first participant recruited, 002 the second and so forth).

Sample ID 'AX/BX/LCX/…' is one or two letters (A/B/LC/RC/H/SD/U/W/WB) to identify the type of sample collected, followed by an one-digit identifier (X) to identify the running number of each type of sample for that worker (e.g. 1 for the first sample, 2 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
Buccal cells	LC (left cheek) or RC (right cheek)
Hair	H
Settled dust	SD
Urine	U
Wipe	W
Wrist band	WB

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

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

E-PT-01-001-W1

E-PT-01-001-W2

7 References

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Annex 2

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

Occupational E-waste study

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This SOP was developed with due consideration of the contents of the "SOP2: Standard operating procedure for completion of company and worker questionnaires" (lead authors Sanni Uuksulainen (FIOH, Finland), Simo Porras (FIOH) and Karen Galea (IOM, UK)) developed for the HBM4EU occupational biomonitoring study on hexavalent chromium and other harmful chemicals (Porras et al., 2019).

This document has been created for the HBM4EU project. HBM4EU has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 733032.

Summary

Most relevant aspects

This SOP defines:

- ✓ Instructions to fill in the workplace questionnaire
- ✓ Instructions for completion of the workers post-shift and controls questionnaire
- ✓ Instructions to provide job descriptions on different activities

1 General introduction

This SOP 2 – “Completion of company and worker questionnaires” is designed to support a targeted occupational study on E-waste 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 stated in SOP 1 (Selection of participants and recruitment, information to the participants, informed consent), the target population are workers from companies performing e-waste management processes located in several European countries. The exact processes used in the companies and by workers will be specified during the collection of contextual information, which is guided on this SOP.

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 (related with workplace contextual information) will not be applicable to the control group (those participants not working in E-waste management processes) and these questions will be clearly identified as such. Additionally, it´s important to clarify that the home address is questioned to guarantee that the participants receive their individual HBM results (as detailed in the participant information leaflet) report when available. Moreover, the questions related with alcohol and tobacco consumption, diet, specific diseases and medical examinations are needed because biomarkers of effect are going to be analysed in the samples of some countries. In this case, the countries that are not going to consider biomarkers of effect should not ask these questions.

It is important that information entered into the hard copy questionnaires is recorded as per the instructions given in this SOP as this information will need to be entered into the data template for the overall E-waste study.

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, further information is provided below should the company representative require additional details. 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 and nature of the business can be described in free text.
  - The researcher will fill the NACE Rev.2 code. Copy corresponding NACE code (with 4 digits) and label text from the link. The classification is available in several EU-languages at the following URL:

    http://ec.europa.eu/eurostat/ramon/nomenclatures/index.cfm?TargetUrl=LST_NOMDTL&StrNom=NACE_REV2&StrLanguageCode=EN&IntPcKey=&StrLayoutCode=HIERARCHIC

Most of the sectors of use in this study belong to the following classes: 38.12 Collection of hazardous waste; 38.22 Treatment and disposal of hazardous waste; 38.31 Dismantling of wrecks and 38.32 Recovery of sorted materials classes.

- **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 - Previous measurements:** This section is to be completed only if the companies have undertaken previous environmental or biomonitoring campaigns to evaluate workers' exposure to chemical substances.
  - Background information about the previous exposure measurements is very important when estimating the risks of 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.

- **Section 2 - Hygiene facilities and procedures:** This section is to be completed with the most detailed information, signalizing the boxes and providing additional information if available.

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. 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 and controls 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 workshift 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
- Job description and personal habits
  - To be completed for the exposed workers and controls
  - Personal habits (cigarette, alcohol consumption, diet) asked are relevant for data analysis.
- 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.

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:**

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

E-waste (E) - Country ID (XX) - Company ID (XX) - Participant ID (XXX)

'E' is to denote that the samples and data relate to the e-waste occupational study.

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).

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Participant ID 'XXX' is a three-digit running number of participants in each country (e.g. 001 for the first participant recruited, 002 the second and so forth).

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

A worker is recruited in Portugal. He is working in the first company recruited and he is the first worker recruited in that company. The worker identification code assigned is therefore: E-PT-01-001

- **Information related to the sample collection:** Fill in the information regarding the samples collected. Pay attention to record 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.

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

E-waste (E) - Country ID (XX) - Company ID (XX) - Participant ID (XXX) - Sample ID (AX/BX/LCX/RCX/HX/SDX/UX/WX/WBX)

‘E’ is to denote that the samples and data relate to the e-waste occupational study. Country ID 'XX', Company ID ‘XX’ and Participant ID 'XXX' are as above.

Sample ID ‘AX/BX/LCX/…’ is one or two letters (A/B/LC/RC/H/SD/U/W/WB) to identify the type of sample collected, followed by an one-digit identifier (X) to identify the running number of each type of sample for that worker (e.g. 1 for the first sample, 2 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
Buccal cells	LC (left cheek) or RC (right cheek)
Hair	H
Settled dust	SD
Urine	U
Wipe	W
Wrist band	WB

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

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

E-PT-01-001-W1

E-PT-01-001-W2

- **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, containing the personal information, should now 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. Copy corresponding ISCO-08 code (with 4 digits) and text label from the link. The classification is available in English, German and French at the following URL:
  http://ec.europa.eu/eurostat/ramon/nomenclatures/index.cfm?TargetUrl=LST_NOM_DTL&StrNom=CL_ISCO08&StrLanguageCode=EN&IntPcKey=&StrLayoutCode=HIERARCHIC

  - **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 chromium, cadmium and lead. 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 3).

- **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 3).

- **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 researcer 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 caffinated 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 may result in exposure to metals and others substances that are going to be studied in the scope of this project. If the respondent indicates that they do have recreational activities that may cause additional exposure, details of what they do and the substances they use should be recorded.

- **COVID-19 Symptoms and diagnosis:** This information is collected since recent viral infection and vaccination will interfere in the inflammatory markers to be analysed.

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 lasting more than 12 months 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.

4. Job descriptions for different e-waste activities (to be completed for only the exposed workers)

Job descriptions in E-waste (interviewed by researcher during the sampling)

- Please select all type of work tasks the worker has been involved in today and not only the main task. Please consider that the information is divided into three different processes, namely E-waste recycling, hydrometallurgical processing and biometallurgical processing.
- Ask the requested contextual information:

    - Duration (hours/min) and frequency of the tasks (times per week)
    - Process type – record manual or automatic.
    - Risk management by personal protection equipment (PPE) and respiratory protective equipment (RPE). Show Flash Card 1 (see Appendix 1) 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 RPE (mask) has been fit tested. To assist the respondent fit testing can be explained as a method of checking that a tight-fitting face piece 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 impact on the level of exposure.

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.

5. Storing and reporting 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 contains 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 from 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. 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. Care must be taken to follow the instructions accompanying the data template. The template must NOT be modified in any way - DO NOT add / remove columns, or alter the drop down lists, or merge cells. In the event that the template is modified or data has been provided which does not follow the instructions, templates will be returned to the data provider for correction.

Both hard copy and electronic data should be archived in compliance with relevant national and European data protection legislation.

4 References

Porras S, Ladeira C, Ribeiro E, Viegas S, Uuksulainen S, Santonen T, Galea K, Cherrie J, Louro H, Ventura C, Silva MJ, Leese E, Jones K, Hanser O, Ndaw S, Robert A, Duca R-C, Poels K, Godderis L, Kiilunen M, Norppa H, Veijalainen H, Parshintsev E, Tuomi T, Ruggieri F, Alimonti A, Koch H, Bousoumah R, Antoine G, Jacoby N, Musgrove D. (2019) HBM4EU occupational biomonitoring study on hexavalent chromium and other harmful chemicals. Standard Operating Procedures (SOPs). WP8 - Targeted field work surveys and alignment at EU level. Available from URL: https://www.hbm4eu.eu/online-library/ (last accessed 22nd February 2021).

Appendix 1: Flash Card 1: PPE and RPE used for e-waste

PPE (Personal protective equipment) worn:

1. Re-usable working clothes (coveralls)
2. Disposable working clothes (protection suit)
3. Working shoes / boots
4. Arm guards
5. Cut resistant gloves
6. Reusable gloves
7. Disposable gloves
8. Safety glasses/goggles
9. Face protection
10. Other (please specify)

RPE (respiratory protective equipment) worn:

1. Reusable particulate respirator (please specify how often the filters are changed)
2. Disposable particulate respirator
3. Other respiratory protection equipment (please specify: e.g. vapors protection besides particles)

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Annex 3

SOP 3:

Standard operating procedure (SOP) for blood sampling, including sample storage and transfer

Occupational E-waste study

WP8

Task 8.5

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1 Introduction

Within the Human Biomonitoring Initiative (HBM4EU project), several priority chemicals were identified, which may be of concern for the European population.

E-waste stream contains many composite materials such as circuit boards, cathode ray tubes, flat screen monitors, batteries, connectors and transformers, plastic casings, and cables. These materials render a broad range of hazardous ingredients, including toxic metals, polybrominated diphenyl ether (PBDE) and organophosphate ester (OPE) flame retardants, phthalates, polychlorinated biphenyls (PCBs), hexabromocyclododecanes (HBCDs), polychlorinated dibenzo-*p*-dioxins (PCDD), polybrominated dibenzo-*p*-dioxins (PBDD) and polychlorinated dibenzofurans (PCDF). Plastic materials may contain chemicals that were legal at the time they were manufactured but are now either restricted or banned, such as lead, PCBs, some phthalates, and flame retardants (Grant et al. 2013). Occupational exposure to a mixture of such pollutants during e-waste handling and recycling is a matter of concern at European workplaces.

In the context of the present occupational study, biomarkers of exposure to chromium (Cr), cadmium (Cd), mercury (Hg), lead (Pb), brominated and organophosphate flame retardants (BFRs and OFRs), polychlorinated biphenyls (PCBs) and phthalates will be analysed in biological specimens of workers involved in different steps in the chain of waste processing. Most of these chemicals can damage DNA, resulting in genome instability, which, in turn, is a crucial event in cell transformation towards malignancy. According to the International Agency for Research on Cancer (IARC) classification, Cr(VI), Cd and PCBs are carcinogenic to humans, while Pb is classified as probably carcinogenic (Bakhiyi et al. 2018). Although the mechanisms underlying the carcinogenetic effect are still unclear for some of these substances (and mixtures), indirect genotoxicity due to oxidative damage to nucleobases, induction of membrane lipid peroxidation, DNA methylation, and dysfunction of DNA repair have been shown (Wang et al. 2018). Accordingly, a significant relationship between the duration of exposure to e-waste and DNA damage in lymphocytes and spermatozoa among recycling e-waste workers was recently reported (Wang et al. 2018). Based on this knowledge, the analysis of biomarkers of early biological effects (effect biomarkers) was also included in this study.

To summarise, blood will be the biological specimen used to analyse the following biomarkers: i) Cr, Cd, Pb, PCBs, and BFRs (exposure biomarkers), and ii) micronuclei (MN) in peripheral blood lymphocytes (PBLs) and reticulocytes, epigenetic markers, oxidative stress, telomere length and inflammation markers (effect biomarkers).

This 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 e-waste occupational study under HBM4EU and is based on a SOP developed within a previous occupational study (Santonen et al., 2019).

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.

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:

Metal atoms can form ionic, covalent, and coordinate bonds. Ligands containing oxygen, nitrogen, or sulfur are preferentially bonded. Consequently, many important biological compounds, such as proteins and nucleic acids, are targets for an interaction with metals. If the ligands are organic molecules with more than one group capable of coordination, metals can form stable complexes known as chelates (examples of chelating agents: BAL, EDTA, DMPS)1 (Greim and Snyder 2008). These properties must be taken into account when choosing the materials used for sample collection and analysis. Moreover, workers can be exposed to common e-waste chemicals through other sources besides occupational ones, e.g., food (Cd, BFRs and PCBs), and water (Cr, Cd, and Pb) (Grant et al. 2013). Furthermore, some of the chemicals, particularly, Cr and Cd, can also be inhaled from tobacco smoke. Therefore, apart from the exposure in occupational settings, also the exposure of workers through the referred sources should be assessed by the questionnaire.

Samples identification will be approached in more detailed below (see 3.4).

b) Interfering factors

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 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 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;
- For Cr, Pb and Cd analyses, appropriate tubes “for trace element” detection, “metal free” or “for lead testing” should be used to collect blood in order to eliminate any possible metals contamination (CDC 2018);
- EDTA anticoagulant is preferable (CDC, 2018);
- 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.

[1] BAL : British Anti-Lewisite, EDTA : Éthylenediaminetetraacetique acid, DMPS : 2,3-dimercapto-1-propanesulphonic acid

3 Blood Sampling

3.1 Blood collection schedule

One blood sample will be collected from each (exposed or non-exposed) worker, following signed informed consent of the participant (see SOP 1: Selection of participants and recruitment, information to the participants, informed consent).

The optimum timing for the sampling would be on the 3rd - 5th day of a working week (assuming a 5-day working week). In addition, because samples have to be processed within 24 h for Cr determination in RBC or to be shipped to the laboratories that will perform the biomarkers analyses, **blood collections should be done between Wednesday and 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 in advance 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: procedure for completion of company and worker questionnaires].

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).

Note: A **Material Transfer Agreement** has to be previously signed between the laboratories that will exchange blood samples for analyses.

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 5** - with sodium heparin (volume: 3 mL per tube) for cytogenetic effect biomarkers (micronuclei);
  - 1 Tube – **tube 2** – with K_2 EDTA (volume: 3 mL) for DNA-based effect biomarkers (epigenetics, telomere length); appropriate for -80ºC (e.g., cryotubes Bio-one);
  - 2 Tube - **tubes 3 and 4** - with K_2 EDTA (volume: 3 mL in tube 3 and 6 mL in tube 4). Tube 3 will be used for analysis of Cd and Pb in total blood while tube 4 will be centrifuged to separate plasma and red blood cells (RBC) for BFRs and PCBs measurement in plasma, inflammation markers in plasma and Cr in RBC (RBC-Cr, only for workers with high U-Cr) (see details in section 4.1). Tubes for trace elements should be used to minimise the background contamination, e.g. *Greiner* Vacuette® Trace Elements, 3 ml or BD Vacutainer® Trace Element tubes (royal blue stopper)

- Vials for plasma and RBC storage must be suitable for trace elements (metal free) (e.g., ICP-MS autosampler tubes) or pre-treated with HNO3 [see 2.b)]; vials for plasma that will be used for BFRs and PCBs determination should be first rinsed with hexane [see 4.1)]
- 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 venepuncture
- Bench centrifuge, refrigerated
- Pipettes for collecting the plasma and buffy coat
- NaCl solution (0.9%)
- Refrigerator for samples storage at +4ºC
- Dry ice
- Freezer samples for storage at -80°C
- Containers appropriate for blood samples shipment (at + 4ºC and -80ºC)

3.3 Instructions for blood sampling

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.

