Post-Emergency, Multi-Hazard Health Risk Assessment in Chemical Disasters
PEC

Deliverable D. E. 4

Guidelines For Environmental and Biomonitoring

ECHO/SUB/2015-713844-PREV11 - PEC: Post-Emergency, multi-hazard health risk assessment in Chemical disasters
Project co-funded by ECHO - Humanitarian Aid and Civil Protection

TABLE OF CONTENT

1 INTRODUCTION 1
2 ENVIRONMENTAL MONITORING 5
2.1 Methodologies for measuring environmental contamination 5
2.1.1 Standard operating procedures for monitoring toxicants in air 5
2.1.2 Standard operating procedures for monitoring toxicants in soil 10
2.1.3 Standard operating procedures for monitoring toxicants in water 11
2.1.4 Long-term environmental monitoring 13
3 BIOLOGICAL BIOMONITORING IN CHEMICAL INCIDENTS 14
3.1 Considerations in the Design of a human biological monitoring campaign 16
3.2 Study Conduct 16
3.2.1 Selection of the compounds 16
3.2.2 Selection of the biomarkers 17
3.2.3 Selecting the target population and preparing an information package 19
3.2.4 Selecting the sample matrices 19
3.2.4.1 Urine 21
3.2.4.2 Blood 21
3.2.4.3 Exhaled breath 22
3.2.4.4 Selection of time and frequency of sampling 22
3.3 Ethical Aspects 23
3.4 Start collection of exposure data and biological media 23
3.5 Sampling and storage 24
3.6 Statistical Analysis 24
3.7 Interpretation of outcome of an HBM campaign 26
3.8 Recommendations for sampling, handling, and storage biological matrices 27
3.8.1 Urine 27
3.8.2 Blood 30
3.8.3 Serum/Plasma 34
3.8.4 Exhaled Breath 35
3.8.5 Exhaled breath condensate (EBC) 43

ECHO/SUB/2015-713844-PREV11 - PEC: Post-Emergency, multi-hazard health risk assessment in Chemical disasters
Project co-funded by ECHO - Humanitarian Aid and Civil Protection

4 CONCLUSIONS 45
5 REFERENCES 46
ANNEX I 54
Inductively Coupled Plasma Optical Emission Spectrometry 59
(EPA, 1997) 59
ANNEX II 61

ECHO/SUB/2015-713844-PREV11 - PEC: Post-Emergency, multi-hazard health risk assessment in Chemical disasters
Project co-funded by ECHO - Humanitarian Aid and Civil Protection

INDEX OF FIGURES

Figure 1: Sequence of stages in human biomonitoring.....2

Figure 2 : Schematic of sample collection and pre-treatment and storage prior to delivery of the samples the laboratory that performs the analysis. 24

ECHO/SUB/2015-713844-PREV11 - PEC: Post-Emergency, multi-hazard health risk assessment in Chemical disasters
Project co-funded by ECHO - Humanitarian Aid and Civil Protection

INDEX OF TABLES

Table 1 : Group of chemicals according to Seveso-III (Directive. 2012/18/EU) 3

Table 2 : Factors affecting the validity and feasibility of biomarker studies: analytical procedures. (Dor, et al., 1999) 18

Table 3 : Factors affecting the validity of biomarkers: intrinsic characteristics of the biomarker.(Dor, et al., 1999) 18

Table 4 : Biological matrices used in human biomonitoring studies. (WHO, 2015) 20

Table 5 : Typical collection devices of human breath and their respective characteristics. (Tang, et al., 2015) 39

Table 6 : Summary of the principal studies using solid-phase microextraction (SPME) as preconcentration technique and devoted to exposure analysis. (Alonso and Sanchez, 2013) 40

Table 7 : Performance characteristics for representative NO analysers (Cristescu, et al., 2013) 42

Table 8 : Commercially available exhaled breath condensate collection systems. (Hunt, 2007) 43

Table 10 : Summarizes the equipment of sampling, the volume air, the preservation, the holding time and the analytical method for the quantification of chemiclas to air samples 54

Table 11 : Summarizes the equipment of sampling, the volume air, the preservation, the holding time and the analytical method for the quantification of chemicals to water samples 57

Table 12 : Summarizes the equipment of sampling, the volume air, the preservation, the holding time and the analytical method for the quantification of chemicals to soil samples. 59

Table 13 : Chemical compounds biomonitoring according to Seveso III – Directive (Directive. 2012/18/EU). 61

1 INTRODUCTION

An acute chemical incident may be defined as an unexpected event with a clear onset during which substance(s) are released into the environment and pose a (potential) hazard to humans, animals, or the environment. The late or follow-up phase starts after the termination of the rapid response activities and health studies may be conducted during this phase. Exposure assessment within the framework of chemical incidents has three basic objectives: (1) Risk assessment (based on existing knowledge of the exposure-response relation); (2) Definition of the population or groups at risk; (3) Providing (individual) exposure estimates for epidemiological studies.

Environmental measurements are critically important, but require well-equipped and skilled personnel to carry them out. It is therefore essential that a systematic plan is developed for the areas and media (including air and water) to be sampled, as well as the required time frame for sampling. The sampling plan should include the time, frequency, sampling methodology, and comparisons to be made, to obtain an accurate representation of conditions. The sampling team should be supported by laboratory facilities that carry out appropriate tests, and that have stringent quality assurance and quality control procedures. Ideally, laboratory facilities should be certified for the specific analyses that they will be conducting. There is only one opportunity to obtain air samples for determination of exposure levels during the emergency itself. While it may not be feasible to collect water or soil samples during the emergency, air sampling in the field is usually possible, even if at locations somewhat distant from the event. If sampling in the plume is attempted, chemical emergency managers may need to coordinate well-protected fire personnel or environmental officers to conduct the sampling; these people need special advanced training to accomplish this task effectively and without endangering themselves. When water or soil has been contaminated, sampling can usually be done in the days following the incident. However, if the chemical incident was such that the site had to be neutralized immediately, for example, by thoroughly rinsing or hosing, samples may not be available. In such cases, biomonitoring, as discussed above, may be a good option for estimating exposure. Monitoring at the source of contamination should continue well beyond the point at which the release has been controlled, to confirm that the release has indeed been controlled. The media likely to be contaminated should be monitored, and personal monitoring should be conducted, determine the concentrations to which populations or individuals are actually being exposed as they go about normal activities. To ensure that environmental sampling is done consistently and in a valid manner, a standard operating procedure (SOP) should be developed that contains protocols for sampling methods.

Human Biological monitoring (HBM) is a useful tool for assessing human systemic exposure to hazardous substances by inhalation, ingestion, and absorption through the skin. HBM monitoring can also help in minor incidents within a workplace to major events where people well beyond the workplace may be exposed. HBM can serve several purposes in the aftermath of a chemical incident: (a) Confirm the presence or absence of an internal exposure in subjects potentially

exposed; (b) Relate clinical symptoms to an exposure or support medical care. The important need to consider the use of biological monitoring in the response phase of an incident was recognized by the World Health Organization. Depending on the type and scale of the incident it may be necessary to assess the exposure of the workers, first responders or the public. Critical to the utility of biological monitoring is the availability of quality assured analytical methods from accredited laboratories and guidance values to put the results in perspective.

It is useful to know when extensive (personal) exposure assessment is needed and when crude exposure indices based on the location of individuals and dispersion of the chemical(s) is sufficient. Some studies may reach conclusive results with a limited amount of data, whereas inadequate exposure assessment may lead to inconclusive studies in other cases. Environmental sampling for risk assessment often takes place close to the source to estimate worst-case scenarios, and these measurements may not accurately represent the exposure of (different) populations.

With respect to legal and public communication issues, the application of HBM calls for a high-quality standard for the entire procedure including specimen sampling, sample preparation, analytical determination, post-analytical evaluation and communication of the HBM results. The request for the ultimate safeguarding of samples to be analysed by HBM allows the generation of exposure data on an individual and group basis to assure appropriate risk communication and respond to legal liability cases. The approach involves two pathways: if the substance is known and an HBM method is available “targeted HBM” may be applied and the appropriate human specimens (for example urine, blood, serum, plasma, erythrocytes) will be collected. If the substance is unknown or an HBM method for a known substance is not available only urine will be collected for “validated HBM” after the development of a new HBM analysis method. Spontaneous urine samples can be easily collected from adults and from children (with the informed consent of their parents) and may be stored deep-frozen until analysis. In addition, ethical considerations ask for the appropriate use of a sample collected in an invasive manner. Although a few national guidelines, recommend the application of HBM for disaster relief forces, most on-scene commanders and many healthcare professionals other than the public health authorities are not aware of HBM as a versatile tool in the aftermath of a chemical scenario.

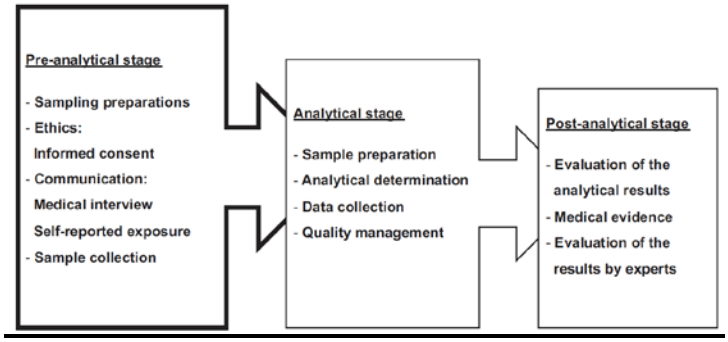

Figure 1: Sequence of stages in human biomonitoring

Group of chemicals

The following table provides detailed information on a variety of chemical agents associated with emergency response. The separation of chemicals was made based on the Seveso III – (Directive. 2012/18/EU). In 2012 Seveso-III was adopted taking into account, amongst others, the changes in the Union legislation on the classification of chemicals and increased rights for citizens to access information and justice. The Directive applies to more than 12 000 industrial establishments in the European Union where dangerous substances are used or stored in large quantities, mainly in the chemical and petrochemical industry, as well as in fuel wholesale and storage sectors. Considering the very high rate of industrialization in the European Union the Seveso Directive has contributed to achieving a low frequency of major accidents. The Directive is widely considered as a benchmark for industrial accident policy and has been a role model for legislation in many countries worldwide. It will be further developed in accordance with this Directive and the categorization of chemicals, the standard operating procedures for sampling during industrial incidents.

Table 1 : Group of chemicals according to Seveso-III (Directive. 2012/18/EU)

Chemical Groups					
Metals	Arsenic pentoxide, arsenic (V) acid and/or salts	Arsenic trioxide, arsenious (III) acid and/or salts	Nickel compounds in inhalable powder form: nickel monoxide, nickel dioxide, nickel sulfide, trinickel disulfide, dinickel trioxide	Lead alkyls	Arsine (arsenic trihydride)
Inorganic Compounds	Phosphine (phosphorus trihydride)	Sulphur dichloride Sulphur trioxide Hydrogen sulfide	Boron trifluoride	Anhydrous Ammonia	Bromine Chlorine Fluorine Hydrogen Oxygen
Aliphatic and Aromatic Organic Compounds	Formaldehyde	Acetylene Ethylene oxide Propylene oxide	Methanol	Methylisocyanate	Carbonyl dichloride (phosgene)
	4′-Methylene bis (2-chloraniline)	Toluene diisocyanate	Polychlorodibenzofurans and polychlorodibenzodioxins	4-Aminobiphenyl and/or its salts, Benzotrichloride, Benzidine and/or salts, Bis (chloromethyl) ether, Chloro-methyl methyl ether, 1,2-	

				Dibromoethane, Diethyl sulphate, Dimethyl sulphate, Dimethylcarbamoyl chloride, 1,2-Dibromo-3-chloropropane, 1,2-Dimethylhydrazine, Dimethylnitrosamine, Hexamethylphosphoric triamide, Hydrazine, 2-Naphthylamine and/or salts, 4-Nitrodip- phenyl, and 1,3 Propanesultone	
Nitrates	Ammonium nitrate	Potassium nitrate			
Amines	Piperidine	Bis(2-dimethylaminoethyl)(methyl)amin	3-(2-Ethylhexyloxy)propylamin		
Petroleum products	gasoline and naphthas,	kerosenes (including jet fuels),	gas oils (including diesel fuels, home heating oils and gas oil blending streams)	heavy fuel oils	alternative fuels serving the same purposes and with similar properties as regards flammability and environ-mental hazards
	Liquefied flammable gases, Category LPG) and natural gas				

2 ENVIRONMENTAL MONITORING

2.1 Methodologies for measuring environmental contamination

This section describes the standard operating procedures for the environmental monitoring (surface waters, air, and soil) in case of an environmental incident. Each standard operating procedure provides information on the following components (where appropriate):

- Introduction
- Apparatus required for sampling
- Procedure of sampling
- Methods for sampling preservation and storage
- Recommended laboratory analysis

2.1.1 Standard operating procedures for monitoring toxicants in air

Introduction

In general, the initial survey is considered to be a relatively rapid screening process for collecting preliminary data at sites where a chemical incident occurs. However, initial surveys may require many hours to complete and may consist of more than one entry. Some information is generally known about the site; therefore, real-time instrumentation for specific compounds (i.e., detector tubes and electrochemical sensors) can be used to identify hot spots. Sufficient data should be obtained with real-time instruments during the initial entry to screen the site for various contaminants. When warranted, intrinsically safe or explosion-proof instruments should be used. An organic vapor analyzer (OVA) typically should be used during the survey. These gross measurements may be used on a preliminary basis to (1) determine levels of personal protection, (2) establish site work zones, and (3) map candidate areas for more thorough qualitative and quantitative studies involving air sampling. In certain conditions, the information obtained may be sufficient to preclude additional monitoring. Materials detected during the initial survey may call for a more comprehensive evaluation of hazards and analyses for specific compounds. Since site activities and weather conditions change, a continuous program to monitor the ambient atmosphere must be established.

Off-Site Monitoring

Typically, perimeter monitoring with the same instruments employed for on-site monitoring should be utilized to determine site boundaries. Because air is a dynamic matrix, physical boundaries like property lines and fences do not necessarily delineate the site boundary or area influenced by a release. Whenever possible, atmospheric hazards in the areas adjacent to the on-site zone should be monitored with direct-reading instruments. Monitoring at the fence line or at varying locations off-site, provides useful information regarding pollutant migration. Three to four locations downwind

of the source (i.e., plume) at breathing-zone height provide a basic fingerprint of the plume. Negative instrument readings off-site should not be interpreted as the complete absence of airborne toxic substances; rather, they should be considered another piece of information to assist in the preliminary evaluation. The interpretation of negative readings is instrument dependent. The lack of instrument readings off-site should not be interpreted as the complete absence of all airborne toxic substances; rather, it is possible that the particular compound or class of compounds to which the monitoring instrument responds is not present or that the concentration of the compound(s) is below the instrument's detection limit.

Apparatus required for sampling

1. **Portable Screening Devices**

Where possible, a datalogger should be used to minimize the length of time required for site personnel to be in a potentially contaminated area. Datalogger cable is available from manufacturers for linear output instruments and some nonlinear output instruments. Below is presented the main categories of sampling devises depending on emissions of gaseous pollutants.

- Total Hydrocarbon Analyzers

Total hydrocarbon analyzers used to detect a variety of volatile organic compounds (VOCs) at hazardous sites principally employ either a photoionization detector (PID) or a flame ionization detector (FID) (Kosch, 2005). Compounds are ionized by a flame or an ultraviolet lamp. FIDs are sensitive to volatile organic vapor compounds such as methane, propanol, benzene, and toluene. They respond poorly to organic compounds lacking hydrocarbon characteristics.

- Oxygen and Combustible Gas Indicators

CGI meters measure the concentration of a flammable vapor or gas in the air and present these measurements as a percentage of the lower explosive limit (LEL) (OSHA, 1990). The measurements are temperature dependent. The property of the calibration gas determines sensitivity. CGIs typically house an electrochemical sensor to determine the oxygen concentration in ambient air.

- Toxic Atmosphere Analyzers

The toxic atmosphere analyzer is a compound-specific instrument, designed and calibrated to identify and quantify a specific compound or class of compounds in either gaseous or vapor form. Cross-sensitivity to air pollutants not of interest may be lead to erroneous results (EPA, 2008).

- Aerosol/Particulate Monitors

A Real-Time Aerosol/Particulate Monitor displays readings for total particulates or particles with diameter 1, 2.5 and 10 μm. The instrument employs a pulse light emitting diode which generates a narrow band emission in conjunction with a photovoltaic cell to detect light scattered from particulates. This provides real-time data without requiring those in personal protective equipment to be constantly present in the plume.

- Gold Film (Hydrogen Sulfide) Analyzer

Hydrogen sulfide (H_2S) monitor operates on the principle that electric resistivity increases across a gold film as a function of H_2S concentration. The monitor provides rapid and relatively low detection limits for H_2S in the air. After extensive sampling periods or high concentrations of H_2S, the gold film must be heated to remove contamination and return the monitor to its original sensitivity.

- Canister Samplers

Evacuated canister sampling systems use the pressure differential between the evacuated canister and ambient pressure to bleed air into the canister. The sample is bled into the canister at a constant rate over the sampling period using a critical orifice, a mechanically compensated regulator, or a mass flow control device until the canister is near atmospheric pressure. Pressure canister sampling systems use a pump to push air into the canister. According to US EPA TO-15, 61 different toxic organic compounds can be identified by using canister samplers (EPA, 1999).

2. Air Sampling Equipment

Below is presented the samplers for particulate pollutants.

- High-Volume, Total Suspended Particulate (TSP) Samplers

High-volume TSP samplers collect all suspended particles by drawing air across an 8- by 10-inch glass or quartz filter. The sample rate is adjusted to 40 cubic feet per minute (CFM), or 1134 liters per minute (L/min), and it is held constant by a flow controller over the sample period. The mass of TSPs can be determined by weighing the filter before and after sampling. The composition of the filter varies according to the analytical method and the detection limit required.

- PM1, PM2.5, and PM10 Samplers

PM samplers collect particulates with a diameter of 10, 2.5 and 1 microns or less from ambient air. Particulates of this size represent the respirable fraction and thus are of special significance. PM samplers can be high-volume or low-volume. The high-volume sampler operates in the same manner as the TSP sampler at a constant flow rate of 40 CFM; it draws the sample through a special impactor head which collects particulates of 10 microns or less. The particulate is collected on an 8- by the 10-inch filter. The low-volume sampler operates at a rate of approximately 17 L/min. The flow must remain constant through the impactor head to maintain the 10-micron cut-off point. The low-volume PM collects the sample on 47-mm Teflon® filters.

- High-Volume PS-1 Samplers

High-volume PS-1 samplers draw a sample through polyurethane foam (PUF) or a combination foam and XAD-2 resin plug, and a glass quartz filter at a rate of 5-10 CFM (144 to 282 L/min). This system is excellent for measuring low concentrations of semivolatile, PCBs, pesticides, or chlorinated dioxins in ambient air. Area Sampling Pumps These pumps provide flow-rate ranges of 2-20 L/min and have a telescopic sampling mast with the sampling train. Because of the higher volume, this pump is suitable for sampling low concentrations of airborne contaminants (i.e.,

asbestos sampling). These pumps are also used for metals, pesticides and PAH sampling which require large sample volumes.

- Personal Sampling Pumps

Personal sampling pumps are reliable portable sampling devices that draw air samples through a number of sampling media including resin tubes, impingers, and filters. Flow rates are usually adjustable from 0.1 to 4 L/min (or 0.01 to .75 L/min with a restrictive orifice) and can remain constant for up to 8 hours on one battery charge or continuously with an AC charger/converter.

Procedure of sampling

The goal of air sampling is to accurately assess the impact of a contaminant source(s) on ambient air quality. This impact is expressed in terms of overall average and/or maximum air concentrations for the time period of concern and may be affected by the transport and release of pollutants from both on- and off-site sources (USEPA, 2016). The location of these sources must be taken into account as they impact the selection of sampling locations. Unlike soil and groundwater concentrations, air concentrations at points of interest can easily vary by orders of magnitude over the period of concern. This variability plays a major role in designing an air sampling plan. Downwind air concentration is determined by the amount of material being released from the site into the air (the emission rate) and by the degree that the contamination is diluted as it is transported. Local meteorology and topography govern downwind dilution. Contaminant emission rates can also be heavily influenced by on-site meteorology and on-site activities. All of these concerns must be incorporated into an air sampling plan. In addition, resource constraints may affect the complexity of the sampling design. An optimal sampling strategy accounts for the following site parameters:

- Location of stationary sources
- Analytes of concern
- Analytical detection limit to be achieved
- Rate of release and transport of pollutants from sources
- Availability of space and utilities for operating sampling equipment
- Meteorological monitoring data
- Meteorological conditions in which sampling is to be conducted

The sampling strategy typically requires that the concentration of contaminants at the source or area of concern as well as background contributions be quantified. It is important to establish background levels of contaminants in order to develop a reference point from which to evaluate the source data. Field blanks and lot blanks, as well as various other types of QA/QC samples, can be utilized to determine other sources. The impact of extraneous sources on sampling results can frequently be accounted for by placing samplers upwind, downwind and crosswind from the subject source. The analytical data from these different sampling locations may be compared to determine statistical differences.

Methods for sampling preservation and storage

All samples are properly stored from the time they arrive at the laboratory to disposal. Samples are refrigerated at 4 ºC prior to analysis unless method SOPs indicate other storage conditions depending on the analytical compound. Preservation, containers, handling, and storage of air samples are discussed in the specific SOPs for the technique selected. In addition, the analytical method (i.e., U.S. EPA, National Institute for Occupational Safety and Health [NIOSH], and OSHA Methods) may be consulted for storage temperature, holding times and packaging requirements. After sample collection, the sampling media (i.e., cassettes or tubes) are immediately sealed. The samples are then placed into suitable containers (i.e., whirl bags, resealable bags or culture tubes) which are then placed into a shipping container.

Variables of Hazardous Site Exposure

Complex environments such as those associated with hazardous sites pose significant challenges to accurately and safely assessing airborne contaminants. Several independent and uncontrollable variables, most notably temperature and weather conditions, can affect airborne concentrations. These factors must be considered when developing an air monitoring program and when analyzing data. Some demonstrated variables include:

- Temperature. An increase in temperature increases the vapor pressure of most chemicals.
- Windspeed. An increase in wind speed can affect vapor concentrations near a freestanding liquid surface. Dust and particulate-bound contaminants are also affected.
- Rainfall. Water from rainfall can essentially cap or plug vapor emission routes from open or closed containers, saturated soil, or lagoons, thereby reducing airborne emissions of certain substances.
- Moisture. Dusts, including finely divided hazardous solids, are highly sensitive to moisture content. This moisture content can vary significantly with respect to location and time and can also affect the accuracy of many sampling results.
- Vapor emissions. The physical displacement of saturated vapors can produce short-term, relatively high vapor concentrations. Continuing evaporation and/or diffusion may produce long-term low vapor concentrations and may involve large areas.
- Work activities. Work activities often require the mechanical disturbance of contaminated materials, which may change the concentration and composition of airborne contaminants.

Table 10 (ANNEX I) summarizes the equipment of sampling, the volume of air, the holding time and the analytical method for the measurement of the chemicals.

2.1.2 Standard operating procedures for monitoring toxicants in soil

Introduction

Soil samples may be recovered using a variety of methods and equipment. These are dependent on the depth of the desired sample, the type of sample required (disturbed vs. undisturbed), and the soil type. Samples of near-surface soils may be easily obtained using a spade, stainless-steel spoon, trowel, or scoop. All sampling devices should be cleaned using pesticide-grade acetone (assuming that acetone is not a target compound) or methanol, then wrapped in clean aluminum foil, and custody sealed for identification. The sampling equipment should remain in this wrapping until it is needed. Each sampler should be used for one sample only. However, dedicated tools may be impractical if there is a large number of soil samples required. In this case, samplers should be cleaned in the field using standard decontamination procedures.

Apparatus required for sampling

Collection of samples from near-surface soil can be accomplished with tools such as spades, spoons, shovels, and scoops. The surface material can be removed to the required depth with this equipment; stainless-steel or plastic scoops can then be used to collect the sample. This method can be used in most soil types but is limited to sampling near-surface areas. Accurate, representative samples can be collected with this procedure, depending on the care and precision demonstrated by the sampling technician. The use of a flat, pointed mason trowel to cut a block of the desired soil can be helpful when undisturbed profiles are required (e.g., for volatile organic analyses [VOAs]). A stainless-steel scoop, lab spoon, or plastic spoon will suffice in most other applications. Care should be exercised to avoid the use of devices plated with chrome or other materials, as is common with garden implements such as potting trowels.

Procedure of sampling

Soil samples are collected using the following procedure (EPA, 1997):

- Carefully remove the top layer of soil to the desired sample depth with a precleaned spade; Using a pre-cleaned, stainless-steel scoop, spoon, trowel, or plastic spoon, remove and discard the thin layer of soil from the area that came into contact with the shovel;
- Transfer the sample into an appropriate container using a stainless-steel or plastic lab spoon or equivalent. If composite samples are to be collected, place the soil sample in a stainless-steel or plastic bucket and mix thoroughly to obtain a homogeneous sample representative of the entire sampling interval. Place the soil samples into labeled containers. (Caution: Never composite VOA samples);
- VOA samples should be collected directly from the bottom of the hole before mixing the sample to minimize volatilization of contaminants;
- Check to ensure that the VOA vial Teflon liner is present in the cap if required. Fill the VOA vial fully to the top to reduce headspace. Secure the cap tightly. The chemical

preservation of solids is generally not recommended. Refrigeration is usually the best approach, supplemented by a minimal holding time;

- Ensure that a sufficient sample size has been collected for the desired analysis, as specified in the sampling plan;
- Decontaminate equipment between samples.
- Fill in the hole and replace grass turf, if necessary.
- QA/QC samples are used to identify error due to sampling and/or analytical methodologies and chain-of-custody procedures. Field duplicates are collected from one location and treated as separate samples throughout the sample handling and analytical processes. These samples are used to assess total error for critical samples with contaminant concentrations near the action level. Field blanks are prepared in the field with certified clean sand, soil, or water. These samples are used to evaluate contamination error associated with sampling methodology and laboratory procedures.

Methods for preservation and storage

The chemical preservation of solids is not generally recommended. Refrigeration is usually the best approach, supplemented by a minimum holding time.

Table 11 (ANNEX I) summarizes the equipment of sampling, the preservation, the holding time and the analytical method for the quantification of each chemical in soil samples.

2.1.3 Standard operating procedures for monitoring toxicants in water

Introduction

This method describes the collection, preservation, and analysis of toxicants in waters, and includes metals, non-metallic inorganics, and organic compounds (such as herbicides, polychlorinated biphenyls, and aromatic hydrocarbons).

Apparatus required for sampling

Plastic acid washed or glass acid washed sampling containers should be used for the sample collection of individual toxicants.

Procedure of sampling

A minimum of 5 samples is recommended.

Method of Surface water sampling

- A sampling of surface waters (i.e. 0-1 m depth) can be carried out from the shore, standing in shallow water, or from a boat (Environmental Protection Authority., 2005).

- In all situations, face into the current for sampling, so that the person sampling (and boat) is downstream (ideally by several meters) from where the sample is to be collected;
- Disposable plastic gloves should be worn to take a water sample if appropriate, and care should always be taken to avoid contamination. Direct contact with the water in the sample, the top of the container, and the inside surface of the lid should always be avoided;
- Unscrew the cap of the container. Where possible, hold the cap while collecting a water sample rather than placing it on any surface;
- The container should be thoroughly rinsed with sample material first – i.e. filled, capped, shaken and emptied - three times before the water sample to be analysed is collected;
- After rinsing, hold the container near its base and plunge it, neck downward, to just below the water surface (typically 0.25-0.50 m depth). Avoid the collection of any surface films that might be present, unless the surface films or slicks are the samples of interest;
- Turn the container until it points slightly upward and the mouth is directed toward the current. If there is no current, create a current artificially by pushing the container forward horizontally in a direction away from the hand;
- Bring the container to the surface once the container is filled, replace the cap immediately and place the sample bottle under the appropriate storage conditions; and,
- Lids on the sample containers must be air-tight to prevent evaporation during storage.
- For a number of parameters, the sampling frequency is recommended for subsequent comparison.

If surface waters are not directly accessed a means of accessing surface waters without entering them is required. This may be achieved in one of three ways. Firstly, a polycarbonate broomstick handle with a frame on one end that may hold a sampling bottle can be used. Secondly, when surface waters cannot be accessed using the polycarbonate broomstick handle, a submersible pump with a length of polypropylene hose attached should be used to sample surface waters. The third sampling method that could be considered is the deployment of an appropriate depth sampler bottle in the surface waters.

The recommended sampling protocol for ultra-trace analysis uses the dirty hand's clean hands approach. This involves two people both wearing (powder free) polyethylene gloves. The double-bagged bottles are sequentially unwrapped. The first (‘dirty hands’) assistant removes the outer bag and hands the bottle to the second (‘clean hands’) assistant, who removes the inner bag. The clean hands assistant then immerses the bottles by hand, or preferably from the end of a Teflon sampling pole, or fills them with the sample collected using a Teflon coated depth sampler. Bottles are usually rinsed with sample material first – filled, capped, shaken and emptied before being refilled with the sample to be analysed, but advice should be sought from the analysing laboratory if organic chemicals are to be analysed.

Table 12 (ANNEX I) summarizes the equipment of sampling, the volume air, the preservation, the holding time and the analytical method for the quantification of the chemicals included in the Seveso III Directive.

2.1.4 Long-term environmental monitoring

Monitoring at the source of contamination should continue well beyond the point at which the release has been controlled, to confirm that the release has indeed been controlled (WHO, 2009). The media likely to be contaminated should be monitored, and personal monitoring should be conducted, determine the concentrations to which populations or individuals are actually being exposed as they go about normal activities.