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 5 tubes and label them with the code number and other relevant information (date, time of collection)
4. Record relevant details in the record form (Annex 1 and Annex 2, 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 from the syringe into the 5 labelled tubes, filling them to the mark to avoid the risk of haemolysis. **Tubes 3 and 4 should be the 1st tubes 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.

		Tube 1	Tube 2	Tube 3*	Tube 4*	Tube 5	Volume of Blood collected
		Na heparin	K_2 EDTA	K_2 EDTA	K_2 EDTA	Na heparin	
Use	**Fraction/ volume**	**3 mL**	**3 mL**	**3 mL**	**6 mL**	**3 mL**	
B-Pb and B-Cd	Whole blood	0	0	**X**	0	0	
PCBs and BFRs	Plasma	0	0	**0**	**X**	0	
RBC-Cr**	RBC	0	0	0	**X**	0	**18 mL**
Micronucleus in PBL	Whole blood	**X**	0	0	0	0	
Micronucleus in RET	Reticulocytes	0	0	0	**0**	**X**	
Oxidative stress	Whole blood	0	**X**	0	0	0	
Epigenetics	Whole blood	0	**X**	0	0	0	
Inflammatory markers		0	0	0	**X**	0	

**__Important:__ Tubes 3 and 4 must be appropriate for trace elements analysis and must be the 1st to be filled; ** store the RBC samples and analyse (or send to the analysing laboratory) only those which had elevated U-Cr (limit value to be defined)*

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

3.4 Sample traceability

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

E-waste (E) - Country ID (XX) - Company ID (XX) - Participant ID (XXX) - Sample ID (AX/BX/LCX/RCX/HX/SDX/UX/WX/WBX)

'E' is to denote that the samples and data relate to the e-waste occupational study.

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).

Country	ISO Alpha-2 country codes
Belgium	BE
Finland	FI
Germany	DE
Latvia	LV
Luxembourg	LU
Poland	PL
Portugal	PT
The Netherlands	NL
United Kingdom	UK

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

Participant ID 'XXX' is a three-digit running number of participants in each country (e.g. 001 for the first participant recruited, 002 the second and so forth).

Sample ID 'BX' where B denotes the type of sample collected (blood), followed by an one-digit identifier (X) to identify the running number of each type of sample for that worker (e.g. 1 for the first sample, 2 for the second and so forth).

The following scenario is provided to illustrate the application of this convention. A worker is recruited in Portugal. He is working in the first company recruited. He is the first worker recruited in that company and is providing a first blood sample. The sample identification code assigned is therefore E-PT-01-001-B1

4 Conservation, transport, and storage of the samples

4.1 Processing and onsite storage of collected blood samples

All tubes should be transported from the site of collection to the nearest laboratory (local laboratory in the country of origin), as soon as possible (preferentially less than 2h after sampling), for further processing and/or expedition to the different partner laboratories involved in biomarkers' analyses.

The above referred transportation should occur at +4ºC (max +10°C) using frozen ice packs placed at the bottom and along the sides of the styrofoam box, but making sure that the samples do not freeze (Annex 2, fig. A.4).Tube **2** can equally be transported at +4 ºC and immediately frozen at –20ºC or -80ºC after arrival to the local laboratory or can be immediately frozen at -80ºC at the site of collection (and transported at that temperature). Please note that tube 2 must be compatible with -80ºC storage (e.g., cryotubes Bio-one).

For onsite storage in the local laboratory until shipment the following procedure must be followed immediately after arrival:

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

- **Tube 1** - keep at room temperature protected from light (wrapped in aluminium foil) until shipment to INSA (Portugal).

- **Tube 2** - keep frozen at -80°C until shipment to KuLeuven with dry ice or until DNA extraction and DNA shipment to KuLeuven.

- **Tube 3** – keep at +4ºC until expedition to the analytical laboratory for analysis of Pb and Cd.

- **Tube 4** - will be used for plasma and RBC separation preferably within 8 h of the specimen collection, maximum 24 h, to avoid haemolysis. The minimum volume of plasma needed for PCBs and BFRs analyses is 2 mL. The remaining shall be criopreserved at – 80°C to analyse inflammation markers. Separation can be done as described by Devoy et al. (2016) to allow the use of RCB for Cr determination. 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. Separate 2 mL of the supernatant (plasma 1) in a PP tube (beforehand washed with hexane and rinsed with purified water) and the remaining volume (plasma 2) in another PP tube; care should be taken to avoid collection of RBC;
    4. Dilute the RBC pellet with NaCl solution (0.9%) up to the initial volume Vi;
    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[2];

[2] The HT2 value 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.

9. After removing the last washing phase, fill the tube containing RBCs with 1% Triton X-100 in deionised water/0.2% HNO33 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.

- **Tube 5** - keep at +4 - 8 ºC (do not freeze) protected from light and send to FIOH (Finland).

4.2 Transportation of the samples to the analytical laboratories

As a general rule, samples should be shipped to the laboratory that will perform analyses as soon as possible. During transportation, the storage conditions recommended above should be maintained (see Annex 2, fig. A.4), as follows:

- **Tube 1** - Pack the whole blood samples at +4°C (max +10°C) while assuring that they do not freeze during transportation to INSA (Portugal). They must arrive within 1–4 days after sampling

- **Tube 2** - Pack the frozen whole blood samples with dry ice (-80°C) and ship them to KuLeuven (KuLeuven will then send whole blood to NIOM, Poland) or send isolated DNA at -20°C.

- **Tube 3** - Pack the whole blood samples at +4°C and ship them to the analytical laboratories, if different from the local laboratory

- **Tube 4** - Following plasma separation and RBC preparation (fractions can be refrigerated up to 3 days) – send RBC to the local laboratory for Cr-RBC measurement and ship the plasma 1 vial (minimum 2 mL) refrigerated (+4ºC) to University of Antwerp for PCBs and BFRs analyses; send plasma 2 at -80 ºC to KuLeuven for inflammation markers.

- **Tube 5** – Deliver the sample to the genotoxicology laboratory, FIOH within one week (preferably 1-4 days) after the sampling. 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 previously 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

[3] The % of HNO_3 must not exceed 0.2%, otherwise the sample coagulates. Prepare the solution (1L) with 10 mL of triton X-100 and 2 mL HNO_3 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_3 must be replaced by 1% Triton X-100/0.2% NH_4OH.

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.

The storage conditions described in the section 4.1. should be maintained until analysis, 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.

5 Data reporting

The data generated respects:

- The total number of workers or controls from whom blood samples were collected and the number stratified per occupational setting and per country.
- The total number of workers or controls that generated data on blood, plasma, or RBC exposure biomarkers
- the number of workers or controls that generated data on blood, plasma or RBC exposure biomarkers stratified per biomarker
- The total number of workers or controls that generated data on blood, plasma, or RBC effect biomarkers
- the number of workers or controls that generated data on blood, plasma or RBC effect biomarkers stratified per biomarker
- Values of haematocrits 1 and 2 that will be used in RBC-Cr determination.

The following information should be obtained from the laboratory undertaking the analysis which is to be entered into the data template:

- Biomarker concentration (µg/L)
  If concentration is below the limit of quantification (LOQ), the result is replaced by **<**LOQ (for example, **<0.23** if 0.23 is the LOQ). Data below LOQ should not be given as an empty cell, zero concentration or free text (i.e. <LOQ, not detected, n.d., LOQ/2) - please ensure that the **<** is used to identify the result as <LOQ.
- Analytical method used
- LOQ of the analytical method (µg/L)
- Method to calculate the LOQ (the laboratory should test that the reported LOQ concentration can be analysed accurately (RSD <20 %))
- Haematocrit 2 (HT2) values

Care must be taken to follow the instructions accompanying the data template. The template must NOT be modified in any way - DO NOT add / remove columns, or alter the drop down lists, or merge cells. In the event that the template is modified or data has been provided which does not follow the instructions, templates will be returned to the data provider for correction.

6 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:

◄◄

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 -

_____

2-

_____

3-

_____

4 -

_____

5 -

_____

Annex 2 – Schemes for blood collection and transportation

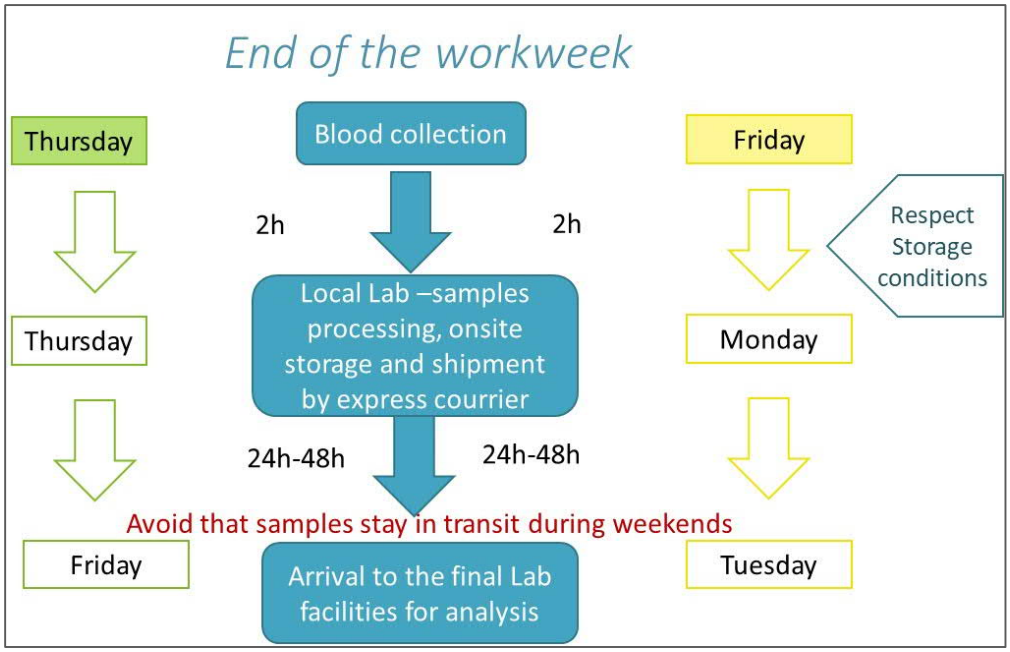
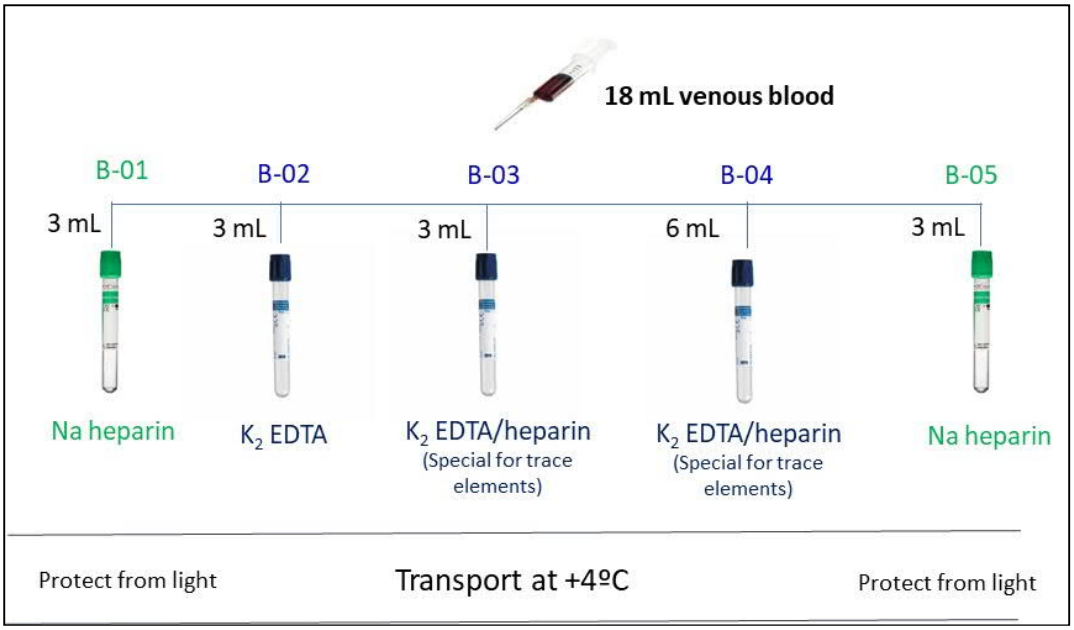


Fig. A.1 -Timing for blood collection: Wednesday or Friday afternoon

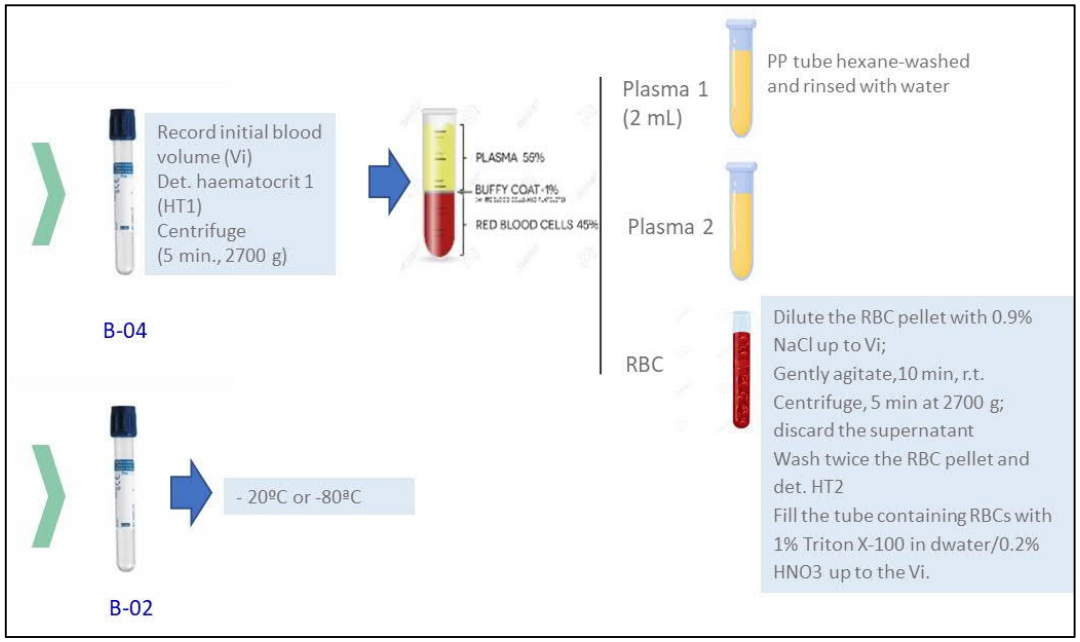
- ☐ *Informed consent* was understood and signed
- ☐ *Questionnaire was filled in and criteria for inclusion were met*
- ☐ *A code was attributed to the worker/control and to each sample*
- ☐ *The Blood Sampling Form was filled in (send to the receiver Lab)*
- ☐ *The Sample Transfer Protocol was filled in (send to the receiver Lab)*
- ☐ According to previous agreement between the samples' provider and the receiver Laboratory, the following biomarkers shall be analysed:
  - ☐ *Metals (prepare tube 3: 3mL)*
  - ☐ *BFRs, PCBs, inflammation and/or RBC-Cr (prepare tube 4: 6 mL)*
  - ☐ *Micronuclei in lymphocytes (prepare tube 1: 3 mL)*
  - ☐ *Micronuclei in reticulocytes (prepare tube 5: 3 mL)*
  - ☐ *Epigenetic markers or telomere lenght (prepare tube 2: 3 mL)*
- ☐ *Collect _____mL of blood*
- ☐ *A* transport box and appropriate containers are available for blood transfer