If the source and nature of contamination remain uncertain, but adverse health effects continue, environmental epidemiological detective work may be able to identify likely types or sources of chemicals. Information regarding characteristics of the affected population, such as geographical residence, water supply, occupation or leisure pursuits, or use of a particular food or product, can be used to generate hypotheses, which can be tested by environmental or biological measurements.

In many cases, once an incident is over, the environment may continue to be contaminated and this contamination may continue to affect people via many contact media and exposure routes. Often, the level of contamination may need to be monitored regularly and over a long period, and the possible effects on people's health assessed regularly. Assessing environmental contamination is an important component of effectively following up after a chemical release incident (or near incident). Data from long-term environmental monitoring following a chemical release can be used to:

- Evaluate variations from baseline environmental conditions.
- Characterize severity and extent of the chemical incident, and therefore, potential pathways for human exposure.
- Design remediation programs, and also evaluate the effectiveness of remediation actions.
- Evaluate potential impacts on human health following incidents using risk assessment techniques. This can be an important alternative to conducting epidemiological studies.

3 BIOLOGICAL BIOMONITORING IN CHEMICAL INCIDENTS

If an incident involves the release of hazardous materials, it is of great importance to know if and to what extent individuals in the population may be or have been exposed (WHO, 2009). In the interest of public health, it is important to be able to verify if short- or long-term health consequences should be anticipated, even if no clinical symptoms are reported in the first hours of the incident. Emergency responders to a chemical incident will collect information on the type and amount of hazardous materials released. If the release or formation of toxic substances is observed or anticipated, attempts are made to perform environmental measurements. It is difficult to find out how, where and when the public may be exposed. Environmental exposure measurements are not very useful for the purpose of assessment of human health risks if air samples are only taken in the close vicinity of the source rather than in those areas were inhabitants may be exposed. Also, substantial time is required to set up environmental sampling equipment and collect ambient air samples. In the meantime, concentrations may already have substantially decreased or even fallen below the limit of detection in a situation where initially the population may have been exposed to values which are relevant in terms of health risk.

Human BioMonitoring (HBM) may provide a better estimate of the actual exposure to emergency chemical exposures than environmental monitoring.

Indeed, HBM integrates exposure from multiple uptake routes and sources over time and is therefore most suitable to quantify direct contact of humans with potentially hazardous chemical substances in the chaotic setting of a chemical incident with potentially complex exposure scenarios. It allows the targeted exposure assessment in identified susceptible subpopulations such as children, elderly, or individuals with previous illnesses. HBM will thus strengthen emergency medical care and support health risk assessment (Wolff et al., 2005; Scheepers, 2015 in press). It will also bring valuable information to improve health impact assessments and support epidemiological follow-up studies (Ackermann-Liebrich et al., 1992; Edelman et al., 2003; Roorda et al., 2004; Carrasco et al., 2007).

In addition, in those cases in which it is not possible to collect environmental samples during an incident, for example, because of administrative or technical problems, the analysis of biological tissues may be the only remaining alternative to support exposure assessment. The use of biomarkers to estimate exposure should therefore always be considered in health studies after disasters(Organization, 1997; WHO, 2009).

HBM is a suitable tool to support follow-up and health risk management at an individual level by:

- confirming internal exposure to one or more chemical substances;
- relating an exposure to observed clinical symptoms;
- supporting optimal medical care (including decisions to be made for treatment);

- providing information to the patient about the relationship between the chemical incident, individual exposure and potential health effects (risk communication);
- helping to establish the cause of death in a post-mortem analysis.

HBM can also have a role in follow-up health surveillance studies at a group level by:

- providing quantitative individual exposure data that can be used for exposure classification in epidemiological studies related to the health impact of the incident;
- monitoring the need for medical attention and support (necessities of screenings, decontamination plans, etc.);
- informing the general public with respect to concerns for health effects and the relationship of these health effects with the incident.

It has to be underlined that not only persons with very serious health effects (who would be admitted to hospital based on such symptoms) should enroll an HBM campaign. Also if no apparent clinical health effects are reported or symptoms do not appear to be life-threatening, individuals who reported direct or indirect signs of exposure (were close to or at a short distance downwind to the incident location, have smelled emissions, have had direct contact with hazardous materials) should have the opportunity to participate to the HBM campaign. The HBM data obtained in such individuals will help to find answers to additional questions such as:

- Is there (any) uptake in the body of a specific (potential) hazardous substance that is a known constituent of the incident emissions?
- Can reported health complaints of an individual be related to the observed biomarker in body tissue of that individual?
- Can the level of exposure be related to the type and gravity of health effects?
- Is a specific treatment possible and required?

Because of the media attention for (even small) chemical incidents, it is useful to anticipate questions from the general public, e.g. expressed by individuals as representative of subgroups in the population, sharing similar concerns as those of the limited number of victims directly involved.

Despite the above advantages, HBM in connection with chemical incidents is a relatively novel tool that needs some expertise in the application, and it has not yet become a standard procedure in disaster response and management plans.

The aim of this section is to provide a guidance on the applicability and feasibility of the use HBM in exposure assessment following a chemical disaster focusing on the four chemicals addressed by the PEC project.

The organization of an HBM campaign entails a tiered approach involving the selection of the appropriate biomarkers, the identification of the time of sampling and of transport and storage

conditions as well as the outcomes interpretation. These recommendations are consistent in their opinion that HBM programs need to rely on suitable and validated analytical methods, established biomarkers, and toxicologically derived assessment value.

3.1 Considerations in the Design of a human biological monitoring campaign

The combination of environmental, biomonitoring and questionnaire/survey data, regarding lifestyle or nutrition, can be used to established relationships between exposure and health outcomes or early signals that are predictive of adverse health outcomes.

The design of all aspects of the study, such as target population, selection of biomarkers, questionnaire content, and data analysis and interpretation, depending on the research questions. Ethical, logistical and financial limitations should be evaluated so as not to be an obstacle during the investigation of the associations of exposure with adverse health outcomes. Other important factors in shaping an efficient study are : (a) the respect of the policy and regulatory framework within which the program would operate, (b) the identification of the chemical compounds to satisfy the needs of policy drivers on the basis of exposure and toxicity information, (c) the identification of the population groups targeted taking into account susceptibility characteristics such as age, gender and socio-economic status, (d) the identification of the appropriate spatial scale of the monitoring program on the basis of exposure or contamination information and the policy framework within which the HBM program would be called to operate, (e) the selection of the appropriate biological matrices for sampling based on the exposure patterns of the targeted population, (f) the ADME characteristics and the bioaccumulation potential of the targeted compounds, which will used for the determination of the periodicity of sampling, and (g) the availability of analytical methods in the context of the physical and chemical characteristics of the biomarkers, as well as the available human and financial resources.

In the following section, the step-by-step organization and implementation of an HBM campaign in the aftermath of a chemical disaster is summarized.

3.2 Study Conduct

Execution of the study includes the collection of samples, taking into consideration the sampling time and quality assurance depending, the transportation from the field to the laboratory, and the analytical processing in the laboratory. If samples are to be saved for future analyses, biobanking may be a consideration.

3.2.1 Selection of the compounds

The selection of the compounds to be biomonitored is the key of each HBM study since it is a criterion for the determination of following factors.

As it has been mentioned in an environmental monitoring session, the presented guidelines for environmental and human biomonitoring concern the chemical agents associated with an emergency response based on the Seveso III – Directive (Directive. 2012/18/EU). The selection of the mentioned compounds (Table 1) derived from the findings of environmental screening of areas close to chemical and petrochemical industry, which indicate the types of compounds that need to be investigated. Although harmonized biomonitoring protocols should be followed, the selection of the chemicals to monitor has to consider area-specific chemical signatures (Krysiak-Baltyn, et al., 2010). Other factors that determined the selection of the compounds to be biomonitored were the dietary patterns in the different parts of Europe, since diet is a key pathway of exposure, e.g. population living near coastal areas is usually exposed to heavy metals due to seafood consumption, the current and the proposed framework and finally, the existing data and the time trends of already on-going biomonitoring schemes provide the information necessary for re-evaluating population exposure burden.

3.2.2 Selection of the biomarkers

A biomarker is any substance, structure or process that can be measured in the body or its products and influence or predicts the incidence of outcome or disease IPCS (2001) has produced a very thorough overview of the use of biomarkers in risk assessment classifying them into biomarkers of exposure, effect, and susceptibility. In case of biomonitoring in chemical incidents, the aim is to complement environmental data with the collection of human biomonitoring which will allow the identification and the analysis of biomarkers of exposure and effects.

Biomarkers of exposure identify and measure chemical residues in tissue or body fluids, metabolites of the xenobiotic or physiological outcomes that are effects of exposure, often unrelated to the toxic effect of concern in humans. For example, a biomarker might be the concentration of a chemical in blood, the excretion of a metabolite of the chemical in urine over twenty-four hours, or the degree of inhibition of an enzyme known to be affected by the chemical. These data provide information on an individual's total exposure from all sources, preceding the time of the analysis. Biomarkers cannot distinguish between the contributions of various absorption pathways to the internal dose that is reflected in the biomarker level. Samples over time are used to identify population trends. Biomarker data can be used to compare exposures in different subpopulations, such as children, adolescents, or the elderly, or residents of different geographical areas. Ultimately, better information about a population's exposure results in better decisions to protect public health and assist in the prioritization of research and intervention programs.

Biomarkers of effect characterize the impact of exposure to chemicals or contaminants on a targeted system such as the blood. As a result, molecular, cellular, or even systemic effects can be observed before clinical symptoms occur. For example, recovery of DNA adducts from blood or urine may reflect the risk of genotoxicity. Not all individuals with a given biomarker of effect will develop the disease, and this distinction is important to communicate to potentially affected groups. Biomarkers

of effect can indicate preclinical effects observed between exposure and disease and ultimately serve as surrogates for the disease for a population.

The selection of the biomarkers should be based on the relevance and the validity. Relevance refers to the appropriateness of biomarkers to provide information on questions of interest and importance to public and environmental health authorities and other decision-makers, while validity refers to a range of characteristics that is the best approximation of the truth or falsehood of a biomarker (Hernberg S, 1987; Schatzkin, et al., 1990) (Schulte and Perera, 1998) (Fu and Boffetta, 1995), (Bernard, 1995), (Dor, et al., 1999). The validity of a biomarker is a function of intrinsic qualities of the biomarker and characteristics of the analytic procedures (Dor, et al., 1999) (see Table 2 and Table 3 for an example of this distinction).

Table 2 : Factors affecting the validity and feasibility of biomarker studies: analytical procedures. (Dor, et al., 1999)

- Sampling constraints (for example, timing requirements)
- Number of samples necessary for an acceptable precision
- Degree of invasiveness of the sampling procedure
- Availability of storage methods after the sample is taken (to avoid the need for immediate analysis)
- Controlling or reducing the contamination of the sample when it is taken and when it is manipulated in the laboratory
- Simplicity, possibility of routine usage, and speed of the procedure
- Trueness, precision, and sensitivity
- Specificity for the component to be detected: interference must be identified to avoid misinterpretation
- Standardization of the procedure

Table 3 : Factors affecting the validity of biomarkers: intrinsic characteristics of the biomarker.(Dor, et al., 1999)

- Significance: exposure, effect, individual susceptibility
- Specificity in relation to the pollutant or pollutant family
- Sensitivity: capacity to distinguish populations with different exposure levels, susceptibilities or degrees of effect
- Knowledge of its background in the general population

- Existence of dose-response curves between exposure level and marker concentration
- Estimation of the inter- and intra-individual variability
- Knowledge of confounding factors that can affect marker

Information on the toxicology of the chemical substance should contribute to the choice of the biomarker(s). If the parent substance is known to be related to the critical toxic effect and the metabolites are products of detoxification pathways, the parent substance is the preferred biomarker. If the parent is not known to be directly involved in the most critical toxicological pathway, products of bioactivation may be preferred as biomarkers.

After the end of exposure, most biomarkers (if they are free metabolites in solution) are eliminated from the body following first order kinetics. Depending on the half-life, the time window for collection of biological media may be possible up to 3–5 days. However, if for the same chemical substance the formation of hemoglobin adducts was described, the time window would become much longer (up to approximately four weeks) due to the zero-order kinetics of elimination. This means that if sample collection cannot be arranged within a week, it is still possible to consider HBM by choosing a suitable biomarker which is eliminated at a slower pace.

3.2.3 Selecting the target population and preparing an information package

The number of the participants or the sample size survey size depends on the survey objectives and research hypothesis. For the estimation of group-specific reference values with sufficient precision and the meaningful comparison of population groups, the randomly choice of at least 120 individuals per population group is recommended (Poulsen, 1997).

Standardized questionnaires need to be developed by to collect information from the victims of an incident or disaster by interview. With this questionnaire information on the chemical exposures can be collected and individuals can be assigned to different exposure classes (e.g. low – medium – high). Also, information can be collected on the duration of exposure (e.g. between the incident and the exact time of evacuation). Those individuals who are selected for participation receive an information package, including a letter to introduce the study, an invitation to participate in the study and an informed consent form.

3.2.4 Selecting the sample matrices

Once the chemical is absorbed in the body it can be excreted without transformation, excreted after metabolization or stored in various tissues or bones. In general, the choice of the suitable specimen has to be based on the specific characteristics of the compound of interest such as the half-life in the human body, the bioaccumulation potential in human tissue, the analytical precision (LOD-LOQ) of

the technique used for the potential analytes in the respective matrices and the prevalent exposure scenario, besides the practical issues and limitations (ethics, invasiveness).

The application of advanced analytical techniques, which have very low limits of quantitation (LOQ), has expanded the possibilities and enabled the use of non-invasive matrices with a relatively low concentration of xenobiotics in human biomonitoring studies. For example, dioxins, which are lipophilic compounds were measured in adipose tissue containing 65–95% fat in the 1980s, but are today measured in serum containing only 0.5–0.6% fat.

The following table summarizes the advantages and limitations of the proposed biological matrices for human biomonitoring to assess health impacts associated with the accidental release of chemicals from industrial sources.

Table 4 : Biological matrices used in human biomonitoring studies. (WHO, 2015)

Matrix	**Population**	**Advantages**	**Limitations**	**Compounds measured in the matrix**
Urine	General	Non-invasive, easy collection, no volume limitation. Allows analysis of metabolite	The composition of urine varies over time.	Metals/trace elements, organic compounds, tobacco smoke. Metabolites of environmental pollutants. e.g.: mercury, cadmium, arsenic, organochlorine compounds, BPA, organophosphate pesticides, parabens, phthalates, PAHs, benzene.
Blood, serum, plasma	General	In equilibrium with all organs and tissues. Well established standard operating procedures (SOPs) for sampling.	Invasive; trained staff and special materials required. Volume limitation. Special conditions for transport and shipment.	POPs, metals/trace elements, organic compounds, tobacco smoke. e.g.: alkylphenols, mercury, lead, BFRs, dioxins, water disinfection byproducts, fluorinated compounds, organochlorine pesticides, organophosphate pesticides, phthalates, PCBs, dioxins.
Exhaled breath	General	Non-invasive. Direct assessment of exposure through the air.	Limited to volatile chemicals. Difficult sampling, transport, and storage.	Metals, VOCs, disinfection byproducts, e.g.: lead, cadmium, trihalomethanes.

3.2.4.1 Urine

Urine has a long, rich history as a source for measuring health and well-being· probably is the most used matrix in which biomarkers are measured. Some of the advantages are that urine is a readily available sample matrix which is easily accessible in large volumes, can be collected directly from the donors which simplify the fieldwork. Problems related to urine as a biological monitoring matrix are related to the specimen influence by the collection method, timing, and handling. There are also problems related to the wide interindividual variability of urinary flow rate, as well as the great temporal variability in urine composition within individuals (Aylward, et al., 2014). Expressing the results per g of creatinine or adjusting the measured values for the specific gravity of the compounds measured practically addresses this problem. Guidelines for creatinine adjustment and proper data interpretation are available in the literature (Barr, et al., 2004).(Barregard, 1993; Mason, et al., 2001)

Urinary biomarkers are used for rapidly metabolized and excreted compounds, such as non-persistent pesticides, bisphenol A (BPA) and other phenols, parabens, phthalates, volatile organic compounds (VOCs) and PAHs. Urine is also used to monitor exposure to some metals, such as arsenic and inorganic mercury. Urinary mercury reflects short exposure effects to inorganic mercury as well as exposure to organic mercury compounds (Barregard, 1993; Mason, et al., 2001).

3.2.4.2 Blood

Blood is one of the most commonly used matrices in human biomonitoring (HBM) surveys. Blood is the preferred matrix for many chemicals as it is in continuous contact with the whole organism and is in equilibrium with the organs and tissues where chemicals are deposited. Furthermore, the procedures for blood sampling are standardized and many people have become used to giving this kind of sample. A disadvantage of using blood in HBM is the need for invasive sampling, which may have an adverse effect on participants response and compliance rates.

Blood is the most appropriate matrix for measuring biomarkers of exposure to certain metals. For example, most of the information on human exposure to lead is based on the blood lead levels, since the findings from several studies confirm that lead in urine primarily reflects the amount of lead absorbed recently but it may not be a reliable biomarker of exposure over a longer time interval (Donohoe, et al., 2012; Lauwerys RR, 2001). Blood has been also used as matrix for measuring the level of mercury in blood, which indicates recent exposure to both organic and inorganic mercury (Branch, 2008) the level of cadmium, which reflects exposure during the past 2–3 months with a contribution from a long-term body burden (Adams and Newcomb, 2014; ATSDR, 2007b). Moreover, blood is usually used for the analyses of persistent, bioaccumulative compounds, such as perfluorinated compounds, organochlorine pesticides, PCBs, dioxins, brominated flame retardants (BFRs), organotins and metals.

3.2.4.3 Exhaled breath

Breath analysis is a biomonitoring method with many advantages, which is applicable to assessments of human exposure to a large number of VOCs. It has the potential to reflect normal and pathologic metabolic processes in a non-invasive and rapid way, and can be carried out with people at any age and as often as it is desirable. It also has great clinical interest for the detection of some chemical agents as biomarkers of diseases like lung cancer, liver disease, myocardial infarction, and diabetes. The exhaled air analysis may also be used to assess the environmental exposure to toxic compounds and the simultaneous quantification of biomarkers of dose and effect in exhaled air may provide new insights into lung damage occurring in people exposed to inhaled toxicants. (Filipiak, et al., 2012)

Exhaled breath is a matrix which facilitates the direct association of inhaled compound concentrations to exhaled concentrations of toxicological relevance. Since a respective biomarker comes directly from the respiratory system, the actual internal dose at the tissue of interest can be assessed. However, information is primarily limited to target tissues in the respiratory tract and the applicability domain covers only specific types of compounds (e.g. volatile compounds) for a short exposure regime.

Breath analysis also presents some challenges, regarding the sensitivity of the sampling procedure and sample preparation, since the chemical substances eliminated through the exhaled air are unchanged in form, not being metabolized, and exhaled compounds are present at extremely low concentrations, i.e. in the nanomolar range. Both sampling and preparation have to be carefully elaborated to avoid contamination during breath collection and loss of target analytes during sample storage. To improve the sensitivity and precision of measurement of the concentration of these substances in exhaled air, the sample usually has to be concentrated before assay by gas chromatography (Amorim and de, 2007). Techniques are being developed to improve the sensitivity and precision of breath analysis based on indirect and direct measurements (Tang, et al., 2015)

3.2.4.4 Selection of time and frequency of sampling

The toxicokinetics of a chemical substance and its biomarkers determine the feasibility of a successful attempt to detect traces of the exposure in biological media in the follow-up to an incident. Therefore, it is useful to be able to predict the time window for collection of biological samples that will reveal detectable levels for the biomarker(s) of choice. This can be done by calculation of the (ultimate) bound of the time period offering a reasonable probability of (still) being able to detect and quantify the biomarker. This ultimate bound is defined as the period of time *ts* in hours following the incident, counting from the approximate time point during the incident when the level of exposure was presumably the highest, until the time point of sample collection.

As part of this decision-making process, a simple algorithm is proposed for calculation of *ts*. When the biomarker follows first-order kinetics the biomarker concentration (*Ct*) at any time-point t after

the exposure can be calculated as:

$Ct = Ce/2^{(ts/t1/2)}$

Ce is the concentration of the biomarker at the end of the exposure and can be calculated from a linear relationship between the average level of inhalation exposure to the substance of interest at the incident location C_{air} and the concentration *Ct* of the biomarker in the biological media.

3.3 Ethical Aspects

It is essential the ethics approval document be in accordance with the ethical and scientific standards subjects that have been developed and established in international guidelines, including the Declaration of Helsinki, the International Ethical Guidelines for Biomedical Research Involving Human Subjects adopted by the Council for International Organizations of Medical Sciences (CIOMS), and the WHO and International Conference on Harmonisation (ICH) Guidelines for Good Clinical Practice. Within Europe, the Oviedo Convention on research on biological material of human origin (Council of Europe, 1997) and the Data Protection Directive (95/46/EC) (WHO, 2015). This document should provide information about the purpose of the study, the overall design plan, possible risks, and benefits, but most importantly about the data protection proposals, according to the Data Protection Directive 95/46/EC, since data obtained in an HBM study are considered "sensitive personal data" (Angerer, et al., 2007; Haines, et al., 2017; WHO, 2015).

3.4 Start collection of exposure data and biological media

For an effective and efficient collection of biological media, contact should be established with the laboratory or hospitals that will support the (final) analysis. This laboratory can provide information on the type and quality of containers to be used in sample collection, the method of sample collection, necessary pretreatment of the samples and conditions for storage and transportation. Depending on characteristics of the study population and the method and location of sample collection, three different pathways for the transportation of biological samples are shown in Figure 2.

In particular cases, it may be needed to follow-up individuals at different time points to support the correct interpretation of the HBM outcome in relation to the exposure event.

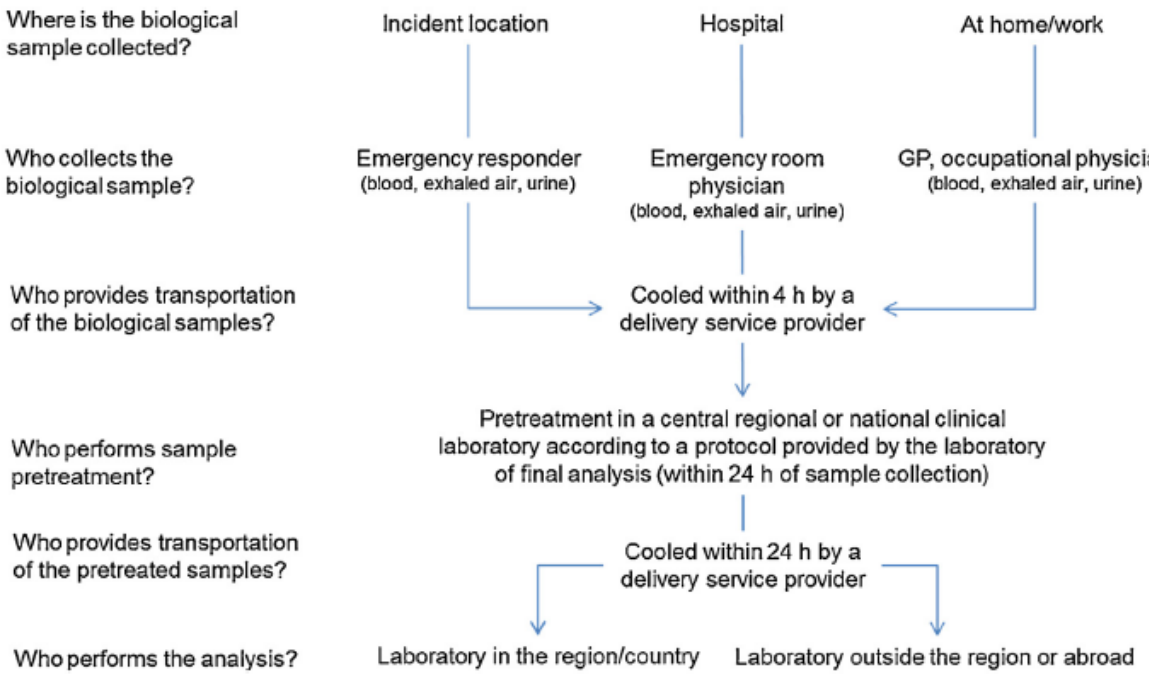

Figure 2 : Schematic of sample collection and pre-treatment and storage prior to delivery of the samples the laboratory that performs the analysis.

3.5 Sampling and storage

Generally, standard procedures are available for the most frequently used matrices such as blood and urine. Many national and international guidelines exist, outlining the proper methodology to adequately sample and store different tissues (TNO, 2005; WHI, 1997). Samples are generally stored frozen between -20°C and -80°C, though matrices containing metals may be stored at 4°C. However, caution needs to be taken so that storage does not alter the concentration of contaminants under study. Since sampling and storage always carry a risk for cross-contamination of samples, appropriate choice of containers and cleaning procedures may be important to take into account. For example, Reid, et al. (2007) showed that common lab equipment and components such as syringes, pipette tips or parafilm may leach out significant amounts of phthalates, thus potentially produce cross-contamination of blood samples.

3.6 Statistical Analysis

The first principle of bioinformatic analysis is that must follow the strategies initiated in study design. Appropriate analysis methodology in biomonitoring studies depends on the target of inference, which is associated with the proper characterization of the sample population that must have been outlined at the beginning of the study, based on specific hypotheses. In order to make it

possible to evaluate the consistency and randomness of the final findings, the data should have different targets of inference with resulting differences in statistical analysis, for example, "exposed" or "not exposed". Closely tied to the target of inference are the measurement process and its precision. In case that only a single population of inference is of interest, the precision of the measurement is the key driver and sample size determinations should reflect the required precision, but if comparisons between two populations are desired, then the magnitude of the difference between the two populations becomes the key driver in the sampling effort. (Alexander, 2015; Banerjee and Chaudhury, 2010; NASEM, 2006; Pfeffermann, 2013)

The first step of the construction of a statistical model is the description of the basic characteristics of each variable, such as the number of observations, mean value, standard deviation, minimum, and maximum values, which will reveal the data below the limits of detection, the missing or miscoded data, the outliers, and the associations among the variables in case of more than three variables. Usually, histograms, scatterplots, and box-whisker plots are used for the exploration of data structures unanticipated from the numerical summaries. Two key considerations, in case of an exploratory study, are the selection bias and confounding. (Axelrad and Cohen, 2011; Axelrad and Cohen, 2011)

The goal of the undergoing and future biomonitoring studies is to provide longitudinal data since that allows the detection of the shifts more precisely. The Achilles heel of longitudinal studies is missing data, due to participant's death, moving from the area, and refusal to continue with the study (NASEM, 2006). Statisticians have developed powerful tools for the analysis of longitudinal data, such as mixed-effect regression models, generalized estimating equations, and logistic-regression models (Locascio and Atri, 2011).

The choice of the statistical model is crucial, so the analyst should keep in mind that at the simplest level, a model fits the data, at the next level, a model predicts the data, at its most useful level, a model shows unanticipated features of the data and the research, and this is the ideal especially for biomarker research. That means that linear models may be easier to be understandable, but they also be only rough approximations of the real world. In case of the investigation of exposure and health effect relationships, the most exquisite characterization of association does not necessarily constitute evidence of causation. (Locascio and Atri, 2011; NASEM, 2006)

In biomonitoring, there are two axes of potential reference around which comparisons may be pertinent and indeed important. The first is in relation to comparison subjects when adequate information exists. For example, it might be reasonable to express lead concentrations as "comparable with concentrations seen in the population not occupationally exposed to lead" "within the range of concentrations seen in workers exposed to lead under well-controlled conditions," or the like. The second axis for interpretation of results is relevant only in the small number of situations where information is available to link a biomarker concentration to a risk or clinical effects, is a comparison with such risk or health-effect concentrations. Those are not assessments of

normality or health effects, merely comparisons with other potentially relevant populations that could lead to research inferences or clinical or public-health interventions. (NASEM, 2006)

3.7 Interpretation of outcome of an HBM campaign

National authorities have established health-based guideline values for acute exposure to chemical substances to support decision making about interventions during the incident (e.g. an instruction to take shelter or evacuate the population at risk) (Bruckner, et al., 2004). Acute exposure guidance levels (AEGLs) and emergency response planning guidelines (ERPGs) are used to intervene in the course of an incident to mitigate the consequences of an incident for health and environment. It is suggested that they may serve as a framework for the process of early decision making on the application of HBM or not.

An alternative to intervention values is the concept of biological equivalents (BEs, Boogaard and Money, 2008; LaKind et al., 2008). BEs may be used for conversion of environmental exposure standards (for the general population) or from occupational exposure standards (for emergency responders) chemical substances to the corresponding biomarker of interest in an HBM campaign. In this way, HBM efforts in the setting of disaster management could benefit from BE values that will be derived from an increasing number of chemical substances.

Another approach would be to compare the outcome of an HBM campaign to the biomarker level found in other populations that can be compared to the study population, especially if the objective of the HBM campaign is to verify if and to what extent the population involved in a chemical incident was exposed at an elevated level compared to the background exposure level for that chemical substance in the general population. Such reference values can be taken from large HBM studies conducted in the US or Germany (Schulz et al., 2007; Joas et al., 2012; Kirman et al., 2012) or from studies in occupational groups.

One might also consider involving a low or no-exposed group in the HBM campaign (as an internal reference). There are certainly methodological reasons in favour of this approach: individuals with no or low exposures could be matched with individuals from the index population classified as potentially highly exposed, e.g. by age, ethnicity, and gender and biological samples could be collected during the same period which rules out possible confounders such as overall changes in the general air quality. However, an important restriction for the involvement of a reference population is that ethics approval may be required in such study as the individuals in this reference group do not have a direct personal (health) benefit that the exposed victims might have.

In addition, for emergency responders and also for other persons who have an employment it is worthwhile to verify with their occupational physician if they have been involved in previous HBM programs targeting the same biomarkers because in that case, it would be possible to use this historic exposure information as a framework of reference.