Fig. A.2 - Check list for blood collection

(A)


18 mL venous blood
B-01
B-02
B-03
B-04
B-05
3 mL
3 mL
3 mL
6 mL
3 mL
Na heparin
K2 EDTA
K2 EDTA/heparin
(Special for trace elements)
K2 EDTA/heparin
(Special for trace elements)
Na heparin
Protect from light
Transport at +4ºC
Protect from light

(B)


Record initial blood volume (Vi)
Det. haematocrit 1 (HT1)
Centrifuge
(5 min., 2700 g)
B-04
PLASMA 55%
BUFFY COAT <1%
RED BLOOD CELLS 45%
Plasma 1 (2 mL)
PP tube hexane-washed and rinsed with water
Plasma 2
RBC
Dilute the RBC pellet with 0.9% NaCl up to Vi;
Gently agitate,10 min, r.t.
Centrifuge, 5 min at 2700 g; discard the supernatant
Wash twice the RBC pellet and det. HT2
Fill the tube containing RBCs with 1% Triton X-100 in dwater/0.2% HNO3 up to the Vi.
- 20ºC or -80ºC
B-02

(C)

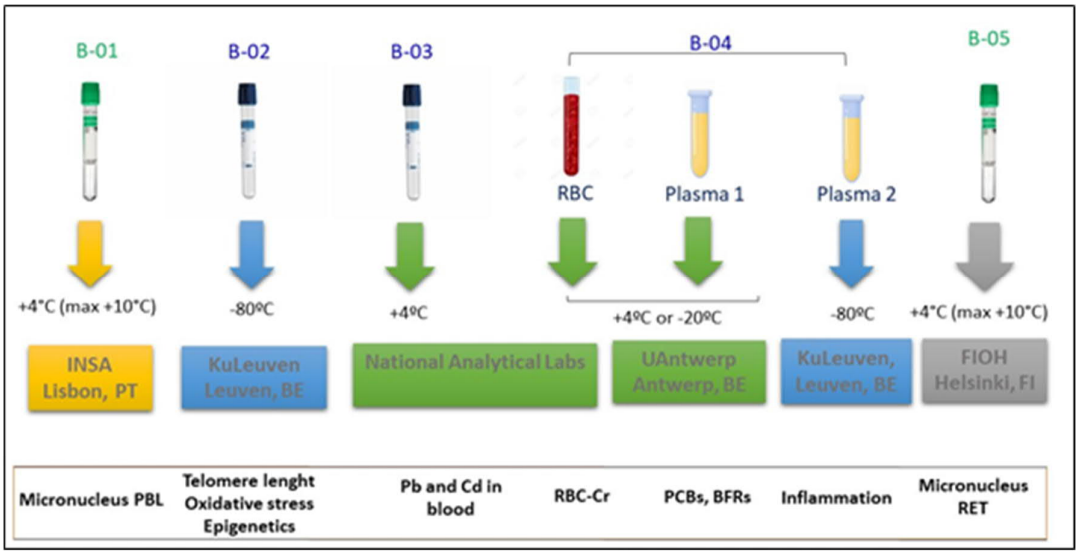


Fig. A.3 - Blood sampling, processing and transportation (A) from the collection site to the local laboratory, (B) onsite processing and (C) from the local laboratory to the laboratories where biomarkers will be analysed.

(A)

(B)

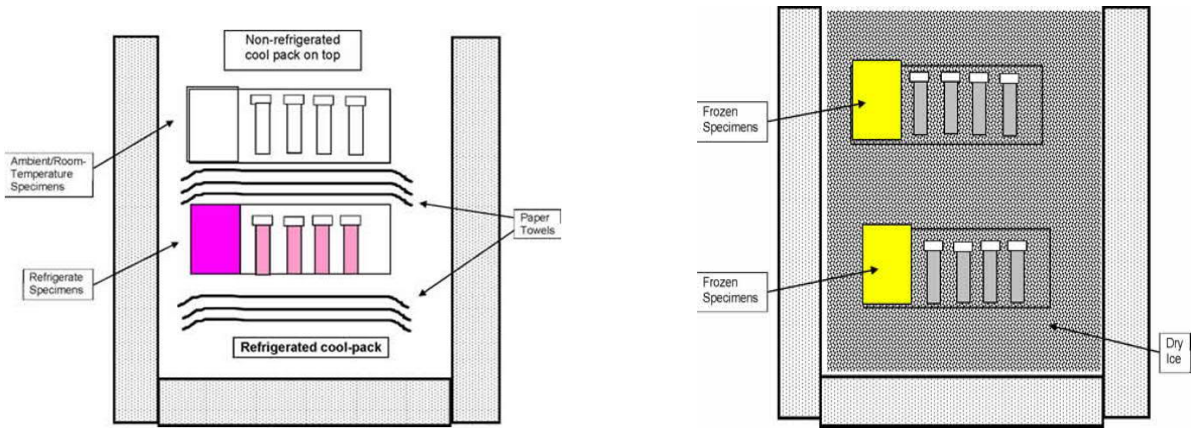
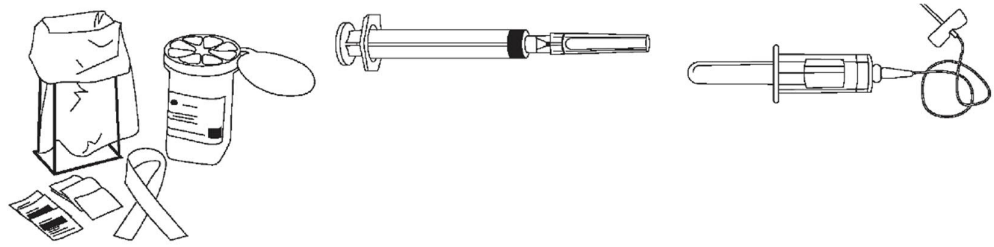
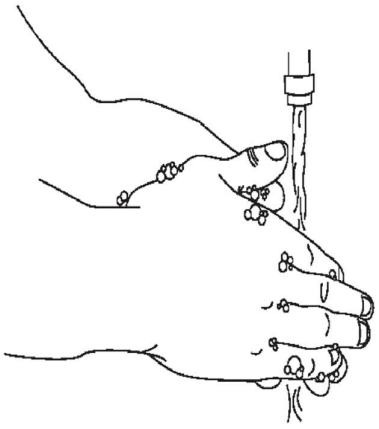


Fig. A.4 – Specimens integrity during transportation - Containers for transportation of samples (A) refrigerated and at room temperature and (B) for transportation of frozen samples (Source: Mayo Clinics)

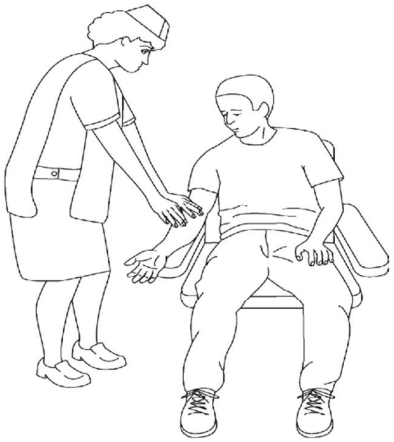
Annex 3 - An illustration of best practices in phlebotomy (WHO, 2010)



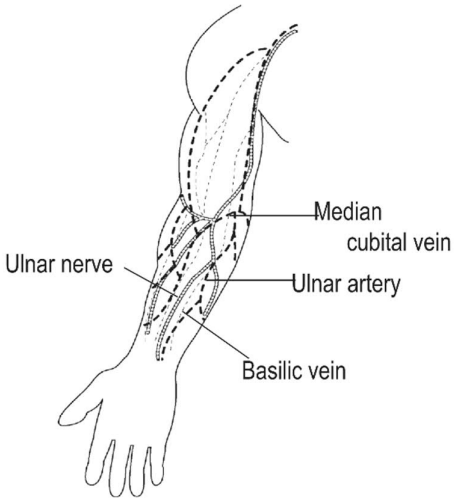
1. Assemble equipment and include needle and syringe or vacuum tube, depending on which is to be used.



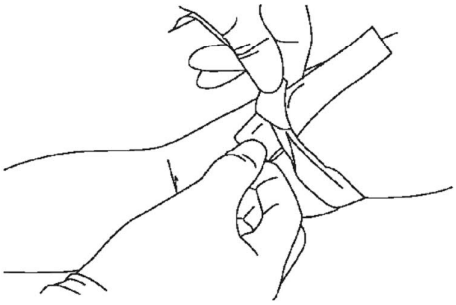
2. Perform hand hygiene (if using soap and water, dry hands with single-use towels).



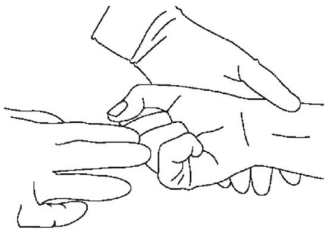
3. Identify and prepare the patient.



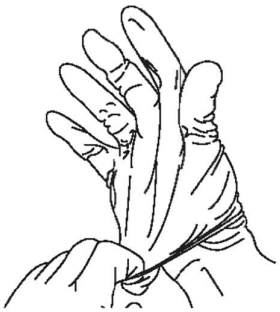
4. Select the site, preferably at the antecubital area (i.e. the bend of the elbow). Warming the arm with a hot pack, or hanging the hand down may make it easier to see the veins. Palpate the area to locate the anatomic landmarks. DO NOT touch the site once alcohol or other antiseptic has been applied.



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

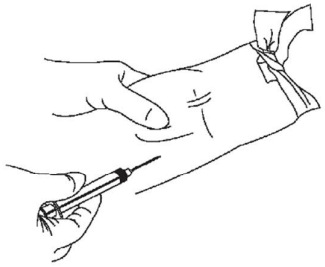


6. Ask the patient to form a fist so that the veins are more prominent.

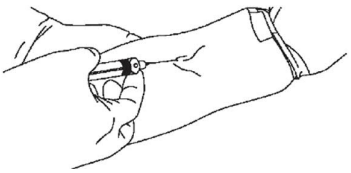


7. Put on well-fitting, non-sterile gloves.

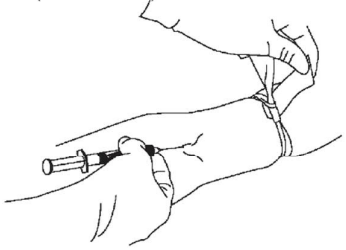
8. Disinfect the site using 70% isopropyl alcohol for 30 seconds and allow to dry completely (30 seconds).



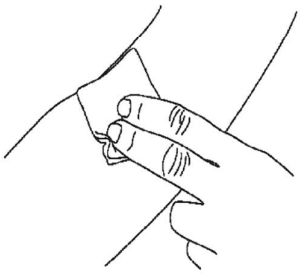
9. Anchor the vein by holding the patient's arm and placing a thumb BELOW the venepuncture site.



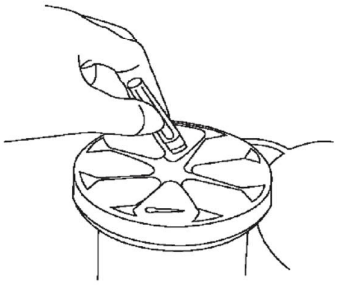
10. Enter the vein swiftly at a 30 degree angle.



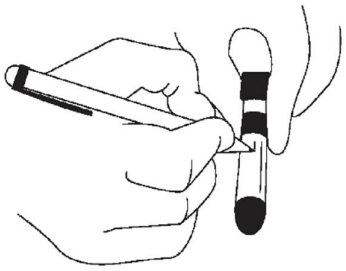
11. Once sufficient blood has been collected, release the tourniquet BEFORE withdrawing the needle.



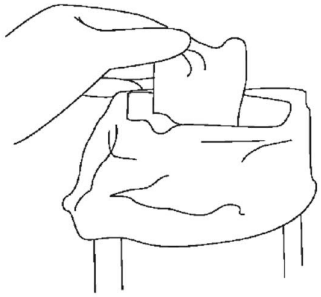
12. Withdraw the needle gently and then give the patient a clean gauze or dry cotton-wool ball to apply to the site with gentle pressure.



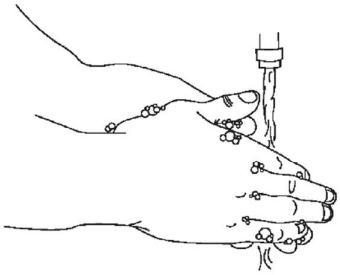
13. Discard the used needle and syringe or blood-sampling device into a puncture-resistant container.



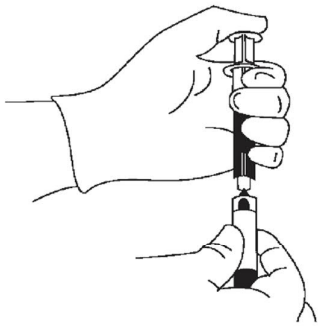
14. Check the label and forms for accuracy.



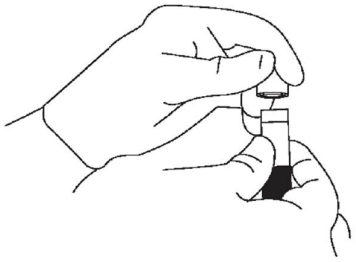
15. Discard sharps and broken glass into the sharps container. Place items that can drip blood or body fluids into the infectious waste.