3.8 Recommendations for sampling, handling, and storage biological matrices

The following sections includes general information and recommendations, as well as the standard operating procedures for the sampling, handling and transport of biological matrices (urine, blood, exhaled breath) in case of environmental incident, since SOPs are an excellent tool to achieve reliable HBM results Each standard operating procedure provides information on the following components (where appropriate): General notes, Types of collection, Sampling Container, Methods for Specimen Preservation, Methods for Specimen Sampling, Handling, and Storage. An extended table based on chemical compounds biomonitoring according to Seveso III – Directive (Directive. 2012/18/EU) is presented in ANNEX II (Table 8) showing for each chemical compound the correct matrix and the proper method for the analysis.

3.8.1 Urine

General Notes

Since urine is one of the most used matrices for biomonitoring purposes, well-standardized procedures for collection, transport, sample preparation, and analysis have become the basis of an effective diagnostic strategy for urinalysis. As reproducibility of urinalysis has been greatly improved due to recent technological progress, preanalytical requirements of urinalysis have gained importance and have become stricter. There are certain recommendations/criteria that need to be met for proper collection and transportation of urine specimens. This will ensure proper stability of the specimen and more accurate test results.

Types of Collection

Laboratory urine specimens are classified by the type of collection conducted or by the collection procedure used to obtain the specimen. The First Morning Specimen should be the specimen of choice for urinalysis, according to the: The Four Corner State Biomonitoring Consortium (4CSBC, 2017). The first-morning void is the most concentrated; it contains higher levels of analytes since it is collected when the patient first wakes up in the morning, having emptied the bladder before going to sleep. This type of collection is preferable for participants who have atypical work/sleep schedules, since the urine can be collected over an eight-hour period. Any voided urine should be pooled, refrigerated and logged, so that a true 8-hour specimen is recorded. In case the participants are infants and small children, a special urine collection bag is adhered to the skin surrounding the urethral area. Once the collection is completed, the urine is poured into a collection cup or transferred directly into an evacuated tube with a transfer straw. Urine collected from a diaper is not recommended for laboratory testing since contamination from the diaper material may affect test results.

Sampling container

First of all, all urine collection and/or transport containers should be clean and free of particles or

interfering substances and they should have a secure lid and be leak-resistant. Leak-resistant containers reduce specimen loss and healthcare worker exposure to the specimen while also protecting the specimen from contaminants. It is recommended to choose one use containers that are made of break-resistant plastic, which is safer than glass, made of a material that does not allow the leach interfering substances into the specimen. The National Committee for Clinical Laboratory Standards (NCCLS) guidelines for urine, GP-16A2, recommend the use of a primary collection container that holds at least 50 mL, has a wide base and an opening of at least 4 cm. The wide base prevents spillage and a 4 cm opening is an adequate target for urine collection. The 24-hour containers should hold up to 3L. NCCLS, also, recommends the use of an amber colored container for specimens, because colorant prevents the degradation of certain analytes (NCCLS, 2001).

Methods for Specimen Preservation

The use of preservatives may be helpful for particular analytes. NCCLS guidelines recommend the use of chemical preservatives if the specimen cannot be processed within 2 hours of collection. Otherwise, these specimens should be refrigerated at 2-8°C. Unfortunately, a universal preservative that allows a complete urinalysis does not (yet) exist. When preservative tubes are used for the transferring, maintaining the correct specimen-to-additive ratio, which will be further used to ensure accurate test results, is especially important. Maintaining the correct ratio can be achieved with the use of an evacuated tube system to achieve proper fill volume because underfilling the tube will leave a high concentration of preservative in the specimen, while overfilling the tube will overly dilute the preservative (NCCLS, 2001). For reducing the risk of errors commercially available vacuum systems have been, also, developed, allowing direct sample aspiration into a secondary container, for reducing the risk of errors and contamination (Delanghe and Speeckaert, 2014). Vacuum systems can only be used for chemical analysis and are not recommended for particle analysis. Evacuated systems also reduce the potential exposure of the healthcare worker to the specimen.

Methods for Specimen Sampling, Handling and Storage

According to the Standard Operating Procedure for urine sample Collection of The Four Corner State Biomonitoring Consortium (4CSBC, 2017), during the initial visit, the Field Technician must inform the participant of biomonitoring, sampling procedure, and test results protocol. Then, he should obtain patient consent. The Field Technician should, also, review the Urine Sample Collection Instruction sheet and the Urine Collection Checklist with the participant, and make the schedule for sample pickup time, as well as, the schedule for the reminder notifications with the participant. Note that reminder notification should be administered 12 to 24 hours prior to scheduled pick-up time, and the notification method should be in accordance with the method of contact on test results protocol. Another responsibility of field technician is to demonstrate proper uncapping, capping and sealing of the container in biohazard Ziploc bag, using an example container. He should match label ID on specimen container to participant ID on forms prior to

handing container, form, and bag to the participant. Before the return, he must complete the First Visit section of Sample Collection Process Checklist. Upon return, the Field technician should verify that all the necessary forms were properly completed and sample procedure was followed appropriately. Then, he should pack refrigerated sample in transport cooler on dry ice. Only, powder-free nitrile gloves must be used for specimen handling.

Four Corner State Biomonitoring Consortium (4CSBC, 2017) recommendations include the best practicing regarding the labeling. Labels must be placed on the urine specimen containers specific to the participant ID for each sample collection. They should, also, include the collection date and time. The information on the container label and the requisition must be matched. If the collection container is used for transport, the label should be placed on the container and not on the lid, since the lid can be mistakenly placed in a different container. Note that some labels are unsuitable for specimens stored under refrigerated conditions because of a lack of adhesion at low temperatures. The global SPREC code, a coding system based on the standard preanalytical options which have recently been published for biospecimen research purposes, could be used. For example, U24-PIX-B-A-N-A-J corresponds to a 24-hour urine (U24) specimen that has been collected in a collection tube with protease inhibitors (PIX), whose pre-centrifugation delay is <2 hours at 3°C to 7°C; centrifugation has been done at an ambient temperature at <3,000 g without braking. Only one centrifugation step was done (N) and the delay between centrifugation and freezing was <1 hour at 3°C to 7°C. Urine was stored in >5 mL polypropylene tubes at a temperature between −85°C and −60°C (Betsou, et al., 2010).

The Laboratory Technician or the Chemist in the laboratory should prepare clean workspace, layout absorbent pad and place all necessary collection samples on the workspace (pipettes, specimen collection vessel, 15 mL metals-free falcon tubes). Then, he should prepare the necessary aliquots based on the analytical techniques will be used for data acquisition, wearing safety glasses, disposable lab coat, and disposable, powder-free nitrile gloves. Note that in case of contamination, the gloves should be changed.

Both the Field Technician and the Laboratory Technician should document the exact sampling time and delays exceeding the specified limits. Information regarding the time lag between sampling and analysis, and the differences in sampling, handling or storage conditions, will be useful during bioinformatic approaches, especially for batch effect correction.

According to the European Consensus Expert Group Report (Yuille, et al., 2010), biobanking procedures for urine should consider the following recommendations:

(i) cells and particulate matter should be removed; A mild centrifugation (pre-centrifugation) at 1000-3000 RCF for 5 min at 4ºC and filtration, before the storage in case that the analysis will be not performed right after the sampling, is preferable,

because fresh urine is characterized by the presence of human cells, bacteria, fungi, sperm counts, and non-cellular components (Bernini, et al., 2011).

(ii) storage of specimens at a temperature of −80 °C or lower; The findings from several studies suggest avoiding shipping urine samples on cool packs or at room temperature for durations of more than 8 h. Also, the recommended storage temperature is at least -20 ºC. It is highly recommended to minimize the number of freezes and thaw cycles to ensure the integrity of urine samples (Rotter, et al., 2017).

(iii) time limits for processing should be experimentally defined;

(iv) specimens should be stored without additives unless specified for a particular downstream analysis.

Unfortunately, for many biomarkers, urine is not the most reliable indicator of exposure because it often contains excreted metabolites instead of parent compounds (Paustenbach and Galbraith, 2006) hence urinary biomarkers are often used for rapidly metabolized and excreted compounds such as non-persistent pesticides, BPA and other phenols, parabens, phthalates, VOCs and PAHs, as well as arsenic and inorganic mercury. Because chemicals are often slowly excreted over the course of hours or days after exposure, also toxicokinetic factors may hamper the usability of urine as a matrix. Although this can be reduced by collecting 24-hr samples rather than single spot samples, the timing of sample collection remains an essential aspect of biomonitoring using urine as a matrix (Barr et al. 2005; Kissel et al. 2005).

3.8.2 Blood

General Notes

The total analytical process comprises of pre-analytical, analytical and post-analytical phases. With increased laboratory instrumentation and automation and technology advancements, the accuracy of results in laboratory (or analytical) phase has become a smaller concern. However, the pre-analytical stage, the way the blood test sample is collected, pre-processed, stored and transported is an important step, which is still neglected. According to some studies, pre-analytical phase accounts for almost 68.2% of the errors in the total testing process, much higher than analytical phase with 13.3% errors. As anyone might conclude, these errors result in higher healthcare costs due to avoidable investigations & inferior patient care. Of all the errors occurring during all the stages, errors in the pre-analytical stage are the hardest to detect and may go un-identified many a times. Therefore, prevention of these errors at the pre-analytical stage is the way forward. The errors in pre-analytical stage can happen either in sample collection, handling, transport, storage, and pre-processing (Plebani and Carraro, 1997). In order to improve the quality of blood specimens and the safety of participants World Health Organization (WHO) produced specific guidelines.

Types of Collection

Depending on the volume of blood needed for the procedure and the type of laboratory analysis to be done, sample collection can proceed through arterial, finger-prick or heel-prick. In case of biomonitoring studies, the proposed sampling method is the arterial, since the obtained sample's volume could reach 7.5 mL, while the sample's volume from a finger could range from 100 to 200 μL (Sarah Fidler, 2017)· The most widely used analytical method for a variety of biomarkers is the liquid chromatography, which demands at least 1 mL for sample preparation. Also, arterial blood comes directly from the lung and should provide a more direct window to events occurring in the lungs, like oxidative stress, perturbations of which have been associated to exposure to air pollutants (Delfino, et al., 2011).

Regarding the choice of the site there are several different arteries can be used for blood collection. The first choice is the radial artery, which is located on the thumb side of the wrist; because of its small size, use of this artery requires extensive skill in arterial blood sampling. Alternative sites for access are brachial or femoral arteries, but these have several disadvantages and in that they may be harder to locate because they are less superficial than the radial artery, they have poor collateral circulation, and they are surrounded by structures that could be damaged by faulty technique.

Arterial blood sampling is the method of choice for blood sampling in case of participants of any age, but particular in term neonates (Ogawa, et al., 2005; Shah, et al., 2008); however, it requires an experienced and trained phlebotomist. If a trained phlebotomist is not available, the physician may need to draw the specimen. The blood from a capillary specimen is similar to an arterial specimen in oxygen content and is suitable for only a limited number of tests because of its higher likelihood of contamination with skin flora and smaller total volume.

Blood sampling from a finger-prick or heel-prick also referred as "capillary sampling" or "skin puncture". Capillary sampling from a finger, heel or (rarely) an earlobe may be performed on participants of any age, for specific tests that require small quantities of blood. The finger is usually the preferred site for capillary testing in an adult patient, while the sides of the heel are only used in pediatric and neonatal patients. Earlobes are sometimes used in mass screening or research studies.

Methods for Specimen Sampling, Handling and Storage

In case of phlebotomy, there are several components of quality assurance. First, the education and the training, which should include an understanding of anatomy, awareness of the risks from blood exposure, and the consequences of poor infection prevention and control, is necessary for all staff carrying out phlebotomy. For blood sampling, after samples have been taken from a patient or a participant in a cohort study, a system of identification and tracking is essential to ensure that the sample is correctly matched with the result and with the patient or participant. Storage and transportation conditions of the sample play important role in the quality of the results. Another element of quality assurance according to WHO, is the existence of a system for reporting all adverse events. A log book or register should be established with accurate details of the incident,

possible causes, and management of adverse events. Last, Standard Operating Procedures (SOPs) are required for each step or procedure. They should be written and be readily available to health workers. This report includes the description of SOPs for arterial and capillary blood sampling, as have been described from WHO in "WHO guidelines on drawing blood: best practices in phlebotomy'', in 2010, since these methods are commonly used in case of biomonitoring studies.

Best practices for the requisite collection, handling and transportation equipment and supplies, in case of arterial blood collection, includes a pre-heparinized syringe, needles, where the choice of length is depending on the chosen site), a safety syringe with a needle cover that allows the syringe to be capped before transport, without manually recapping, a bandage to cover the puncture site after collection, a container with crushed ice for transportation of the sample to the laboratory (if the analysis is not done at the point of care), and where applicable, local anesthetic and an additional single-use sterile syringe and needle (WHO, 2010).

As in every specimen sampling procedure, the first step for sampling from the radial artery using a needle and syringe includes the introduction of the phlebotomist to the participant, and vice versa. Then, the phlebotomist should inform the participant of biomonitoring, sampling procedure, and test results protocol. Next, the participant should be placed carefully on their back, lying flat. An Allen test must be performed for collateral circulation. The test has been modified since 1929 when was first described by Edgar V. Allen, to evaluate the adequacy of collateral circulation or to locate the artery. To perform the modified Allen's test, the examiner compresses both arteries while the participant's fist is clenched (Fuhrman, et al., 1992). Once a site is identified, the examiner should note anatomic landmarks in order to be able to find the site again. After performing hand hygiene, and preparing a bedside work area and the necessary supplies, the phlebotomist should put on an impervious gown or apron, and face protection, and start the sampling procedure by disinfecting the sampling site on the patient with 70% alcohol and allow it to dry. Holding the syringe and needle like a dart, the examiner should use the index finger to locate the pulse again, inform the patient that the skin is about to be pierced then insert the needle at a 45-degree angle, approximately 1 cm distal to the index finger, to avoid contaminating the area where the needle enters the skin. The examiner must advance the needle into the radial artery until a blood flashback appears, and then allow the syringe to fill to the appropriate level, without a pull back the syringe plunger. In the final step of sampling from the radial artery the examiner must withdraw the needle and syringe; place a clean, dry piece of gauze or cotton wool over the site and have the patient or an assistant apply firm pressure for sufficient time to stop the bleeding, and check after 2-3 minutes if the bleeding has stopped. Five minutes or more may be needed for patients who have high blood pressure or a bleeding disorder or are taking anticoagulants. The examiner must activate the mechanisms of a safety needle to cover the needle before placing it in the ice cup. He should, also, expel air bubbles, cap the syringe and roll the specimen between the hands to gently mix it, and then cap the syringe to prevent contact between the arterial blood sample and the air, and/or to prevent leaking during transport to the laboratory, and label the sample. Note that labels should include participant ID for

each sample collection, and the collection date and time. After disposing, appropriately of all used material and personal protective equipment, remove gloves and wash hands, the examiner should check the patient site for bleeding or any other malaise and thank the patient. The sample should immediately transport to the laboratory, following laboratory handling procedures (WHO, 2010).

There are several potential complications related to arterial blood sampling, including arteriospam, haematoma, nerve damage, fainting or a vasovagal response, and other problems like a drop in blood pressure, complaints of feeling faint, sweating or pallor that may precede a loss of consciousness. Arteriospasm or involuntary contraction of the artery may be prevented simply by helping the patient relax; this can be achieved, for example, by explaining the procedure and positioning the person comfortably. Haematoma or excessive bleeding can be prevented by inserting the needle without puncturing the far side of the vessel and by applying pressure immediately after blood is drawn. Due to the higher-pressure present in arteries, pressure should be applied for a longer time than when sampling from a vein and should be supervised more closely, to check for cessation of bleeding. Nerve damage can be prevented by choosing an appropriate sampling site and avoiding redirection of the needle. Fainting or a vasovagal response can be prevented by ensuring that the patient is supine (lying down on their back) with feet elevated before beginning the blood draw (WHO, 2010).

Inappropriate collection and handling of arterial blood specimens can produce incorrect results. Reasons for an inaccurate blood result include the presence of air in the sample, a collection of venous rather than arterial blood, an improper quantity of heparin in the syringe, or improper mixing after blood is drawn, and a delay in specimen transportation. In order to avoid this collection and handling errors, the staff should follow SOPs procedures and the best practices as these have been described by European and international organizations, and/or medical laboratories (WHO, 2010).

In case of pediatric and neonatal blood sampling venepuncture is the proposed method of choice for biomonitoring; however, it requires an experienced and trained phlebotomist, and probably the parent. As has been described in SOPs a winged steel needle, usually 23 or 25 gauges, with an extension tube (butterfly), is more preferable. Note that the examiner should keep the tube and needle separate until the needle is in the vein. The first step includes the collection of the equipment and the supplies, following the hand hygiene. Then the parent should immobilize the baby or the child, in order the examiner be able to put the tourniquet on the patient about two finger widths above the venipuncture site. After that, the examiner has to attach the end of the winged infusion set to the end of the vacuum tube and insert the collection tube into the holder until the tube reaches the needle, remove the plastic sleeve from the end of the butterfly and finally, disinfect the collection site and allow to dry. Then the examiner must use a thumb to draw the skin tight, about two finger widths below the venipuncture site, and push the vacuum tube completely onto the needle. Blood

should begin to flow into the tube. After the required amount of blood has been collected, the examiner should release the tourniquet (WHO, 2010).

3.8.3 Serum/Plasma

Blood derivatives like serum and plasma are also very common biofluids in biomonitoring studies. The following protocol, suggested by (Dunn, et al., 2011), describes the separation of whole blood to serum and plasma.

The serum is obtained by taking the blood sample and allowing it to clot naturally. The clot is then removed to leave the serum, while plasma is prepared by mixing blood with an anticoagulant followed by centrifugation at 4 °C to separate the plasma from the formed components of the blood (red and white blood cells and platelets). A number of anticoagulants are available, including potassium EDTA, citrate and lithium heparin. Both citrate and EDTA can interfere with subsequent metabolic profiling, either by introducing interfering peaks or, in the case of citrate, by obscuring the endogenous analyte. For this reason, the preferred use of lithium heparin is recommended for preparing plasma samples for general analysis. EDTA is also commonly used as an anticoagulant, although we do not recommend it. Following the preparation of serum and plasma, aliquots (0.5 ml) should be rapidly frozen and stored at − 80 °C until analyzed.

Another protocol suggests for the collection of the whole blood the red-topped tubes available from Becton Dickinson a covered test tube should be used (Puskas, et al.). BD's trade name for the blood handling tubes is Vacutainer. After the collection of the whole blood, the researcher should allow the blood to clot by leaving it undisturbed at room temperature for 15–30 minutes, and then remove the clot by centrifuging at 1,000–2,000 x g for 10 minutes in a refrigerated centrifuge. The resulting supernatant is designated serum. Following centrifugation, it is important to immediately transfer the liquid component (Amiot, et al.) into a clean polypropylene tube using a Pasteur pipette. The samples should be maintained at 2–8°C during the handling procedure. If the serum is not analyzed immediately, should be apportioned into 0.5 ml aliquots, stored, and transported at –20°C or lower. It is important to avoid freeze-thaw cycles because this is detrimental to many serum components. Samples which are hemolyzed, icteric or lipemic can invalidate certain tests.

For plasma separation, the whole blood should be collected into commercially available anticoagulant-treated tubes e.g., EDTA-treated (lavender tops) or citrate-treated (light blue tops). Heparinized tubes (green tops) are indicated for some applications; however, heparin can often be contaminated with endotoxin, which can stimulate white blood cells to release cytokines. Cells are removed from plasma by centrifugation for 10 minutes at 1,000–2,000 x g using a refrigerated centrifuge. Centrifugation for 15 minutes at 2,000x g depletes platelets in the plasma sample. The resulting supernatant is designated plasma. Following centrifugation, it is important to immediately transfer the liquid component (Babaknejad, et al.) into a clean polypropylene tube using a Pasteur pipette. The samples should be maintained at 2–8°C while handling. If the plasma is

not analyzed immediately, the plasma should be apportioned into 0.5 ml aliquots, stored, and transported at –20°C or lower. It is important to avoid freeze-thaw cycles.

In order to choose the preferable anticoagulant, you have to know the scientific question you want to answer or in other words, the metabolites you want to identify. For example, EDTA as an anticoagulant is poorly suited for the analysis of polar metabolites (Jorgenrud, et al., 2015; Lopez-Bascon, et al., 2016).

Regarding storage conditions, metabolite and lipid stability in plasma samples that have been stored for up to 4 days at room temperature demonstrate the importance of immediate cold storage of blood samples. It appears that metabolites and lipid degradation commence after only a few hours. Small, polar metabolites are more stable than lipids and amino acids and long-chain fatty acids have approximately the same levels after 4 days. Clotting temperature in serum samples does not have such a significant effect, although lower molecular weight carboxylic acids tend to have higher levels when samples are left clotting at room temperature (Jorgenrud, et al., 2015).

3.8.4 Exhaled Breath

Breath samples can be used in two important fields: (1) clinical diagnosis to analyse volatile compounds generated in the organism and eliminated through exhaled breath (endogenous compounds); and, (2) exposure analysis in order to have fast, accurate information regarding the levels of potentially noxious inhaled volatile organic compounds (VOCs) reaching the blood stream (exogenous compounds). Breath tests cover the fraction of nitric oxide in expired gas (FeNO), volatile organic compounds, variables in exhaled breath condensate (EBC) and other measurements. For EBC and for FeNO, official recommendations for standardised procedures are more than 10 years old and there is none for exhaled VOCs and particles in exhaled breath (WHO, 2015) . The aim of this document is to provide recommendations for standardisation of sample collection and evaluation of different analytic approaches.

General Notes

The chemical substances that exist mainly in the gas phase at room temperature are eliminated unchanged, principally through the lungs. According to Henry's law, the amount of a given substance eliminated through the lungs is proportional to its vapor pressure. Breath analysis is based on the equilibrium between alveolar air and pulmonary capillary blood. The compounds present in exhaled breath are proportional to their blood concentrations because of rapid gas exchange at the blood–gas interface in the lungs. No specialized transport systems have been described for the excretion of toxic substances by the lungs. These substances seem to be eliminated by simple diffusion. The elimination of gas is, in general, inversely proportional to its absorption by the lungs. This behavior is true for a gas or a vapor that does not have a special affinity for certain blood components. When it is inhaled, gas molecules diffuse from the alveolar space into the blood and then dissolve. The uptake of a gas by a tissue usually involves a simple physical dissolution. This

dissolution facilitates the partition of gas molecules between the air and blood during the absorptive phase and between blood and other tissues during the distribution phase. The chemical substances present in the alveoli remain there sufficiently long to reach equilibrium with the blood. The contact of inhaled gas with blood is continued in the alveoli, and equilibrium occurs easily and quickly. At equilibrium, the ratio of the concentration of the chemical in the blood to that in the gas phase is constant. This is called the blood–gas partition coefficient, and it is unique for each gas (Amorim and de, 2007).

Exhaled breath can be influenced by environmental exposures based on direct inhalation, dermal exposure or consumed food. A part of inhaled substances will be retained in the upper airways, while another part enters the lung for further mass transfer, distribution, and metabolism. The water solubility and partial pressure are probably the most important properties affecting the mass transfer of organic compounds between aqueous phase and gas phase; thus, this determines the partition between blood stream and alveolar capillary membrane and so the transfer rate of VOCs from blood to alveolar breath (Filipiak, et al., 2012).

The interpretation of biological monitoring of VOC using breath analysis as a biomarker requires that special attention is paid to factors that influence pulmonary excretion: ventilation-perfusion, diffusion–adsorption-desorption, metabolism, breathing technique, temperature, blood composition and time.

Types of Collection

It is necessary to distinguish between two different types of samples when we refer to exhaled breath: (1) exhaled breath vapor (EBV); and, (2) exhaled breath condensate (EBC). Only volatile compounds form EBV. The main fraction (>99%) comprises a mixture of nitrogen, oxygen, CO_2, water vapor, and inert gases. The remaining fraction (<100 ppmv) is formed by a mixture of hundreds of VOCs in a wide range of concentrations (ranging from a few ppmv to pptv). EBC is more complex, as it is a mixture of the breath expired from the lungs and the aerosolized droplets emerging with the breath. EBC contains both volatiles and non-volatile, and these must be recognized as separate entities with different properties. But, it has been found that conventional EBC-collection methods yield significantly lower sensitivity in the analysis of VOCs than specific EBV-concentration methodologies (e.g. solid-phase micro-extraction (SPME)). When VOCs are the analytes of interest, EBV sampling is preferred (Alonso and Sanchez, 2013).

Breath analysis may rely on both direct (on-line) and indirect (off-line) reading methods: in the on-line method, breath analysis is immediately available, whereas the use of indirect methods generally involves collecting, trapping and pre-concentration of the breath sample and subsequently transferring it to an analytical instrument for analysis. Various kinds of breath samples have been used in biological monitoring, including mixed expired air and end-expired air: end-exhaled-air

represents the alveolar air concentration and mixed-exhaled-air represents the gas mixture coming from the dead space of the bronchial tree and the alveolar gas-exchange space (Corradi and Mutti, 2005).

Direct sampling methodologies integrate sampling and pre-concentration into one single step, which can avoid the problems related to storage in containers. These methodologies are based on the direct collection of target VOCs on a sorbent material, which presents better stability and permits longer storage times (Alonso and Sanchez, 2013).

Indirect methods involving collection devices to obtain and to transport the samples are less expensive, and, at the moment, seem to be the most appropriate methodology for obtaining on-site breath samples. There are many different sampling techniques for breath analysis. Several containers are used to sample exhaled air, such as glass tubes and plastic bags, from which a sub-sample is transferred directly to the analysis system by means of syringes, or solid adsorbents, from which the components are thermally desorbed. This is a time-consuming process consisting of a number of steps that may lead to loss of compounds. Compounds may also be adsorbed onto the surface of the containers. Therefore, several sampling devices have been proposed to guarantee efficient sampling that will truly represent the content of exhaled air during the exhalation time. The device must necessarily have a safe storage system to prevent the loss of the analyte between sampling and the analysis itself (Amorim and de, 2007).

One of the main problems when dealing with breath analysis is the limited volume of sample that can be obtained. Moreover, breath needs to be collected under careful conditions that include monitoring the breathing. The average total lung capacity of an adult human male is about 6 L of air, but only a small amount of this capacity is used during normal breathing. In each expiration, almost 500 mL of breath is expired. The first portion is ‘‘dead space air’’, which comes from the mouth, trachea, and bronchi, so it does not involve a gaseous exchange between air and blood. The remaining fraction is ‘‘alveolar air’’, which comes from the lungs, so it does include a gaseous exchange between air and blood. Exhaled breath is a mixture of dead space and alveolar air. The volume of breath sample that is usually collected currently ranges from a few mL when VOCs are directly retained into a sorbent device to 1 L when the breath is collected in a gas-sampling container. To collect more than 0.5 L of the sample, it is necessary to use forced-expiratory sampling or to collect samples from tidal breathing over several expirations. This methodology has been proposed to obtain a steady flow of representative alveolar air, but it has many drawbacks:

(1) it is highly dependent on the volunteer’s cooperation and effort;

(2) breath-holding with the lung full or partially emptied gives different results;

(3) there is no control of the volunteer’s breathing.

This should not be recommended as a sampling procedure for quantitative analysis (Alonso and Sanchez, 2013).

Sampling by collecting different exhalations during tidal breathing would seem to be the most reliable methodology, despite breathing patterns being irregular and the existence of random fluctuations in breathing frequency and intensity, making it necessary to collect breath samples from a series of cycles. In tidal breathing, different types of samples can be collected depending on the aim of the study:

(1) mixed expiratory or total breath sampling;

(2) time-controlled sampling (i.e. sampling over a predetermined time after the beginning of the expiration);

(3) alveolar or end-tidal sampling.

In mixed expiratory sampling, there is no need to identify each fraction and the whole expired air is directly collected into an appropriate device. In clinical work, this sampling procedure should not be used, as it is subject to dilution and contamination by exogenous substances from the dead space air. However, the analysis of this fraction is required when substance concentrations in the airways are of interest, as is the case in exposure analysis (Alonso and Sanchez, 2013)

The best results are obtained by the simultaneous determination of CO_2 in an expired breath as a corrective factor. A CO_2 controller is commonly used as CO_2 concentrations are higher and practically constant in the alveolar phase. When CO_2 levels increase and plateau out, portions of breath can be obtained by using a syringe (single breath sample) or connecting a collection device to the system (several breaths) (Alonso and Sanchez, 2013).

Most methods have two common limitations: (1) the surface of the plastic, glass and metal containers may be adsorbents, and the losses may be significant, especially when low concentrations are to be determined; (2) the sample must be transferred to an analytical instrument, generally by using syringes. Despite its simplicity and low cost, the use of syringes leads to injection repeatability errors and to possible problems with leakage. Sampling, storage, transport, and transference of the final air sample to the analysis system are critical elements for the successful analysis of exhaled air. Many of these problems may be solved by direct analysis methods such as infrared spectroscopy and mass spectrometry. Furthermore, the use of portable equipment enables field work and minimizes sample processing and laboratory transport losses (Amorim and de, 2007).

Sampling Container

The most common methodology for breath collection is to use polymer sampling bags, due to the ease with which they can be manipulated, their reduced cost and the possibility of reuse. These bags

must be made of inert materials to avoid both diffusion and reactions between the compounds and the bag. The most commonly used material is Tedlar but other materials such as Teflon, FlexFoil, and Nalophan are also used (Alonso and Sanchez, 2013).