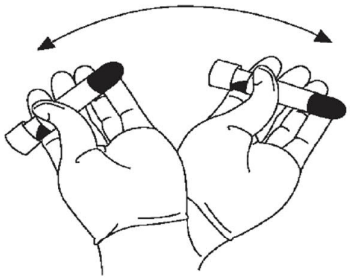
16. Remove gloves and place them in the general waste. Perform hand hygiene. If using soap and water, dry hands with single-use towels.



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.

HBM4EU science and policy for a healthy future

HORIZON2020 Programme
Contract No. 733032 HBM4EU

Annex 4

SOP 4:

Standard operating procedure for urine sampling, including sample storage and transfer

Occupational E-waste study

WP 8

Task 8.5

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1 Scope

This Standard Operating Procedure (SOP) is focused on the collection of urine samples for the e-waste study.

2 When should urine sample be collected?

Urine samples will be collected from participants during the same working week.

Two urine samples will be collected from exposed workers:

- (1) before the start of the first shift of the work week (for example pre-shift on day 1).

- (2) post-shift sample towards the end of the workweek (preferably day 4 or day 5).

Controls will collect one post-shift urine sample during the working week.

3 Material required for urine collection and storage

Below is a list with different materials to collect and store urine samples:

- Wide-mouth polypropylene bottle (100 ml or more) for urine collection
- 5 mL polyethylene storage tube
- Labels
- Nitrile or similar disposable gloves
- Biological hermetic bags or plastic bags with a zipper
- Pipettes and tips (3 mL)
- Modesty bags
- Tube containers
- Freezers
- Cool boxes
- Posters with the hand washing and sampling procedures (Appendix 1)
- Trash cans
- Urine sample record sheet (Appendix 2)

The research team should ensure that bottles, tubes and tips are free from background contamination by metals, flame retardants or phthalates.

4 Participants instruction

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 before urine sampling:

- Participants will be requested to remove their work clothes (overalls) before the urine collection;
- Participants will be asked to wash their hands thoroughly with soap and water; 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.
- That they will be required to include details of their name, the date and the hour of urine collection on the bottle label.
- That the urine sample must be immediately returned to the research team.

5 Researcher precautions

When handling each urine sample from the participants, researchers much always wear a new pair of disposable nitrile gloves.

Care should be taken to avoid cross contamination of samples. It is recommended that urine samples are handled in an area considered to be free of potential contamination e.g. office space, medical room etc. It is also important that researchers ensure that the sampling location itself does not become contaminated.

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.

6 Urine sampling procedure

The following procedures should be used to collect urine samples:

- Distribute labelled wide-mouth bottle to the participant, along with biological hermetic bags to place the bottle inside.
- Ask the participant to complete the label with the required information (name, date, hour).
- Advise the participant to remove their work clothes (overalls) and wash their hands with soap and water in accordance with the provided instructions (Appendix 1) before collecting their urine.
- Advise the participant to collect the sample of the void (does not have to be ‘midstream’ such as sometimes requested in hospitals)
- Advise the participant to screw firmly and to place the bottle in the biological and hermetic bag provided to avoid any leak after collecting its urine.
- The participants should return the urine sample to the research team straight away. They can use a modesty bag, left at disposal by the research team, for the transport.
- The researcher checks that the required label details are recorded and that these are correct and legible.

7 Urine samples processing and traceability

After collection, urine samples are homogenized and distributed in 7 aliquot tubes for analysis and storage:

- 3 aliquots of 4 mL of urine in a 5 mL tube, for
  - metals and creatinine analysis;
  - organophosphate flame retardants analysis;
  - phthalates analysis;
- 2 aliquots of 2 ml of urine in a 5 ml tube for metabolomic studies
- 2 aliquots of 4 mL of urine in a 5 mL tube, for storage.

To ensure that the aliquot tubes are properly labelled, a standardised convention will be used to assign unique identification codes for all samples collected. The identification code convention is as follows:

E-waste (E) - Country ID (XX) - Company ID (XX) - Participant ID (XXX) - Sample ID (AX/BX/LCX/RCX/HX/SDX/UX/WX/WBX)

'E' is to denote that the samples and data relate to the e-waste occupational study.

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).

Country	ISO Alpha-2 country codes
Belgium	BE
Finland	FI
Germany	DE
Latvia	LV
Luxembourg	LU
Poland	PL
Portugal	PT
The Netherlands	NL
United Kingdom	UK

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

Participant ID 'XXX' is a three-digit running number of participants in each country (e.g. 001 for the first participant recruited, 002 the second and so forth).

Sample ID 'UX' where U denotes the type of sample collected (urine), followed by an one-digit identifier (X) to identify the running number of each type of sample for that worker (e.g. 1 for the first sample, 2 for the second and so forth).

The following scenario is provided to illustrate the application of this convention. A worker is recruited in UK. He is working in the first company recruited. He is the first worker recruited in that company and is providing a first urine sample. The sample identification code assigned is therefore E-UK-01-001-U1

The urine sample record sheet should be completed by the researcher (Appendix 2). Following item are recorded:

- Sample code
- Date
- Hour
- Name of the participant
- Number of aliquot tubes for analysis and storage.

Used tips, used washing solution and urine collection containers are thrown in the trash can. The researchers collect all the used trash cans to dispose of them according to their standard practice.

Urine aliquot tubes are then stored on site in freezer at -20°C. If immediate urine processing on site is not possible, urine samples can be transferred 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 and processing should be scheduled for the same day.

8 Urine sample storage

Urine aliquot tubes are stored at -20°C in each participant laboratory until analysis or transfer to another laboratory for analysis.

9 Urine sample transfer

To ensure samples transportation at -20°C samples shall be immersed in dry ice using an adequate box.

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.

For the analysis of organophosphate flame-retardants biomarkers, samples will be transferred to IPASUM.
For the analysis of phthalates biomarkers, samples will be transferred to the University of Antwerpen.
For the analysis of effect biomarkers, sample will be transferred to NIOM.

10 Data reporting

The following information should be obtained from the laboratory undertaking the analysis which is to be entered into the data template:

- Urinary concentration of samples (µg/L)
    - If concentration is below the limit of quantification (LOQ), the result is replaced by **<**LOQ (for example, **<0.23** if 0.23 is the LOQ). Data below LOQ should not be given as an empty cell, zero concentration or free text (i.e. <LOQ, not detected, n.d., LOQ/2) - please ensure that the **<** is used to identify the result as <LOQ.
- Analytical method used
- LOQ of the analytical method (µg/L)
- Method to calculate the LOQ. The laboratory should test that the reported LOQ concentration can be analysed accurately (RSD < 20%).
- Creatinine concentration (g/L) of each urine sample
- Method to determine creatinine and LOQ (g/L) of the method

Care must be taken to follow the instructions accompanying the data template. The template must NOT be modified in any way - DO NOT add / remove columns, or alter the drop-down lists, or merge cells. If the template is modified, or data has been provided which does not follow the instructions, templates will be returned to the data provider for correction.

Below is the list of the biomarkers to be analysed in urine samples.

Chemicals	Biomarkers	Comments
Metals	chromium	
	cadmium	
	mercury	
	lead	
Organophosphate flame retardants	DPHP (diphenyl hydrogen phosphate) BCIPP (bis(2-choropropyl) phosphate) BDCIPP (bis(1,3-dichoro- -propyl) phosphate) BCEP (bis(2-choroethyl) phosphate)	
Phthalates	Monoethyl phthalate (MEP) Monobenzl phthalate (MBzP) Monoisobutyl phthalate (MiBP) Mono-n-butyl phthalate (MnBP) Mono(2-ethylhexyl) phthalate (MEHP) Mono(2-ethyl-5-hydoxyhexyl) phthalate (5OH-MEHP) Mono(2-ethyl-5-oxo-hexyl) phthalate (5oxo-MEHP) Mono(2-ethyl-5-oxo-hexyl) phthalate (5cx-MEPP) Mono-hydroxy-isononyl phthalate (OH-MiNP) Mono-carboxy-isononyl phthalate (cx-MiNP) Mono-hydroxyl-isodecyl phthalate (OH-MiDP) Mono-carboxy-isodecyl phthalate (cx-MiDP) cyclohexane-1,2-dicarboxylic mono hydroxyisononyl ester (OH-MINCH) cyclohexane-1,2-diarboxylic mono carboxyisononyl ester (cx-MINCH)	
Metabolomics in urine / effect markers		To be completed by NIOM

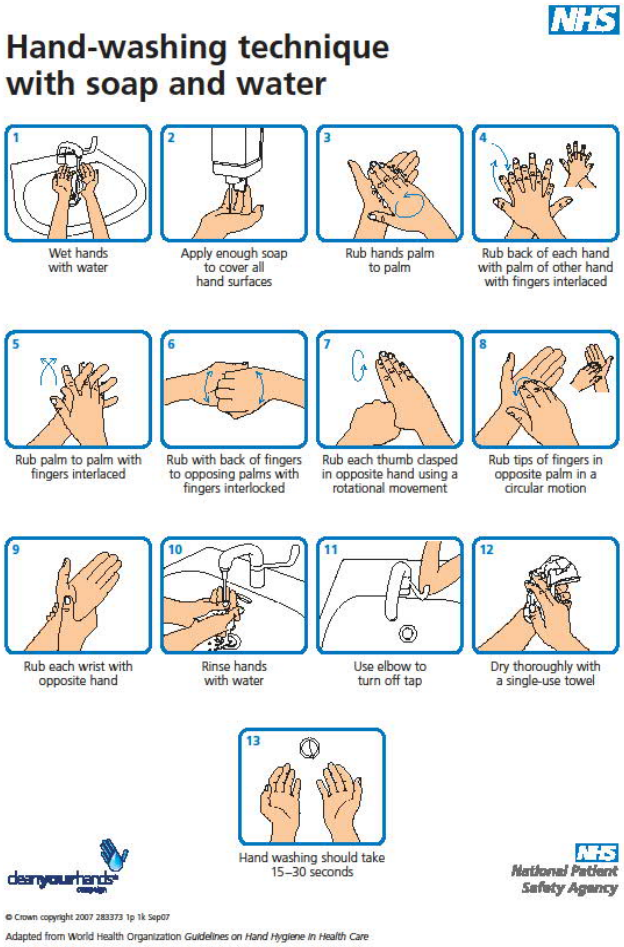
Appendix 1: Urine sampling procedure

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



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

Appendix 2: sample record sheet

Company Name:	
Company ID:	

Researcher(s):	
Research Organisation:	

Sample ID	**Date**	**Hour**	**Name**	**Observations**	**Aliquots collected for further analysis / storage**				
					1 x 4 mL metals/creatinine	1 x 4mL Flame retardants	1 x 4mL phthalates	1 x 4mL effect markers	2 x 4mL preservation

HORIZON2020 Programme
Contract No. 733032 HBM4EU

Annex 5

SOP 5:

Standard operating procedure for obtaining settled dust samples

Occupational E-waste study

WP 8

Task 8.5

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1 Authors and Acknowledgements

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2 Introduction

This Standard Operating Procedure (SOP) is focussed on the collection of settled dust samples to measure several HBM4EU priority compounds, including metals (lead, inorganic mercury, cadmium, chromium), phthalates and flame retardants in the workplaces of volunteers participating in the workplace biomonitoring studies.

In the scope of this SOP, a settled dust sample is considered to be surface/soil dust adhering to floor surfaces, normally removable by vacuum cleaners.

The purpose of this SOP is to establish a uniform procedure for collecting settled dust samples in the workplaces and is based on internal SOPs employed by IOM to evaluate exposure to metals in the home environment (Loh et al., 2015).

The collection and assessment of these occupational hygiene samples will provide information on the level of contamination present in the workplaces and will allow a connection with the exposure found on workers through the use of human biomonitoring (HBM) samples to be made. To sum up, the results will provide information concerning the role of the workplace environment on the workers' exposure to the substances being studied.

2 Summary of the method

Settled dust samples are collected by field researchers during workplace visits done to collect contextual information and/or other environmental and biological samples. Vacuumed dust samples will be analyzed for metals (lead, inorganic mercury, cadmium, chromium), phthalates and flame retardants (brominated and organophosphate). Samples are to be taken from the workplace where the participating workers spend the majority of their time. This is usually the location where workers perform most of their work tasks and activities. If more than one similar exposure group (SEG) is identified in a specific company, involving different tasks and waste, this might imply the need to collect a sample for more than one workplace/area. Therefore, depending on the participating workers, type of activities develop and risk management measures in place, it may imply collecting separate settled dust samples from more than one workplace area in the same company.

A minimum of 4 grams of coarse dust fraction per settled dust sample is required for analysis. The composite sample will be split and aliquoted in HBM4EU laboratories. One aliquot will be analyzed for metals and then other two aliquots will be distributed for specific HBM4EU laboratories to be analyzed for phthalates and flame retardants.

3. Materials required for the collection of settled dust samples

To undertake the collection of settled dust samples, researchers should ensure that they have the following:

1. Vacuum Supplies

- A Museum Vac (Figure 1)

- Dust collector - Filter bags (Figure 2).

- The field kit which contains:

  - ✓ Pre-weighed filter bags (that should be used once for each workplace/area)
  - ✓ Sample model already weighed with 4 grams
  - ✓ Field sheet (Annex 1) and custody records
  - ✓ A 4 m long chain or other suitable devices for measuring area for vacuuming
  - ✓ Indelible labelling pen
  - ✓ Clean and dry wipes
  - ✓ Thermometer and Hygrometer

2. Other Collection Supplies:

- ✓ Disposable nitrile gloves
- ✓ Timer/ stop watch
- ✓ Measure tape
- ✓ Masking tape
- ✓ Zip tie
- ✓ Ziploc bag

3. Cleaning supplies

- ✓ Reagent grade isopropanol and deionized water to clean the vacuum and accessories
- ✓ Laboratory glassware detergent
- ✓ Disposable nitrile gloves
- ✓ Sheet of clean plastic (garbage bag)/Plastic tray
- ✓ Lab brushes to clean the vacuum if needed
- ✓ Toothbrush
- ✓ Tweezers

- ✓ Wide mouth glass jar or stainless-steel bucket
- ✓ New, clean, large plastic garbage bags

Figure 1. Museum Vac

(https://www.universityproducts.com/museum-vac-vacuum-with-dial-suction-control.html)

Figure 2. HEPA Filter bags

4. Procedure for defining the sampling strategy and samples collection

4.1 Sampling strategy

- Samples should be collected near the end of a full shift in a workplace occupied by workers engaged in the biomonitoring campaign and are from a specific workplace area.
- At each workplace/area sampled relevant contextual information should also be collected, namely: tasks undertaken in that area, type of waste processed and number of workers that occupied that workplace and workplace cleaning procedures (e.g. when last cleaned, type of cleaning process). This should be recorded in the Field sampling sheet (Annex 1).