Table 5 : Typical collection devices of human breath and their respective characteristics. (Tang, et al., 2015)

	Collection devices	Advantages	Disadvantages
Initial ones	Tedlar bags, enact cartridges Sampling tube/bulb with two end opening	Simple	Time-consuming; hard to control; losses; contamination; other technic issues
Modified ones (for alveolar breath)	Spirometer system Adsorption tube Single breath canister (SBC)	Rapid recycle sampling Losses controlled Dead space component minimized	Complex Relatively poor efficiency Complicated processing procedure before use; adsorption on the canister surface
	Haldane-Priestly tube	Simple; portable	Potential adsorption capability of the tube
	Bio-VOC sampler	Good preservation of original sample; convenience; commercial available	No control of subject's breathing and CO_2 level

For human biomonitoring, the preferable device is the Bio-VOC® sampler, which is commercially available and is considered simple and affordable for exposure monitoring. The sampler captures the final portion of an exhalation, the end-expired air. This sample is then transferred into a stainless-steel tube packed with an adsorbent material. Any solvents present in breath are trapped in the tube, which is sent to the laboratory for analysis (Kwak, et al., 2014). The Bio-VOC breath sampler has three components: a mouthpiece, a volumetric sampler (tube/syringe) that retains around 100 mL air, and a plunger. The subject is asked to blow through a disposable mouthpiece into a cylinder with an open end. Only the last portion (100–150 mL) of breath is retained by the Bio-VOC sampler, which is all regarded as coming from the alveolar portion of lungs. Next, the sample is steadily pushed with a piston into a sorbent tube (which is capped immediately) or Tedlar bag via a connection to the previously opened end of the cylinder (Tang, et al., 2015). The gas sample only remains in the container for a few seconds with this device and no losses are expected.

Although the manufacturers state that only alveolar air is collected, there is no control of the subject s breathing and CO_2 levels.

The low concentrations of compounds in exhaled breath make the use of a pre-concentration technique prior to analysis necessary. Pre-concentration onto a solid sorbent followed by thermal desorption is the most frequently indicated method for the analysis of exhaled air samples. Sorbent traps present the advantages that they can be prepared on a microscale and coupled on-line with a GC system to allow near real-time measurements, and the sorbent configuration can be easily changed to adapt to different compounds. Solid-phase microextraction (SPME) has been demonstrated to have a great potential in the analysis of VOCs in exhaled air and has been applied to the analysis of chemical substances present in human expiration in the nanomolar range (Amorim and de, 2007).

Collection of exhaled air using SPME may be applied either passively or actively. Passive sampling requires the collection of breath in a plastic bag or some other kind of sample container for extraction at a later time. In the case of active sampling, the individual expels breath directly onto the fiber. The SPME fiber can be directly exposed in the mouth of a subject through an SPME device adapted with a Teflon tube with a small opening to the coated fiber (Amorim and de, 2007).

Table 6 : Summary of the principal studies using solid-phase microextraction (SPME) as preconcentration technique and devoted to exposure analysis. (Alonso and Sanchez, 2013)

Coatings	Target VOCs	LOD	Sampling collection device
CAR/PDMS	Isoprene	17 ug/m^3 (SPME) 1 ug/m^3 (sorption)	8 L Tedlar bag SPME inside bag, 10 min at 40°C
PDMS/DVB	Acetone	0.1 ug/m^3	3 L Tedlar bag (max. storage 6 h) SPME inside bag, 4 min at 40°C
PDMS	Tetrachoroethylene	300 ug/m^3	125 mL glass bulb (exposed 1 min)
PA PDMS CW/PDMS PDMS/DVB	Ethanol Acetone Isoprene	276 ug/m^3 116 ug/m^3 20 ug/m^3 (PDMS/DVB coating)	Fiber directly to mouth (10 s)
PDMS CW/DVB PDMS/DVB DVB/CAR/PDMS	Benzene	6 ug/m^3	Fiber directly to mouth (30 s

Coatings	Target VOCs	LOD	Sampling collection device
CW/PEG	2-aminoacetophenone	7 ug/m^3	1 L glass bulb (24 h fiber)
CAR/PDMS	Acetone Acetonitrile Benzene n-butane Dimethylsulfide Furan 2-methylfuran Isoprene Limonene Toluene	5 ug/m^3 25 ug/m^3 0.2 ug/m^3 12 ug/m^3 10 ug/m^3 6 ug/m^3 7 ug/m^3 0.6 ug/m^3 11 ug/m^3 0.4 ug/m^3	3 L Tedlar (20 mL vials, 10 min at 37°C)

In-tube SPME is a more robust and reproducible SPME technique and needle trap devices (NTD) are particularly practical. An NTD is an extraction trap that contains sorbents within a needle, which can be transported and stored easily. NTDs are inexpensive, reusable, and suitable for the sampling and analysis of VOCs from different sample matrices because of its convenient operation and handling, as well as high concentration efficiency. In particular, benzene, toluene, xylene, and ethyl benzene (BTEX) have good storage stability using NTD. Compared with fiber SPME, this method also has better selectivity.

Methods for Sampling, Handling, and Storage

When sampling the final exhaled air, the first flow of air is discarded and only the final part of the exhalation is collected. The alveolar air is generally about two-thirds of the total volume of exhaled air. Exhaled air consists of a mixture of alveolar air diluted with room air retained in the dead space of the respiratory tract (mouth, nose, trachea, and bronchi). Different breath types, integration of the concentration over different expired volumes and different possible ventilatory states of the subject, such as hypoventilated, hyperventilated or breathing normally before expiration, can lead to results that are very difficult to interpret and are sometimes quite far from the real blood concentration (Amorim and de, 2007).

According to the proposed SOP procedure for the exhaled breath collection, the participant should take a breath, exhale excess inspired air and the lung tidal volume (until abdomen is tight) and fill the bag with the remaining air from the lungs (the functional residual capacity). This procedure is repeated until the bag is full (Filipiak, et al., 2012; Harshman, et al., 2016).

All exhaled breath VOCs are concentrated on preconditioned stainless steel Tenax TA thermal desorption tubes, selected due to its hydrophobic properties, fitted with brass caps and polytetrafluoroethylene (PTFE) ferrules as recommended in the US EPA TO-17 method. Briefly, 550 mL of exhaled breath (total volume) is pulled through an inline Tenax TA TD tube using a

calibrated MultiRae Pro pump. The maximum observed percent CO2 is recorded via real-time sensor, to confirm an end-tidal portion of the exhaled breath is collected, for each bag sample. Samples with % CO2 less than 3.5% are removed from the analysis. All sample TD tubes are capped (brass caps with PTFE ferrules) and stored at 4 ° C (unless otherwise noted) until analysis. Cold storage is the optimum storage condition for exhaled breath on Tenax TA. Additionally, the results suggest analysis by day 14 in storage will minimize a potential 1– 2 standard deviation gain or loss of VOC concentration.

The above-mentioned sampling, handling, and storage procedure may be different depending on the measured biomarker. The following paragraphs provide a description of the proposed SOP procedures for sampling, handling, and storage of exhaled breath samples, in case of nitric oxide.

Nitric oxide is an important endogenous messenger and inflammatory mediator that is widespread in the human body, functioning, for example, to regulate peripheral blood flow, platelet function, immune reactions, and neurotransmission and to mediate inflammation. Exhaled NO is typically measured during single breath exhalations. First, the subject inspires nitric oxide-free air via a mouthpiece until total lung capacity is achieved, followed immediately by exhalation through the mouthpiece into the measuring device. There is a consensus that the fractional concentration of exhaled nitric oxide (FeNO) is best measured at an exhaled rate of 50 mL per second (FeNO 50 mL/s) maintained within 10% for more than six seconds at an oral pressure between five and 20 cm H_2O. Results are expressed as the NO concentration in parts per billion (ppb), based on the mean of two or three values. A 2011 clinical practice guideline from the American Thoracic Society (ATS) recommended FeNO cutoff values for predicting the presence of eosinophilic inflammation. The guidelines recommended that FeNO less than 25 ppb (<20 ppb in children) be used to indicate that eosinophilic inflammation is less likely and that FeNO greater than 50 ppb (>35 ppb in children) be used to indicate that eosinophilic inflammation is more likely (Harnan, et al., 2015).

Table 7 : Performance characteristics for representative NO analysers (Cristescu, et al., 2013)

Characteristic	**Chemiluminescence**	**Electrochemical**	**Laser-based**
Sensitivity	< 1 ppb in 1 s	>5 ppb in 30 s	100 ppt in 1 s
Response time	< 1 s	>10 s	~1 s
Advantages	Real-time breath monitoring Multiple breath flow rates	Easy to operate Portable device/hand held	Real-time breath monitoring Multiple breath flow rates
Limitations	Need for frequent calibration	Not suitable for multiple flows analysis	Need to be miniaturized
Purchase price	~50 kEUR	<4 kEUR	125 000 USD
Maintenance costs	1800 EUR/year	18 EUR/test	Depends on the laser life time

3.8.5 Exhaled breath condensate (EBC)

Exhaled breath condensate (EBC) is a promising source of biomarkers of lung disease. EBC is obtained by cooling exhaled breath through contact with a cold surface or condenser. Samples are collected as fluid or frozen material and analysed immediately or later for volatile and non-volatile macromolecules. EBC is composed essentially of water, but it also contains slightly volatile and non-volatile compounds which are expired as a bio-aerosol. Bio-aerosols consist of small droplets joining the vapor stream during its passage over the mucous layer lining the lung. Exhaled air is almost in equilibrium with water vapor at the body temperature. Owing to the very large surface area of the lungs, approximately 400 mL of water are lost by evaporation each day. Because the saturation of the exhaled air is nearly complete, the rate of ventilation effectively determines the amount of water lost from the lungs, which is relevant for airway heat transfer. Exhaled water vapor condenses onto a surface when that surface is cooler than its temperature. Therefore, EBC consists almost completely of condensed water vapor. Water vapor does not behave as a solvent of non-volatile solutes, but rather it acts as a vehicle of exhaled molecules joining the vapor stream. Non-volatile molecules are expired as small particles, which are aerosolized and dispersed into condensed water vapor. These particles could be formed at a variety of sites, including the airways, upper respiratory tract, and even the upper gastrointestinal tract (Mutti and Corradi, 2006).

It is important to note that EBC is not a biomarker, but rather a matrix in which biomarkers may be identified, in that way equivalent to blood, sweat, tears, urine, and saliva. EBC may be thought of either as a body fluid or as a condensate of exhaled gas (and therefore not a body fluid). This issue is relevant because of potential government regulatory issues involved with laboratory assessment of “body fluids”. Entirely non-invasive, the collection of EBC takes as little as 5 breaths to collect sufficient sample for assay, although in research practice, substantially longer collection times are often used to assure sufficient sample is available for repeated analysis of multiple biomarkers. Ten minutes of tidal breathing yields 1–2 milliliters of the sample, and is well tolerated (Hunt, 2007).

As EBC mainly consists of water that is practically free of potentially interfering solutes, it is an ideal biological fluid for elemental determinations; EBC elemental analysis may be used to assess target tissue levels of pneumotoxic metals and essential trace elements, and hence the probability of local effects resulting from highly reactive or poorly soluble species retained by the lung for a long time (Mutti and Corradi, 2006).

Table 8 : Commercially available exhaled breath condensate collection systems. (Hunt, 2007)

EBC collection system	Manufacturer	Advantages
ECoScreen I and ECoScreen II	Viasys, USA, Europe	Most commonly published EBC collection system. More common in European centers. Optional package
RTube	Respiratory Research, USA	Total EBC collections performed using RTube than other systems. Multiple collections can be performed

EBC collection system	Manufacturer	Advantages
Anacon	Biostec, Spain	The controllable temperature of the collection. Designed for use on ventilated patients
TurboDeccs	Italchil, Italy	Has both non-disposable and disposable portions. Controllable collection temperature. Moderately portable

Considering that EBC is water practically free of potentially interfering solutes, it represents an ideal biological matrix for elemental determination by relatively common techniques, such as electrothermal atomic absorption spectroscopy (ETAAS) or less commonly available instruments, such as inductively coupled plasma – mass spectrometry (ICP-MS). EBC elemental analysis may be used to assess target tissue levels of pneumotoxic metals and essential trace elements, and hence the probability of local effects resulting from highly reactive or poorly soluble species retained by the lung for a long time. To our knowledge, there are no previous reports considering elements excreted in exhaled air, too. Considering that almost 500 mL of water is expired per day, the amount of metals which are expired every day is not negligible. For several elements, it may represent about 20 % of total daily excretion (Corradi, 2005).

The following issues related to EBC must be resolved:
• Standardization of collection and storage techniques
• Effect of dilution of respiratory droplets by water vapor
• Effect of contamination from oral and retropharyngeal mucosa
• Variability in EBC assays for certain substances, including assay kits for the same biomarker and kit lot numbers from the same manufacturer
• Lack of criterion standard for determining absolute concentrations of airway lining fluid non-volatile constituents to compare with EBC
• Lack of normative values specific to each potential EBC biomarker.

4 CONCLUSIONS

A major component of an effective chemical incident is to establish a routine environmental monitoring programme in high-risk areas. This programme should include periodic measurements of chemical concentrations in various exposure media (i.e. water, soil, air) in areas surrounding potential sources of chemical release, such as industrial sites. Systematic collection and analysis of environmental data, will be important for several reasons. These data should make it possible to:

- detect trends in the types of chemicals commonly involved in incidents
- stimulate epidemiological research likely to lead to control or prevention
- identify risk factors associated with the occurrence of chemical incidents
- lead to improvements in the practice of health and environment officials who are involved in responding to an incident
- perform analyses to pinpoint what additional expertise, training and facilities are needed to deal with incidents
- and finally to stimulate governments to initiate proper incident control mechanisms.

HBM has a long tradition of human exposure assessment in occupational health care. HBM is requested by law in occupational health and is used in public health survey. Even if there is no official definition of HBM it is commonly understood as a method for assessing human exposure to exogenous substances by measuring the substances or their metabolites, or the product of an interaction between a xenobiotic agent and some target molecule or cell in human tissues or specimens. HBM relies on the use of biomarkers in human tissues or specimens.

HBM can be performed for many chemical substances which are in the focus of the worldwide discussion of environmental health, but this number is still small in comparison with the number of chemicals and contaminants in the environment. Depending on biomarkers (BM) used, HBM does not only allow to monitor exposure but also to detect early health effects. BM of biological effect is more closely related to adverse health effects but are more difficult to validate. Protein adducts specifically and sensitively measure exposure as well as biochemical effect. High throughput technologies (Omics technologies) have recently revolutionized the monitoring of BM effect.

HBM implies the development of a study protocol, recruitment of study persons, informed consent, sampling, and sample processing, sample analysis and interpretation, data reporting and communication. HBM programmes can be cross-sectional or vertical in design. Given the wide range of BMs, biological matrices, analytical methods and other influencing factors, the study design is a critical aspect of the efficient use of HBM in risk assessment. This comprises the planning of which BM(s) should be measured, in which biological fluid or tissue, when and how many samples should be collected and from which study populations.

5 REFERENCES

4CSBC (2017) Standard Operating Procedure for Sample Collection of Heavy Metals (As, Hg, U, Cd, Mn, Se), Creatinine, Phthalate Metabolites, 2,4-DCP, 2,5-DCP and Pyrethroids for Biomonitoring.

Abdel Mohsen, M.A., *et al.* (1999) Biomonitoring of n-nitroso compounds, nitrite and nitrate in the urine of Egyptian bladder cancer patients with or without Schistosoma haematobium infection, *Int J Cancer*, **82**, 789-794.

Adams, S.V. and Newcomb, P.A. (2014) Cadmium blood and urine concentrations as measures of exposure: NHANES 1999-2010, *Journal of exposure science & environmental epidemiology*, **24**, 163-170.