4.2 Samples collection

- Make sure the vacuum crevice head tool is cleaned before every sample collection. If the team member goes from one workplace to another workplace without returning to the laboratory, the crevice head tool must be cleaned in the field.
- Clean the crevice head tool using first isopropyl alcohol (IPA) and then dry with paper towels. Use the wipes to dry both the outer and accessible inner surfaces of the sampling apparatus and allow the vacuum inlet to air dry.
- Filter bags are prepared under controlled conditions in the laboratory prior to the company visit. Filter bags are pre-weighed. This information is recorded for the entire filter (with the pre-assigned sample ID number).
- Assembly of the vacuum is performed in the company.

The following collection procedure should be followed:

- Once in the workplace, put on a pair of disposable gloves.
- Insert the pre-weighed filter bag and make sure the filter is well adjusted.
- Place the 4 m long (1 m^2) chain on the floor and FORM A SQUARE with approximately 1 m long sides. Keep the perimeter and the chain taut.
- If there is insufficient room on the floor to form a 1m^2, fold the chain to serve as the perimeter of a 0.25 m^2 square and sample multiple locations in the available space in the room, or, lay out the chain in the shape of a rectangle. NOTE: This rectangle will not provide an area of 1 m^2, thus the sides of the rectangle must be recorded to compute the true area of the rectangle. Record the dimensions (length X width) of the sampled area under “comments” on the field sheet.
- Plug in the vacuum cleaner and check for proper operation (if not doing strange noises and if the vacuum is constant). Record the temperature and relative humidity before sampling.
- Start the timer/stop watch and the vacuum simultaneously.
- Vacuum the area within the chain thoroughly. Sweeping with vacuum must be slow and deliberate but plan to cover the entire 1 m^2 in 2 minutes. Thorough coverage is essential.
- Sweep across the 1 m^2 surface with firm even pressure once in the first minute. Then change your position and sweep across the same 1 m^2 (within the chain boundary) at an angle. Sampling at orthogonal/perpendicular directions is preferable; however, when not possible due to space constraints, diagonal sampling is acceptable.
- Repeat previous steps in the same area if more dust is needed to obtain 4 grams (e.g. by visual checking and comparing using a sample model already weighed in the lab).
- Ensure the vacuum crevice head is pointing up and turn off the vacuum cleaner and move to an area where the air current is minimal. Remove the head of the crevice head and estimate that at least 4 grams of dust have been collected and if not, sample additional area.

- If the yield is sufficient, remove the filter carefully and close the open end by rolling it down. Then secure the rolled end with a piece of scotch tape and place in the Ziploc bag.
- Place the sample in the sample container (already identified with the sample numbers) and seal it. Complete the Field Sheet and Chain-of-Custody Record after placing the sampler in the transport container.
- Change the filter and repeat the procedures for other workplace/area in the same company if needed.
- Transport the sample to the laboratory protected from the light.
- Keep the dust samples at room temperature (about 20-25ºC) until analysis.

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:

E-waste (E) - Country ID (XX) - Company ID (XX) - Sample ID (SDX).

'E' is to denote that the samples and data relate to the e-waste occupational study.

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).

Country	ISO Alpha-2 country codes
Belgium	BE
Finland	FI
Germany	GE
Latvia	LV
Luxembourg	LU
The Netherlands	NL
Poland	PL
Portugal	PT
United Kingdom	UK

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

Sample ID 'SDX' where SD denotes the type of sample collected (settled dust), followed by an one-digit identifier (X) to identify the running number of sample (e.g. 1 for the first sample, 2 for the second and so forth).

The following scenario is provided to illustrate the application of this convention. A settled dust sample is collected in Portugal. It is the first sample taken in the first company recruited. The sample identification code assigned is therefore E-PT-01-SD1.

The unique sample identification code will be clearly stated on the labelled self-seal bag. A Field sheet (Annex 1) should be completed when collecting the settled dust samples.

6. Storage of collected settled dust samples

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

7. Quality control

To check for contamination during the sampling procedure, transportation and storage, field blanks should be collected for each sampling survey. In this case the Filter Packet will be taken to the workplace sampling site but it will remain sealed in the Ziploc freezer bag, and experience the same transportation conditions as other valid samples both pre- and post-sample collection.

These samples should be treated in the same way as the exposure samples, using the same procedures as previously described, but omitting the vacuum procedure. 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 samples.

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

8. Transportation of settled dust 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 can be transported at room temperature 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 samples are analysed as soon as possible by the receiving laboratory.

9. Reporting

It is important that information entered into the sample record sheet (Appendix 1) is recorded as per the instructions given in this SOP as this information will need to be entered into the data template for the overall e-waste study.

Care must be taken to follow the instructions accompanying the data template. The structure, drop down lists etc of the data template must NOT be modified in any way. In the event that the template is modified or data has been provided which does not follow the instructions, templates will be returned to the data provider for correction.

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Field sheet (Annex 1)

Company Name:		
Company ID:		
Workplace ID:		
Job title/SEG:		
Cleaning procedures	**Mode of cleaning** Vacuum Water based Sweeping with a slot Other (specify) _____ _____ _____ _____	**Date/hour of last cleaning:** Date: ___/___/___ Hour:_____ : _____

Researcher(s):	
Organisation:	
Date sampling:	

Sample ID	Filter Weight	Collection duration (00:00)	Area sampled	Temperature	Relative humidity	Tasks undertaken in the workplace sampled	Type of waste processed

HORIZON2020 Programme
Contract No. 733032 HBM4EU

Annex 6

SOP 6:

Standard operating procedure (SOP) for air sampling of inhalable and respirable dust fraction

Occupational E-waste study

WP8

Task 8.5

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Authors and Acknowledgements

The contents of the current SOP draw heavily from “SOP6: Standard operating procedure for air sampling of inhalable and respirable dust fraction and (hexavalent) chromium) developed for the HBM4EU occupational biomonitoring study on hexavalent chromium and other harmful chemicals (Porras et al., 2019). As such the contributors listed here as those that contributed to this original SOP.

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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 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). 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 though 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 (cassette in the instance of the IOM sampler), the filter (cassette in the case of the IOM sampler) is re-weighed and the concentration of particulate matter is calculated from the mass difference and the sampled air volume. The metals (i.e. Cr, Cd, Hg, Pb) determination can be performed on this same filter, depending on the agreed analytical laboratory.

Depending on the agreements made with the project team and the analytical laboratories assigned to the air sample analysis, the following methods can be used for further metals analysis:

- OSHA Method ID-125G ‘Metal and metalloid particulates in workplace atmospheres (ICP analysis)’ (OSHA, 2002)
- NIOSH Method 7302 'ELEMENTS by ICP (Microwave Digestion)' (NIOSH, 2014)

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 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.
- Higgins-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
- MCE (mixed cellulose ester) filter, diameter 25 mm, pore size 0.8 µm (SKC-filter SKC 225-1930) should be used or alternatively PVC membrane filter, GLA-5000, Pall 66466, diameter 25 mm, pore size 0.8 µm, 100/package (VWR 514-0466).
- 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 Higgins-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). If other samplers are used, researchers also need to check that the pumps are capable at running at the required sampler flow rates for a period of up to 8 hours.
- 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)

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.
- 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 plastic IOM cassettes and cyclone cassettes are used these should be treated as disposables (single use only), in order to avoid any possible contamination.
- IOM and cyclone samplers 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 Sampler Instructions', 'IOM O-Ring Fitting Instructions', 'Cyclone Samplers for Respirable Dust-Operating Instructions')
- Tightness of IOM-samplers should be tested secured
- 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. The flow rates of the pumps should be checked during this period.
- Spare pumps, sampling heads and cassettes must always be provided so that planned samplings are not compromised.

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.

Setting up sampling equipment

- Ensure that IOM and cyclone 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

IOM-sampler

- Remove IOM cassette (this will have been pre-weighed with the loaded 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).

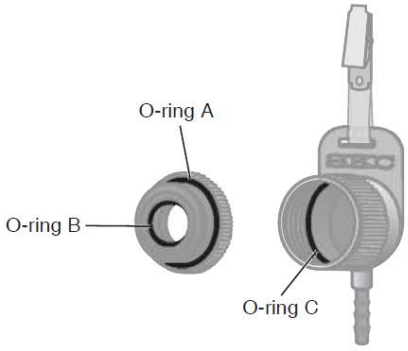

Figure 1. O-ring placement for plastic IOM sampler

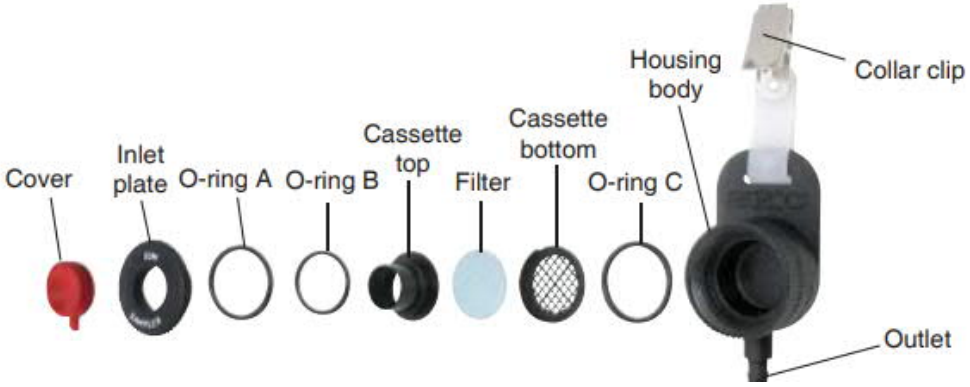

Figure 2. Configuration for plastic IOM sampler and cassette

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 as way of example but please refer to the manufactures instructions of the cyclone selected for use by your institution).
- 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

- 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.
- The 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)
- Set the flow rate within ± 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
    - In the event that other samplers are being used, check the manufacturer's instructions to ensure that the correct flow rates are being used.
- 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 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).
- Make sure the opening of the IOM cassette is not directed upwards
- Cyclone sampler should be attached with the grit pot pointing downwards
- When ready to begin sampling, remove protective cap from the IOM 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, being adjusted as necessary.

Figure 6. IOM sampler and pump on worker

Figure 7. Cyclone sampler and pump 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.
- 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 example.

  08:30 IOM sampler placed on worker and started, flow rate = 2000 ml/min
  09:45 flow rate check rate, flow = 1900 ml/min (flow rate reset to 2000 ml/min)
  12:00 Sample off and stopped for lunch (flow rate = 2000 ml/min)
  12:45 lunch break over, sample back on and restarted (flow rate = 2000ml/min)
  13:45 End of sample, sampler stopped (flow rate = 1950ml/min)

  Sample period 1; 08:30 – 09:45 (75 mins); average flow rate=(2000+1900)/2 = 1950
  Sample period 2; 09:45 – 12:00 (135 mins); average flow rate=(2000+2000)/2 = 2000
  Sample period 3; 12:45 – 13:45 (60 mins); average flow rate=(2000+1950)/2 = 1975

  Total sample volume = (75 x 1950) + (135 x 2000) + (60 x 1975) = 534750 ml = 534.75 litres

- 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).

8hr TWA for the workers will be calculated at the data analysis stage, using the information provided in the sample record form. It is therefore important that this is fully completed.

Cleaning the IOM and Cyclone Sampler

- Disassemble the IOM and Cyclone Sampler.
- Place parts in an ultrasonic cleaner with water and a wetting agent such as a mild soap. IOM and Cyclone components may also be cleaned with a solvent such as isopropyl alcohol. O-rings should be cleaned separately using water only.
- Clean the components using a clean lint-free paper, cloth, or soft brush. Allow components to dry completely.

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:

E-waste (E) - Country ID (XX) - Company ID (XX) - Participant ID (XXX) - Sample ID (AX/BX/LCX/RCX/HX/SDX/UX/WX/WBX)

'E' is to denote that the samples and data relate to the e-waste occupational study.

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).

Country	ISO Alpha-2 country codes
Belgium	BE
Finland	FI
Germany	DE
Latvia	LV
Luxembourg	LU
Poland	PL
Portugal	PT
The Netherlands	NL
United Kingdom	UK

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

Participant ID 'XXX' is a three-digit running number of participants in each country (e.g. 001 for the first participant recruited, 002 the second and so forth).

Sample ID ‘AX’ where A denotes the type of sample collected (air), followed by an one-digit identifier (X) to identify the running number of each type of sample for that worker (e.g. 1 for the first sample, 2 for the second and so forth).

The following scenario is provided to illustrate the application of this convention. A worker is recruited in Luxembourg. He is working in the first company recruited. He is the first worker recruited in that company and is providing a first air sample. The sample identification code assigned is therefore E-LU-01-001-A1

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.

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.

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.

9 Data reporting

It is important that information entered into the sample record sheet (Appendix 1) is recorded as per the instructions given in this SOP as this information will need to be entered into the data template for the overall e-waste study.

With respect to the air samples results, the following information should be obtained from the laboratory undertaking the analysis which is to be entered into the template:

- Air concentration (this must be in µg)
    - If concentration is below the limit of quantification (LOQ), the result is replaced by **<**LOQ (for example, <0.23 if 0.23 is the LOQ). Data below LOQ should not be given as an empty cell, zero concentration or free text (i.e. <LOQ, not detected, n.d., LOQ/2)
      - please ensure that the **<** is used to identify the result as <LOQ.
- Air volume (this must be in m^3)
- Analytical method used
- LOQ of the analytical method (this must be in µg)
- Method to calculate the LOQ (the laboratory should test that the reported LOQ concentration can be analysed accurately (RSD <20 %))

Care must be taken to follow the instructions accompanying the data template. The template must NOT be modified in any way - DO NOT add / remove columns, or alter the drop down lists, or merge cells. In the event that the template is modified or data has been provided which does not follow the instructions, templates will be returned to the data provider for correction.

10 References

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Appendix 1: Sample record sheet

Identification code:		**Company Name:**	
Sampling date:		**Aerosol fraction:**	Inhalable / respirable
Sampling head used:		**Filter media used:**	
Shipping date:		**Company ID:**	
Researcher:		**Participant Name:**	
Organisation:		**Participant ID:**	
Duration of worker shift (mins)		**Were samples left on and running during worker breaks? If so, what was the duration of this sampled break time**	Yes / No
Duration of worker breaks during shift (mins)			..... mins

Pump ID	**Start time (00:00)**	**End time (00:00)**	**Sampling duration (min)**	**Flow rate (L/min) *before***	**Flow rate (L/min) *after***	**Average flow rate (L/min)**	**Air volume (L)**	**Location of the sampler (left or right side)**	**Activity**
Period 1									
Period 2 (if required)									
		Overall sample duration:			**Overall average flow rate:**				

HBM4EU science and policy for a healthy future

HORIZON2020 Programme
Contract No. 733032 HBM4EU

Annex 7

SOP 7:

Standard operating procedure for obtaining hair samples

Occupational E-waste study

WP8

Task 8.5

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1 Introduction

The purpose of this Standard Operating Procedure (SOP) is to establish a standardised procedure for collecting head hair samples for the assessment of exposure in the e-waste workplace biomonitoring study.

The collection and analysis of this biological matrix will provide information on the level of occupational exposure to metals (and some organic compounds) for e-waste workers. Indeed, hair analysis has become a very useful tool for human biomonitoring [1, 2, 3] since this matrix reflects the level of exposure during a wider time window than urine or blood [4]. Hair analysis offers the potential for evaluation of chronic exposure during several months (depending on the length of the hair strand analysed), while blood and urine represents only recent exposure (from a few hours to several days). Since the hair growth is considered to be 1 cm/month [4,5], segmentation of the hair sample could also provide a detailed history of the exposure for the last months before sampling [4].

This SOP is based on the SOP for scalp hair sampling already employed for the COPHES project [6] and the Society of Hair Testing (SoHT) guidelines for drug testing in hair [4].

The hair samples collected are to be analysed for metals by the Laboratoire National de Santé (LNS, Luxembourg) to investigate the suitability of hair matrix in occupational health studies. Depending on the amount of sample collected and on the feasibility, the University of Antwerpen (UA) may also determine some organic compounds such as brominated flame retardants and PCBs.

2 Materials required for the collection of hair samples

To ensure a uniform collection of hair samples for the whole study, researchers should ensure that they have the following materials:

- Powder-free disposable nitrile gloves
- Titanium scissors (at least with undamaged titanium blade coating)
- Alcohol solution prepared using 70% of isopropanol and 30% of deionised water (v/v)
- Paper tissues (or cotton)
- Ruler or measuring tape
- Hair clip
- Kitchen twine or surgical rope
- Permanent marker
- Small paper envelopes (90x140 mm) and cardboard sheets (105x218 mm)
- Sample labels
- Ziplock plastic bags
- Sample record sheets (Appendix 1): one form per sample
- Pens
- A couple of mirrors

3 Information to communicate to the participants

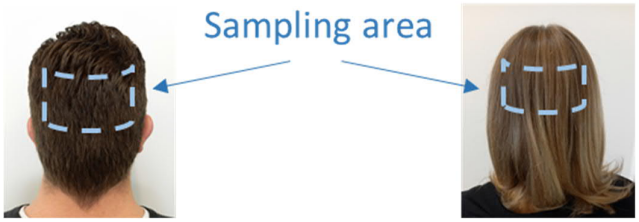
Participants should be fully informed of the sampling procedure. They will be advised that the sampling has to be done before the workshift and preferably before the workweek in order to limit external contamination of the hair shaft. They will also be informed in advance that one or a few strands of hair will be collected by cutting as close to the scalp as possible.

4 Researcher precautions

When collecting each hair sample from the participants, researchers must always wear a new pair of powder-free disposable nitrile gloves.

To avoid cross-contamination between samples and to ensure each participant safety, the scissors and the ruler used for the samplings have to be cleaned/ disinfected with alcoholic solution before and after each sampling and the collection has to be done in an area considered to be free of potential contamination (as far as possible). Since chromium element has to be determined in the collected hair samples, direct contact of hair fibers with stainless steel should be avoided so the scissors material has to be carefully selected and checked (full titanium or undamaged titanium-coated blades).

Researchers should also ensure to collect a sufficient amount for the analysis, i.e. a mass of minimum 500 mg of hair (section diameter of the strand: 1 cm almost equivalent to a big pencil/marker thickness). To avoid “invasive” sampling, i.e. a more aesthetic aspect for the participants, it is possible to combine different small strands cut in the posterior vertex region (see picture below).



In the case of hair samples longer than 6 cm, it is also particularly important to keep the hair fibers aligned with the root end clearly identified and secured, as far as possible.

5 Sampling procedures

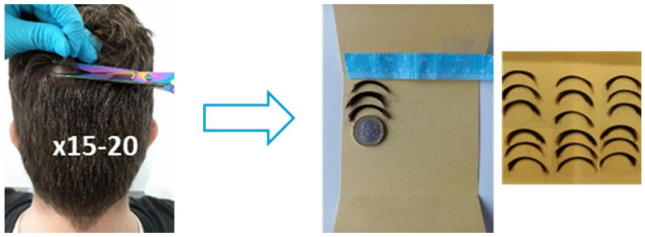
Participants must be seated in order to facilitate and secure the hair sampling.

Before each sampling, put on a pair of powder-free disposable nitrile gloves and clean/disinfect properly the scissors with a paper tissue moistened with alcohol solution. Measure the length of the hair of the back of the head using a ruler or a measuring tape cleaned/disinfected using the procedure previously mentioned.

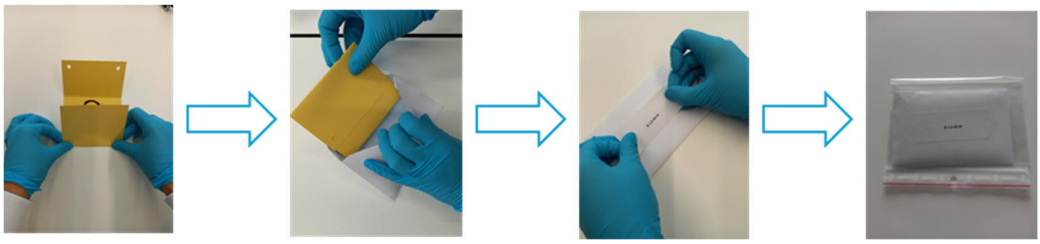
After that, two different procedures have to be applied depending on the hair length:

5.1. **Hair shorter than or equal to 6 cm (2.36 inch)**

a. Cut 15-20 strands of hair as close as possible to the scalp from different places on the middle of the back of the head and put them directly on the cardboard sheet. The minimum mass of sample required is 500 mg (equivalent to 18 strands like the ones presented on the picture (see below).



b. Fold the cardboard sheet in three and place it inside the paper envelope. Label the envelope with the sample identification code and place the envelope in a plastic bag (see pictures below).



5.2. **Hair longer than 6 cm (2.36 inch)**

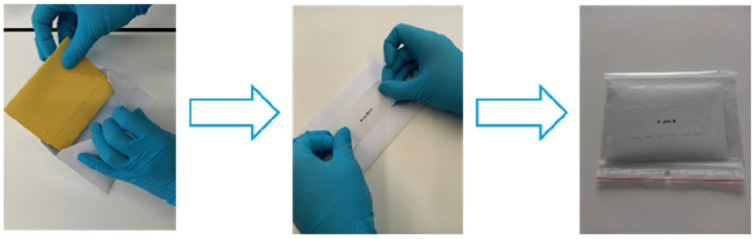
a. Grasp and lift the hair to access the middle of the back of the head. A hair clip may be helpful for this.

b. Select a lock of hair and fasten it with the string at 1-2 cm to the scalp. Cut it as close as possible to the scalp.

c. Place it on the carboard sheet and identify the root end on the sheet. Repeat until obtaining the desired amount of sample and add the strands on the sheet (for this size of lock, two strands are necessary).

d. Fold the sheet to secure the strands and keep them well aligned until analysis in the agreed analytical laboratory.

f. Place the cardboard sheet in the paper envelope, which you will label with the sample identification code.



After each sampling, clean/disinfect the scissors and the ruler, dispose of the gloves and fill in the sample record sheet with the participant (Appendix 1). The researcher may also suggest the participant to look in a mirror how much was cut to be reassured.

6 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:

E-waste (E) - Country ID (XX) - Company ID (XX) - Participant ID (XXX) - Sample ID (AX/BX/LCX/RCX/HX/SDX/UX/WX/WBX)

‘E’ is to denote that the samples and data relate to the e-waste occupational study.

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).

Country	ISO Alpha-2 country codes
Belgium	BE
Finland	FI
Germany	DE
Latvia	LV
Luxembourg	LU
Poland	PL
Portugal	PT
The Netherlands	NL
United Kingdom	UK

Company ID ‘XX’ is a two-digit running number of companies in each country (e.g. 01 for the first company recruited, 02 the second and so forth).

Participant ID 'XXX' is a three-digit running number of participants in each country (e.g. 001 for the first participant recruited, 002 the second and so forth).

Sample ID ‘HX’ where H denotes the type of sample collected (hair), followed by an one-digit identifier (X) to identify the running number of each type of sample for that worker (e.g. 1 for the first sample, 2 for the second and so forth).

The following scenario is provided to illustrate the application of this convention. A worker is recruited in Luxembourg. He is working in the first company recruited. He is the first worker recruited in that company and is providing a first hair sample. The sample identification code assigned is therefore E-LU-01-001-H1

The unique sample identification code has to be clearly written on the sample envelope. A sample record sheet and questionnaire with the same identification code should be completed when collecting the hair sample (Appendix 1).

7 Storage of collected samples

After the collection and before the shipping to the concerned laboratories, the envelopes containing the collected hair samples have to be stored at room temperature in a dry and dark environment, so preferably in a non-transparent cardboard box.

8 Transportation of 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.

Since hair is a particularly stable biological matrix (no degradation or any loss of the incorporated compounds is expected), the samples can be sent to the agreed analytical laboratory once the complete collection necessary for the study is done. 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.

9 Reporting

It is important that information entered into the sample record sheet (Appendix 1) is recorded as per the instructions given in this SOP as this information will need to be entered into the data template for the overall e-waste study.

Care must be taken to follow the instructions accompanying the data template. The structure, drop down lists etc of the data template must NOT be modified in any way. In the event that the template is modified or data has been provided which does not follow the instructions, templates will be returned to the data provider for correction.

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Appendix 1: Sample record sheet

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Occupational Biomonitoring
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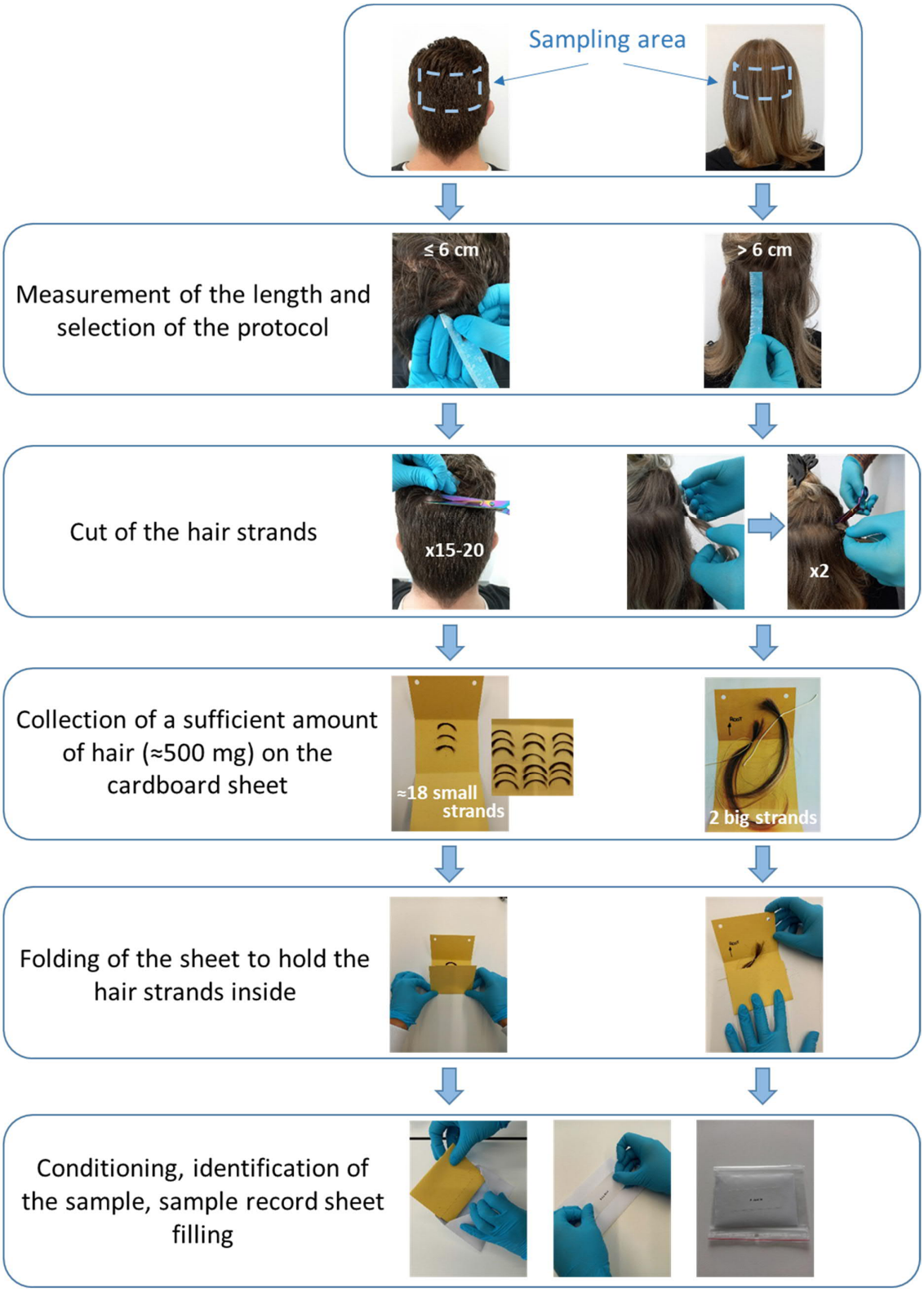
HAIR SAMPLE RECORD SHEET

Company Name:	
Company ID:	
Worker ID:	
Worker name:	
Job title:	

Sample ID:	
Sampling date:	
Researcher(s):	
Organisation:	

Participant authorization and sample obtained:	◯ Yes ◯ No → details: _____
Collection at the beginning of the workweek:	◯ Yes ◯ No → details: _____
Collection before the workshift:	◯ Yes ◯ No → details: _____
Sample length (cm):	
Natural color of the hair:	◯ Black/dark brown ◯ Brown/brunette ◯ Blond ◯ Red ◯ Grey ◯ White
Cosmetic treatment applied on hair within the last 6 months:	◯ None ◯ Dye/Tinting → _____ months ago ◯ Chemical hair structure treatment (perm or hair straightening) → _____ months ago
Use of curling or straightening iron:	◯ Yes → frequency: _____ times / month ◯ No

Appendix 2: Hair sampling guide



HORIZON2020 Programme
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Annex 8

SOP 8:

Procedure for obtaining dermal sampling using wipes and wristbands

Occupational E-waste study

WP8

Task 8.5

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This SOP was developed with due consideration of the contents of the “SOP7: Standard operating procedure for obtaining dermal wipe samples” developed for the HBM4EU occupational biomonitoring study on hexavalent chromium and other harmful chemicals (Porras et al., 2019).

This document has been created for the HBM4EU project. HBM4EU has received funding from the European Union’s Horizon 2020 research and innovation programme under grant agreement No 733032.

1 Introduction

The purpose of this Standard Operating Procedure (SOP) is to establish a uniform procedure for collecting wipe and wristband samples for the evaluation of dermal exposure in the e-waste workplace biomonitoring study. 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. Low-cost silicone wristbands are a non-invasive passive sampling technique that has been used to assess cumulative exposure over several days (e.g. Aerts et al, 2018; Wong S et al., 2019).

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^2 per male hand, 445 cm^2 per female hand (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, and the protocol presented by Aerts et al, 2018 Environ. Sci. Technol. 2018, 52, 298−307 for the use of wristband passive samplers, respectively. It involves the use of SKC Ghost sampling wipes, which can also be used to collect samples of several metals including Cr, Cd, Pb and Hg, as specified in OSHA Method ID-125G, Addendum B[1] and of Silicon wristbands, which can be used to collect samples of different organic compounds including flame retardants. It should however be noted that recovery, sampling and storage efficiency of the wipe and wristbands sampling method has not been validated within the HBM4EU project.

The goal is to provide a uniform methodology to collect representative samples to determine the study participants' dermal exposure in a standardized manner.

The Ghost wipes are to be analysed for metals using OHSA Method ID125G ‘Metal and metalloid particulates in workplace atmospheres (ICP analysis)’ by laboratories experienced in this analysis.

The Silicon wristbands are to be analysed for flame retardants by laboratories experienced in this analysis, in accordance for instance with the protocol proposed by Wong S et al., 2019.

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)
- Silicon wristbands, adult-size (202 mm L × 12 mm W × 2 mm T; weight 5.33 g, SD = 0.10 g) with HBM4EU debossed text (e.g. https://www.promofit.nl/promo-premiums/polsbandjes-bedrukken/siliconen-polsbandjes-bedrukken/
- The wristbands will be distributed uncleaned. So prior to utilization the wristbands need to be cleaned (30 min with 1:1 ethyl acetate/hexane (v/v) (125ml of each for n=5 wristbands) followed by a second 30 min cleaning with 1:1 ethyl acetate/methanol (v/v)

[1] http://www.skcltd.com/products2/9-uncategorised/310-surface-and-skin-1

(125ml of each for n=5 wristbands) in an overhead shaker and subsequently dried under N_2 at 40°C (consider to be modified to a higher temperature). Each wristband will be stored in aluminium/LDPE ziplock bags (see Figure 3) at -20°C until further use. Keep 5 cleaned wristbands as blanks to be returned with the field samples for the laboratory to verify the background).

Figure 1: Wristband. The colour will be changed to yellow and the text to *'HBM4EU'.*

- Digestion tubes with screw caps (sufficient number for the wipe samples).
- Aluminium/LDPE ziplock bags for wristband storage
- Sample labels
- Supply of powderless disposal nitrile gloves
- Sample record sheets (Appendix 1)
- Supply of disposable paper towels
- Pens

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/work week:

a. For wipe sample collection

- Rings should be removed (wherever possible) and stored securely.
- 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 the dominant hand, prior to the participant commencing their work activity using the standardised wiping procedure.
- Further wipe samples will be collected from the participants dominant hand prior to their refreshment/comfort and lunch and before they finish their work shift. Participants must not wash their hands before the wipe samples are collected.
- The participant should also be informed that if for any reason they do wash their hands between any two wipe sampling periods that they should inform the researcher that this is the case.

b. For wristband samples collection:

- Wristbands will be worn for the entire work-week period (starting on the 1st morning of the working week) only during working activities.
- From the end of each working day to the following work morning wristbands must be placed in their original ziplock bags and stored overnight in a clean area.

- At the end of working week (end of the working day), wristbands must be placed in their original ziplock bags for transport to the lab. Keep cool during transportation and stored at -20°C until shipment.

4 Researcher precautions

When collecting each wipe or wristband 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 or wristbands are collected from the participants in an area considered to be free of potential contamination e.g. office space, medical room etc. Wipes or wristbands should not be collected from participants in the physical work area where, for example, where e-waste dismantling 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 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 Sampling procedures

Ensure have a sufficient number of prepared wristbands (one for each participating worker), stored in ziplock bags with a unique number (see Section 7).

Prepare a sufficient number of digestion tubes (for the wipe samples) 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 the participant details on the sample record form (Appendix 1).

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

- 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 palmer and dorsal area and fingers of the dominant hand:
    - Ask the participant what their dominant hand is
    - The wiping will be done only on the dominant 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 2).
- Wipe twice the palm of each finger, from the top to the fingertip. Repeat, to sample the back of the fingers.

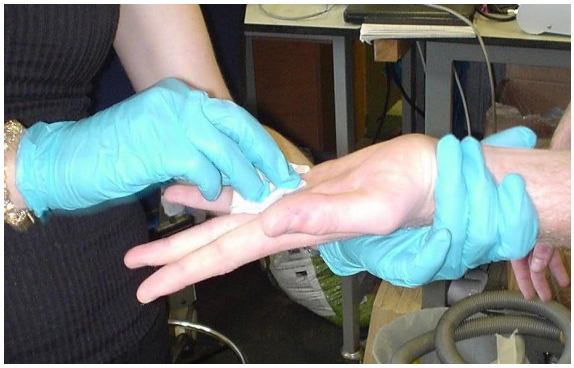

Figure 2: Dermal sample collected from the fingers

- After wiping, fold the wipe again with the contaminant side inward. Place the wipe immediately in the labelled digestion tube and securely seal using the screw cap.
- Submit one blank wipe for every ten hand wipe samples, treated in the same fashion, but without wiping (See Section 9).

The following procedures should be used to collect the wristband samples

- Remove the pre-cleaned wristband from its ziplock bag (Fig. 3). The wristband is to be worn on the wrist of the dominant hand and must be worn for the entire working-week (starting on the 1st morning of the working week) only during the working hours.
- From the end of each working day to the following work morning wristbands must be placed in their original ziplock bags and stored overnight in a clean area.
- Upon the end of the sampling work-week period the wristbands will be placed in the original ziplock bag and returned to the researcher.
- Submit one blank wristband for every ten wristbands worn at the site. These should be stored in their ziplock bag for the entire working week (See Section 9).

Figure 3: Wristband with ziplock bag

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. The researchers should use their judgment to decide on the numbers of samples to be collected. For example, if the participant works a full shift on e-waste activities and has two rest breaks and one lunch break, there will be five sample collection periods: pre-shift, first break period, lunch, second break period and post-shift, resulting in 5 samples being collected per participant.

A single wristband sample per worker will be taken for the entire work-week period.

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:

E-waste (E) - Country ID (XX) - Company ID (XX) - Participant ID (XXX) - Sample ID (AX/BX/LCX/RCX/HX/SDX/UX/WX/WBX)

'E' is to denote that the samples and data relate to the e-waste occupational study.

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).

Country	ISO Alpha-2 country codes
Belgium	BE
Finland	FI
Germany	DE
Latvia	LV
Luxembourg	LU
Poland	PL
Portugal	PT
The Netherlands	NL
United Kingdom	UK

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

Participant ID 'XXX' is a three-digit running number of participants in each country (e.g. 001 for the first participant recruited, 002 the second and so forth).

Sample ID. 'WX' denotes the collection of a wipe sample, followed by an one-digit identifier (X) to identify the running number of each type of sample for that worker (e.g. 1 for the first sample, 2 for the second and so forth). 'WB' denotes the collection of a wristband sample.

The following scenario is provided to illustrate the application of this convention. A worker is recruited in Luxembourg. He is working in the first company recruited. He is the first worker

recruited in that company and is providing a first wipe sample. The sample identification code assigned is therefore E-LU-01-001-W1. The code for this workers wristband sample is E-LU-01-001-WB1.

The unique sample identification code will be clearly stated on the labelled digestion tube / wristband vial. A sample record sheet should be completed when collecting the wipe samples (Appendix 1).

8 Storage of collected samples

The collected wipe samples can be stored at room temperature in a clean box before transportation to the laboratory. Since no standard recomandations are available for wristbands, a more strict approach is recommended for their storage after the sampling period. Thus, the collected wristband samples can be kept preferably cool (or at room temperature but not more than 24h). If longer storage period is required the samples should be kept at -20°C prior to the transport to laboratory as well as in the lab prior to analysis.

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. 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. It is recommended that at least one wipe blank is collected, with further wipe blanks to be collected for every 10 wipe samples obtained. This recommendation also applies to the collection of blank wristband samples.

10 Transportation of 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 samples are analysed as soon as possible by the receiving laboratory and certainly within no more than 14 days of collection.

11 Reporting

It is important that information entered into the sample record sheet (Appendix 1) is recorded as per the instructions given in this SOP as this information will need to be entered into the data template for the overall e-waste study.

Care must be taken to follow the instructions accompanying the data template. The structure, drop down lists etc of the data template must NOT be modified in any way. In the event that the template is modified or data has been provided which does not follow the instructions, templates will be returned to the data provider for correction.

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Appendix 1: Sample record sheet

Company Name:	
Company ID:	
Worker ID:	
Name (family name, initial):	
Job title:	
Dominant hand of worker:	

Researcher(s):	
Organisation:	
Date sampling:	

Wipe Sample collection

Collection round	Sample ID	Time collected (00:00)	Gloves worn prior to collection (Y/N)	Type gloves worn	Hands washed prior to collection (Y/N)	Hands observed to be wet (Y/N)
Pre-shift						
Break 1						
Lunch						
Break 2						
End shift						
Blank						

Wristband sampler

Sample ID	Placed on dominant hand? (Y/N)	Date wristband donned (dd:mm:yyy)	Time donned (00:00)	Day wristband collected (dd:mm:yyyy)	Time collected (00:00)

Questions for the participant when collecting the wristband

Did you encounter any problems or any deviations from the wearing instruction for the wristbands over the work-week?	Yes / No
If yes, please provide more detail.	Reason:

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Annex 9

SOP 9:

Standard operating procedure (SOP) for Buccal cells sampling including sample storage and transfer

Diisocyanate and E-waste occupational studies

WP 8

Task 8.5

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1 Introduction

Genomic Instability has been observed in workers involved in processing electronic waste (Wang et al, 2018; Li et al., 2014; Liu et al., 2009).

The buccal micronucleus assay is a minimally invasive approach for measuring DNA damage, cell proliferation, cell differentiation and cell death in exfoliated buccal cells (Bolognesi et al., 2015). It offers a great opportunity to evaluate in a clear and precise way the appearance of genetic damage whether it is present as a consequence of occupational or environmental risk, being reliable, fast, relatively simple, cheap, and minimally invasive and causes no pain (Torres-Bugarín et al., 2014). Previous studies suggested that this effect biomarker can be related to waste exposure. One work reported micronuclei and other nuclear anomalies in exfoliated buccal cells from urban solid waste collectors and recyclers in southern Brazil (Brina et al 2017), and other works indicated higher frequencies of buccal micronuclei and other nuclear abnormalities such as karyolytic and karyorrhectic cells in waste pickers women (Franco de Diana et al., 2018). More importantly, increased frequencies of micronucleus, karyolysis, and pycnosis in the exfoliated buccal cells in scavenging teenagers at Alaba International market has been related to E-waste indiscriminate disposal and primitive recycling processes, possibly due to elevated Serum Pb, Ni, Cd, and Cr Levels (Alabi et al., 2020). Thus, this effect biomarker seems to be suitable to assess local effects from e-waste exposure. On the other hand, studies on workers exposed to diisocyanates utilized in the manufacture of polyurethane foam have reported increased genotoxic effects (Lindberg et al., 2011). Both toluene diisocyanate- and 4,4′-methylenediphenyl diisocyanate- exposed workers showed increased frequencies of micronuclei in peripheral blood lymphocytes (Norppa et al., 2000; Bilan, 2004) and in buccal epithelial cells (Norppa et al,, 2000), confirming the suitability of this assay to assess diisocyanates.

This guideline is intended to be used in the framework of the Human Biomonitoring Initiative (HBM4EU). The Standard Operating Procedure (SOP) for buccal cells sampling provides the general procedure for the collection, storage and transfer of buccal cell samples to be analysed within the diisocyanate and the e-waste occupational studies.

2 Precautions in the pre-analytical phase

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

a) Influencing factors:

   Alcohol consumption, medication intake and smoking or diet, are influencing factors that can modify the levels of MN in buccal cells. This information will be collected through questionnaire (SOP 2) and should be taken into account when analysis the results.

b) Interfering factors

   Buccal cell samples should be immediately fixed after collection and sent to the Laboratory in fixative medium to avoid cells degradation or morphology alteration. Tubes have to be appropriately coded to avoid misidentification.

3 Buccal cells Sampling

3.1 Sampling schedule

Post-shift collection is preferred.

Buccal cell collection takes approximately 10 minutes per worker.

3.2 Sampling material

The following materials and equipment will be necessary for sampling

- Small-headed toothbrushes (2-cm head length) or cytologic brushes are preferential or you can use standardised swabs (e.g.: https://isohelix.com/products/isohelix-dna-buccal-swabs/)
- 30–50 mL polystyrene containers or test tubes labeled LC (left cheek) and RC (right cheek), 2 per worker
- Saccomanno's fixative (50% alcohol which contains approximately 2% of Carbowax 1540), 20 ml per worker

3.3 Instructions for buccal cells sampling

The method presented here is described in detail by Bolognesi and Fenech (2019), and is standardized and widely used by others (Thomas and Fenech, 2011; Bolognesi et al., 2013; 2017 Thomas et al., 2009). At the end of this section, an alternative procedure is provided for samples that will be processed up to 24h after collection, without being transported.

1. For each participant prepare two 30-ml polystyrene containers or test tubes, labeled with individual code and LC (left cheek) and RC (right cheek), each containing 10 ml of Saccomanno's fixative.

2. Before buccal cell collection, the mouth of the subject should be rinsed twice thoroughly with 30 ml of water to remove excess debris.

> *! CAUTION Human samples should be considered as infectious and the appropriate safety precautions should be taken.*

3. Gently but firmly rotate a small-headed toothbrush (2-cm head length) 10 times against the inside of the cheek wall in a circular motion starting from the middle and gradually increasing in circumference to produce an outward spiral effect.

> *Use a different toothbrush for each cheek.*
>
> *! CAUTION It is important to remember not to revisit the mouth with the same toothbrush, so as to avoid the introduction of the fixative to the mucosal lining. Use a new toothbrush for resampling.*

4. The head of the brush is then placed into the fixative container and rotated such that the cells are dislodged and released into the suspension.

> *The cell sampling is performed on the inside of both cheeks to maximize cell collection and to obtain an homogeneous cell suspension, avoiding unknown biases that may be caused by sampling one cheek only.*

5. The sampling brush should be discarded as risk waste after sampling.

6. Tightly seal the tops of the fixative containers and cover in parafilm to prevent leakage during transit from the remote collection location to the laboratory.

7. The containers are then returned to the laboratory for analysis by a courier service, and the laboratory should be informed of their shipment and anticipated arrival date, so that they can be processed as soon as possible after receipt.

8. Buccal cell suspension fixed in Saccomanno's solution can be stored at 4º C for months, but need to be washed with buccal buffer and centrifuged one or two times to be rehydrated before proceeding with next steps.

Alternative procedure:

In step 1, use Buccal cell buffer instead of Saccomanno's fixative for collecting the cells.

Buccal cell buffer preparation: 1.6 g of Tris–HCl (0.01 M), 37.2 g of ethylenediaminetetraacetic acid (EDTA) tetra sodium salt (0.1 M), and 1.2 g of NaCl (0.02 M). Weigh and dissolve in 600 mL of Milli-Q water. Make up the volume to 1000 mL. Adjust pH to 7.0 using 5 M HCl and autoclave at 121 ºC for 30 min. The buffer will last for up to 3 months when stored at room temperature.

Proceed with buccal cells washes and cell spreading within 24h (Thomas et al., 2009).

4 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:

E-waste (E) / Diisocyanate (D) - Country ID (XX) - Company ID (XX) - Participant ID (XXX) - Sample ID (LCX/RCX)

'E' is to denote that the samples and data relate to the e-waste occupational study.

'D' is to denote that the samples and data relate to the diisocyanate occupational study.

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).

Country	ISO Alpha-2 country codes
Belgium	BE
Finland	FI
France	FR
Germany	DE
Latvia	LV
Luxembourg	LU
Poland	PL
Portugal	PT
The Netherlands	NL
United Kingdom	UK

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

Participant ID 'XXX' is a three-digit running number of participants in each country (e.g. 001 for the first participant recruited, 002 the second and so forth).

Sample ID 'LCX' where LC denotes the type of sample collected (buccal cells, left cheek), followed by an one-digit identifier (X) to identify the running number of each type of sample for that worker (e.g. 1 for the first sample, 2 for the second and so forth).

The following scenario is provided to illustrate the application of this convention. A worker is recruited in Portugal. He is working in the first company recruited for the E-waste study. He is the first worker recruited in that company and is providing first two samples of buccal cells. One sample from the left cheek (LC) and one from the right cheek (RC). The sample identification codes assigned are therefore:

E-PT-01-001-LC1

E-PT-01-001-RC1

5 Conservation, transport and storage of the samples

5.1 Processing and storage of collected buccal samples at the local laboratory

Buccal cells fixed in Saccomanno's solution can be stored at 4ºC, allowing preservation of the cell suspensions at 4 ºC for months before processing (Bolognesi and Fenech, 2019).

5.2 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.

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.

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.

5.3 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 slides 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.

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Annex 1 – Buccal cells Sampling form

Confidential Data – do not send with sample

Study Identification (Diisocyantes/ E-waste): _____

Worker Identification:

Country: _____

Company name and name of department: _____
Worker name: _____
Position: _____
Control? _____(Yes/No) Exposed? _____ (Yes/No)

Date: _____
Code Number: _____

Send with sample for analysis

***Questionnaire*:**

Date: _____

Code Number: _____

Buccal Sample:

Date: _____ Time of sampling: _____

Buccal Code Number: _____

Number of Samples collected from this individual: _____

Notes/Observations:

_____

_____

_____

HORIZON2020 Programme
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Annex 10

SOP 10:

Standard operating procedure for comparing occupational hygiene measurements with exposure estimates generated using the Advanced REACH Tool via the TREXMO model

Diisocyanate and E-waste occupational studies

WP 8

Task 8.5

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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, ART 1.5 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 occupational studies 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 a selected REACH model, ART 1.5 via the TREXMO (TRanslation of EXposure MOdels) tool with participants being asked to estimate inhalation exposure. TREXMO is a tool primarily developed to efficiently and reliably assess the wide variety of exposure situations using the available occupational exposure models. TREXMO integrates six commonly used occupational exposure models: ART v.1.5, Stoffenmanager® version 4.0, ECETOC TRA v.3, MEASE v.1.02.01, EMKG-EXPO-TOOL and EASE v.2.0, although we will only be using the outputs of ART 1.5 (the higher exposure assessment tool)**.** 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 other environmental samples are collected.

3 Generation of exposure scenarios for use in modelling exercise

The competed proformas will be returned to KG (karen.galea@iom-world.org) and SV (susana.viegas@ensp.unl.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 generated exposure scenarios 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).

4 Model to be used

Participants will be asked to generate inhalation exposure estimates for the various scenarios using the higher tier REACH model, ART 1.5. This will be via the TREXMO tool, http://trexmo.chuv.ch/.

5 Who should complete the modelling exercise?

Each participating country where air samples will be collected as part of the biomonitoring campaigns, are invited to participate. 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 and other environmental samples.
- A member of the project team who did not visit the sites.
- An individual experienced in exposure assessment but who has no direct experience of the projects or sites where exposure to the chemicals being studied was assessed.

6 The modelling exercise

6.1 Overview

Participants will be asked to complete a short background questionnaire. They will then be provided electronically with a pack containing simple instructions for completing the exercise, instructions on how to use TREXMO (ART 1.5), the exposure scenarios and supporting worksheets. Details of how the assessments are to be returned will also be provided.

6.2 Background questionnaire

A short background questionnaire will be administered to collect key information on participants' experience in relation to the measurement campaign and also their use of 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 measurement campaigns, e.g. if they personally collected the contextual information / air and other environmental samples at the sites; if they did not attend any site visits; had no involvement with the studies being developed.
- Previous experience with the use of the model.

6.3 How to use TREXMO

A simple guide to gaining access to TREXMO and how to use the ART 1.5 model for the purposes of the exercise will be provided. 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 once.

For each exposure scenario, participants will be instructed to undertake an inhalation exposure assessment using ART 1.5, even where the scenario may be outwith the scope for the tool. For each exposure scenario they will be asked to complete a worksheet to record their results .

For each exposure scenario issued, participants will be required to document systematically the following contextual information on the worksheet:

- Previous experience of the given exposure scenario.
- 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.

Participants will be asked to complete the given exposure scenario and return the completed worksheet 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.

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Appendix 1: Contextual information proforma

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

General working environment	
Location	Indoor / Outdoor
Room temperature	°C
Approximate size of room where the participant works	m^3
Ventilation in room	Natural / Mechanical / Both
Air change rate	air changes per hour
Free text description of how well room is ventilated	
General impression of cleanliness and tidiness of workplace	Poor / Good / Excellent
Frequency of cleaning per day and how	

General hygiene 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:	

Training	
Workers received previous training on the health risks associated with their work?	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:	
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 task: Time spent on each task / activity during the day
Approximate working distance of worker from the exposure source	…….m If different distances for different workers please specify
Any observed differences in working behavior between workers involved in same activity?	
Were the tasks /activities	Yes / No

observed on the day typical of usual work activities?	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 hazardous substances being assessed 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	
RPE 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:

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^{o}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/ gmol^{-1}	Vapour pressure at 20^{o}C/ Pa	Concentration of Nickel in Product R (%)
Product R	Supplier R	Nickel	7440-02-0	59	1 (Negligible)	100

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Annex 11

SOP 11:

Communication plan for the occupational studies (E-waste and diisocyanates)

WP 8

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1 General introduction

In the scope of HBM4EU project two further occupational studies will be developed.

The first is focused in diisocyanate exposure in the manufacturing and repair of large vehicles (non-booth spraying of e.g. boats/planes), the use of diisocyanate based hot-melt glues in different sectors, and construction sector, which includes different sources of diisocyanates exposure (floorings/screeds, insulation). Its main aims are to provide new data on the exposure to diisocyanates in specific sectors based on harmonized sampling protocols, test the usability of different biomarkers in the assessment of exposure to diisocyanates, and use the collected data to validate the PBPK model developed. The study will be conducted during 2020-2021 in five countries: Belgium, Finland, France, The Netherlands and United Kingdom.

The second study is dedicated to occupational exposures in the E-waste handling sector. The main aim is to contribute to awareness of potential hazards and stimulate good work practices that will lead to further improve protection of the worker's health from the risk of exposure to toxic components, including that of combined exposures. The study will include the assessment of exposure to several HBM4EU priority compounds, including metals (lead, inorganic mercury, cadmium, chromium), phthalates, and flame retardants. The study will be performed in Belgium, Finland, Germany, Hungary, Latvia, Luxembourg, The Netherlands, Poland, Portugal and United Kingdom.

This Communication Plan SOP is designed to ensure that all the relevant results of these studies are communicated to all study participants (companies, workers and equally to controls) and national and EU stakeholders. Dissemination of information to the scientific community is also covered. The results will be presented in different manner dependent of the type of participant (e.g. individual levels for workers and or aggregated levels to companies). Effective and consistent communication with participants and stakeholders will help ensure that the results obtained are accessible to support organizational learning and used for decision-making. This will happen at the companies and workers level but also at regulatory level. To sum up, it is CRUCIAL to consider that communication is very relevant and it is a way to guarantee that the tools and data being developed in the scope of HBM4EU will be available to be used by risk assessors.

This SOP has been created with the premise in mind that every participating country is obliged to follow the HBM4EU documents and guidance concerning ethical aspects and also the national ethical committees.

2 Communication Plan

Considering the above a communication plan is proposed for both occupational studies (Table 1). This communication plan describes the different target audiences, the objectives/actions desired, the message content for each stakeholder/group, the method of choice to convey the message and, finally, the best moment in time for the message to be communicated. Additionally, it is important to consider that this is a living document and it will be reviewed and revised as necessary throughout the full duration of the occupational studies.

Table 1 – Current communication plan

Stakeholder/group	Objectives/actions desired	Data to be communicated	Delivery methods/venue	When
Study participants (workers and controls)*	Fulfill conditions set in informed consent procedure. Inform the workers about their individual results to support behavioral change if needed	Individual results of exposure as per study information leaflet and consent form. In some countries they will only be provided to the study persons on request. Extra care will be given to the communication of unexpected results (procedure as specified in approved study protocol).	Depending on the country, for example via occupational physician of the company, or directly by the responsible researcher/physician of the national research group. Workers are given an opportunity to discuss on their results and on their meaning.	When the results are available for each company engaged in the project.
Participating companies**	Inform companies about the exposure results to support the risk assessment, definition of priorities and improve risk management measures.	Results of the occupational hygiene (air and wipe/settled dust) samples). Results of the exposure biomarkers specified in information leaflet and informed consent form will be provided in an aggregated manner guaranteeing that workers identities are not perceived; Recommendations for RMMs if needed.	Technical report be issued to the authorised person who consented to the company participating (unless otherwise specified)	When the results are available for each company engaged in the project.
HBM4EU partners	Provide study results and recommendations with respect to the outlined study aims and objectives. This intends to be a continuing process.	Exposure data obtained in each country will be combined with the other countries and statistical analysis will be performed	Deliverable report, presentations in consortium meetings and training school (lessons learned)	When the results are ready, by the due date of Deliverable report to HBM4EU.
HBM4EU partners and other interested partners	Inform about the methodology to be employed in the occupational exposure monitoring campaigns	Developed finalized SOPs	HBM4EU training school Website Publications Linkedin	When SOPs have been finalized.
Scientific and professional community	Inform about the most suitable biomarkers for each substance, exposure levels,	Exposure data obtained in each country will be combined with the	Scientific publications in peer reviewed journals;	Begins already when the methodology has been

Stakeholder/group	Objectives/actions desired	Data to be communicated	Delivery methods/venue	When
	variables that influence exposure, occupational settings with higher exposure, RMMs with higher efficacy	other countries and statistically analysis will be performed.	presentations at conferences and seminars (at national and international level) and online webinars	established, and continues when the results are ready.
HBM4EU stakeholders (ECHA, EU-OSHA and national contact points via their focal contact point network, DG Santé, DG Employment) and other interested parties (industry associations, workers unions, national authorities, ISES-Europe and European industrial hygiene associations)	Inform about more suitable biomarkers, occupational settings with higher exposure, evaluate the impact of the regulatory actions already in place, the support of new regulatory actions if needed.	Aggregated exposure data	Articles in HBM4EU newsletter Policy brief HBM4EU webpages Presentations in HBM4EU stakeholder forum. Deliverable report Webinar/seminar on the results targeted to key stakeholders. Slides from the Webinar published in web. Information is shared also using social media channels.	Dates to be defined. Communication begins already when the studies start and continues throughout the study.
Targeted communication to ECHA Committees if relevant (e.g. RAC).	Evaluate the impact of the regulatory actions already in place, the support of new regulatory actions if needed.	Aggregated exposure data	Presentation in ECHA	When the results and deliverable report are ready
National industry stakeholders, workers unions (optional, depending on country)	Inform about the exposures in different occupations, recommendations for the assessment and management of exposure.	Aggregated exposure data	Press release in national language, description of the study published in the website of the participating institutes. Possibly short articles in national journals targeted to relevant industry fields. Use of social media to distribute information also nationally.	This is the responsibility of each participating institute (dates do be defined)

* Communication starts in the first contact companies/workers and information about why they should participate in the study and what are the hazards related to their exposures should be provided since the beginning of the study.

** Preferably done before any reporting to HBM4EU, stakeholders and scientific community.