Agilent (2012) Using the Agilent 7000B Triple Quadrupole GC/MS for Parts per Trillion Detection of PAH Metabolites in Human Urine, *Application Note*.

Alexander, N. (2015) What's more general than a whole population?, *Emerging Themes in Epidemiology*, **12**, 11.

Alonso, M. and Sanchez, J.M. (2013) Analytical challenges in breath analysis and its application to exposure monitoring, *TrAC Trends in Analytical Chemistry*, **44**, 78-89.

Amiot, A., *et al.* (2015) (1)H NMR Spectroscopy of Fecal Extracts Enables Detection of Advanced Colorectal Neoplasia, *Journal of proteome research*, **14**, 3871-3881.

Amorim, L.C. and de, L.C.Z. (2007) Breath air analysis and its use as a biomarker in biological monitoring of occupational and environmental exposure to chemical agents, *Journal of chromatography. B, Analytical technologies in the biomedical and life sciences*, **853**, 1-9.

Angerer, J., Ewers, U. and Wilhelm, M. (2007) Human biomonitoring: State of the art, *International Journal of Hygiene and Environmental Health*, **210**, 201-228.

ASTM D6303-98. (1998) Standard Test Method for Formaldehyde in Water. ASTM International.

ATSDR (2007b) Toxicological Profile for Lead. Atlanta, GA: Agency for Toxic Substances

and Disease.

Axelrad, D.A. and Cohen, J. (2011) Calculating summary statistics for population chemical biomonitoring in women of childbearing age with adjustment for age-specific natality, *Environmental research*, **111**, 149-155.

Axelrad, D.A. and Cohen, J. (2011) Calculating summary statistics for population chemical biomonitoring in women of childbearing age with adjustment for age-specific natality, *Environmental Research*, **111**, 149-155.

Aylward, L.L., Barton, H.A. and Hays, S.M. (2008) Biomonitoring Equivalents (BE) dossier for toluene (CAS No. 108-88-3), *Regulatory toxicology and pharmacology : RTP*, **51**, S27-36.

Aylward, L.L., *et al.* (2014) Sources of variability in biomarker concentrations, *Journal of toxicology and environmental health. Part B, Critical reviews*, **17**, 45-61.

Babaknejad, N., *et al.* (2016) The Relationship between Zinc Levels and Autism: A Systematic Review and Meta-analysis, *Iranian journal of child neurology*, **10**, 1-9.

ECHO/SUB/2015-713844-PREV11 - PEC: Post-Emergency, multi-hazard health risk assessment in Chemical disasters
Project co-funded by ECHO - Humanitarian Aid and Civil Protection

Bailey, E., *et al.* (1993) Monitoring exposure to 4,4'-methylene-bis(2-chloroaniline) through the gas chromatography-mass spectrometry measurement of adducts to hemoglobin, *Environmental health perspectives*, **99**, 175-177.

Banerjee, A. and Chaudhury, S. (2010) Statistics without tears: Populations and samples, *Industrial Psychiatry Journal*, **19**, 60-65.

Barman N. B. (2000) Chromatographic Techniques for Petroleum and Related Products, *Critical Reviews in Analytical Chemistry*.

Barr, D.B., *et al.* (2004) Concentrations of dialkyl phosphate metabolites of organophosphorus pesticides in the U.S. population, *Environmental health perspectives*, **112**, 186-200.

Barregard, L. (1993) Biological monitoring of exposure to mercury vapor, *Scandinavian journal of work, environment & health*, **19 Suppl 1**, 45-49.

Bernard, V.L. (1995) The Feltham-Ohlson Framework: Implications for Empiricists*, *Contemporary Accounting Research*, **11**, 733-747.

Bernini, P., *et al.* (2011) *Standard operating procedures for pre-analytical handling of blood and urine for metabolomic studies and biobanks*.

Betsou, F., *et al.* (2010) Standard Preanalytical Coding for Biospecimens: Defining the Sample PREanalytical Code, *Cancer Epidemiology Biomarkers & Prevention*, **19**, 1004.

Bettinelli, M., *et al.* (2000) Determination of heavy metals in soils and sediments by microwave-assisted digestion and inductively coupled plasma optical emission spectrometry analysis, *Analytica Chimica Acta*, **424**, 289-296.

Boogaard, P.J., Rocchi, P.S. and van Sittert, N.J. (1999) Biomonitoring of exposure to ethylene oxide and propylene oxide by determination of hemoglobin adducts: correlations between airborne exposure and adduct levels, *International archives of occupational and environmental health*, **72**, 142-150.

Branch, U.C. (2008) *The Global Atmospheric Mercury Assessment: Sources, Emissions and Transport*. UNEP Chemicals Branch, Châtelaine.

Bruckner, K.L., *et al.* (2004).

Buszewski, B., *et al.* (2012) New approaches to extraction techniques in determination of 4,4′-methylenebis(2-chloroaniline) in air and water solutions, *Talanta*, **93**, 117-121.

Cheever, K.L., DeBord, D.G. and Swearengin, T.F. (1991) 4,4'-Methylenebis(2-chloroaniline) (MOCA): the effect of multiple oral administration, route, and phenobarbital induction on macromolecular adduct formation in the rat, *Fundamental and applied toxicology : official journal of the Society of Toxicology*, **16**, 71-80.

Corradi, M. and Mutti, A. (2005) Exhaled breath analysis: from occupational to respiratory medicine, *Acta bio-medica : Atenei Parmensis*, **76 Suppl 2**, 20-29.

CPCB (2011) Guidelines for the Measurement of Ambient Air Pollutants. CENTRAL POLLUTION CONTROL BOARD.

Cristescu, S.M., *et al.* (2013) Methods of NO detection in exhaled breath, *Journal of breath research*, **7**, 017104.

Delanghe, J. and Speeckaert, M. (2014) Preanalytical requirements of urinalysis, *Biochemia Medica*, **24**, 89-104.

Delfino, R.J., Staimer, N. and Vaziri, N.D. (2011) Air pollution and circulating biomarkers of oxidative stress, *Air Quality, Atmosphere & Health*, **4**, 37-52.

Directive. 2012/18/EU of the European Parliament and of the Council *Control of major-accident hazards involving dangerous substances, amending and subsequently repealing Council Directive 96/82/EC*

Dodson, R.E., *et al.* (2014) Urinary biomonitoring of phosphate flame retardants: levels in California adults and recommendations for future studies, *Environ Sci Technol*, **48**, 13625-13633.

Donohoe, D.R., *et al.* (2012) The Warburg effect dictates the mechanism of butyrate-mediated histone acetylation and cell proliferation, *Molecular cell*, **48**, 612-626.

Dor, F., *et al.* (1999) Validity of Biomarkers in Environmental Health Studies: The Case of PAHs and Benzene, *Critical Reviews in Toxicology*, **29**, 129-168.

Dunn, W.B., *et al.* (2011) Procedures for large-scale metabolic profiling of serum and plasma using gas chromatography and liquid chromatography coupled to mass spectrometry, *Nat. Protocols*, **6**, 1060-1083.

Dyne, D., Cocker, J. and Wilson, H.K. (1997) A novel device for capturing breath samples for solvent analysis, *The Science of the total environment*, **199**, 83-89.

El-Haj, B.M., *et al.* (2000) A GC-MS method for the detection of toluene and ethylbenzene in volatile substance abuse, *Journal of analytical toxicology*, **24**, 390-394.

Environmental Protection Authority. (2005) MANUAL OF STANDARD OPERATING PROCEDURES For Environmental Monitoring against the Cockburn Sound Environmental Quality Criteria. Arizona.

EPA (1995) METHOD 525.2 DETERMINATION OF ORGANIC COMPOUNDS IN DRINKING WATER BY LIQUID-SOLID EXTRACTION AND CAPILLARY COLUMN GAS CHROMATOGRAPHY/MASS SPECTROMETRY. NATIONAL EXPOSURE RESEARCH LABORATORY OFFICE OF RESEARCH AND DEVELOPMENT U.S. ENVIRONMENTAL PROTECTION AGENCY.

EPA (1996) METHOD 8315A DETERMINATION OF CARBONYL COMPOUNDS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC).

EPA (1996) SW-846 METHOD 0100 SAMPLING FOR FORMALDEHYDE AND OTHER CARBONYL COMPOUNDS.

EPA (1997) METHOD 1640 DETERMINATION OF TRACE ELEMENTS IN WATER BY PRECONCENTRATION AND INDUCTIVELY COUPLED PLASMA-MASS SPECTROMETRY. U.S. Environmental Protection Agency Office of Water Office of Science and Technology Engineering and Analysis Division

EPA (1997) Soil Sampling. In Document, U.E. (ed).

EPA (1999) Determination Of Volatile Organic Compounds (VOCs) In Ambient Air Using Specially Prepared Canisters With Subsequent Analysis By Gas Chromatography. Center for Environmental Research Information.

EPA (1999) Method IO-3.5 DETERMINATION OF METALS IN AMBIENT PARTICULATE MATTER USING INDUCTIVELY COUPLED PLASMA/ MASS SPECTROMETRY (ICP/MS).

Center for Environmental Research Information Office of Research and Development U.S. Environmental Protection Agency.

EPA (1999) PARTICULATE MATTER (PM2.5) SPECIATION GUIDANCE Final Draft. U.S. Environmental Protection Agency Monitoring and Quality Assurance Group.

EPA (2001) Ammonia -N in water and biosolids by automated coloeimentrt with preliminary distillation. Method 1690. Environmental Protection Agency, Washington.

EPA (2007) EPA Guidelines: Regulatory monitoring and testing Water and wastewater sampling. EPA Guidelines: Regulatory monitoring and testing

EPA, U.S. (2008) Standard operating prosedure of general air sampling guidlines.

Ernstgard, L., Shibata, E. and Johanson, G. (2005) Uptake and disposition of inhaled methanol vapor in humans, *Toxicological sciences : an official journal of the Society of Toxicology*, **88**, 30-38.

Filipiak, W., *et al.* (2012) Dependence of exhaled breath composition on exogenous factors, smoking habits and exposure to air pollutants, *Journal of breath research*, **6**, 036008.

Force, C.B.T. (2005) CEN/BT/Task Force 151 – Horizontal Standards in the Field of Sludge, Biowaste and Soil.

Fu, H. and Boffetta, P. (1995) Cancer and occupational exposure to inorganic lead compounds: a meta-analysis of published data, *Occupational and Environmental Medicine*, **52**, 73-81.

Fuhrman, T.M., *et al.* (1992) Evaluation of collateral circulation of the hand, *Journal of clinical monitoring*, **8**, 28-32.

Gallardo, H., *et al.* (2016) Bromine and bromide content in soils: Analytical approach from total reflection X-ray fluorescence spectrometry, *Chemosphere*, **156**, 294-301.

Haines, D.A., *et al.* (2017) An overview of human biomonitoring of environmental chemicals in the Canadian Health Measures Survey: 2007–2019, *International Journal of Hygiene and Environmental Health*, **220**, 13-28.

Harnan, S.E., *et al.* (2015) Measurement of exhaled nitric oxide concentration in asthma: a systematic review and economic evaluation of NIOX MINO, NIOX VERO and NObreath, *Health technology assessment (Winchester, England)*, **19**, 1-330.

Harshman, S.W., *et al.* (2016) Storage stability of exhaled breath on Tenax TA, *Journal of breath research*, **10**, 046008.

Hawke, L. (2012) Ammonia (plasma, blood). Association for Clinical Biochemistry and Laboratory Medicine (ACB).

Hernberg S, A.A. (1987) *Validation of biological monitoring tests.* . Occupational and environmental chemical hazards: cellular and biochemical indices for monitoring toxicity. . Ellis Horwood, Chichester, England, .

Hunt, J. (2007) Exhaled Breath Condensate—an overview, *Immunology and allergy clinics of North America*, **27**, 587-v.

IEHIAS, I.E.H.I.A.S. (2009) Arsenic (As).

IEHIAS, I.E.H.I.A.S. (2009) Lead (Pb).

ECHO/SUB/2015-713844-PREV11 - PEC: Post-Emergency, multi-hazard health risk assessment in Chemical disasters
Project co-funded by ECHO - Humanitarian Aid and Civil Protection

Jorgenrud, B., *et al.* (2015) The influence of sample collection methodology and sample preprocessing on the blood metabolic profile, *Bioanalysis*, **7**, 991-1006.

Kimura, K., *et al.* (1988) Gasoline and kerosene components in blood--a forensic analysis, *Human toxicology*, **7**, 299-305.

King, J., *et al.* (2010) *Dynamic profiles of volatile organic compounds in exhaled breath as determined by a coupled PTR-MS/GC-MS study*.

Klotz, K., Weistenhofer, W. and Drexler, H. (2013) Determination of cadmium in biological samples, *Metal ions in life sciences*, **11**, 85-98.

Kosch, J. (2005) Total Hydrocarbon Analysis Using Flame Ionization Detector. In, *Environmental Instrumentation and Analysis Handbook*. John Wiley & Sons, Inc., pp. 147-156.

Krysiak-Baltyn, K., *et al.* (2010) Country-specific chemical signatures of persistent environmental compounds in breast milk, *International journal of andrology*, **33**, 270-278.

Kwak, J., *et al.* (2014) Evaluation of Bio-VOC Sampler for Analysis of Volatile Organic Compounds in Exhaled Breath, *Metabolites*, **4**, 879-888.

Ladeira, C., *et al.* (2011) Genotoxicity biomarkers in occupational exposure to formaldehyde--the case of histopathology laboratories, *Mutation research*, **721**, 15-20.

Larson T. E. and Sollo P. W. (1970) DETERMINATION OP FREE BROMINE IN WATER U.S. ARMY MEDICAL RESEARCH & DEVELOPMENT COMMAND Office of the Surgeon General, Washington.

Lauwerys RR, H.P. (2001) Industrial Chemical Exposure: Guidelines for Biological Monitoring, Third Edition. In Raton, B. (ed). FL: Lewis Publishers.

Locascio, J.J. and Atri, A. (2011) An Overview of Longitudinal Data Analysis Methods for Neurological Research, *Dementia and Geriatric Cognitive Disorders EXTRA*, **1**, 330-357.

Lopez-Bascon, M.A., *et al.* (2016) Influence of the collection tube on metabolomic changes in serum and plasma, *Talanta*, **150**, 681-689.

Lu, Y., *et al.* (2015) Alkali dilution of blood samples for high throughput ICP-MS analysis—comparison with acid digestion, *Clinical Biochemistry*, **48**, 140-147.

Malgorzata Trzcinka-Ochocka, R.B., and Beata Janasik (2016) Useful and Fast Method for Blood Lead and Cadmium Determination Using ICP-MS and GF-AAS; Validation Parameters, *Journal of Clinical Laboratory Analysis* 30–139.

Mason, R.P., Lawson, N.M. and Sheu, G.R. (2001) Mercury in the Atlantic Ocean: factors controlling air–sea exchange of mercury and its distribution in the upper waters, *Deep Sea Research Part II: Topical Studies in Oceanography*, **48**, 2829-2853.

Mochalski, P., *et al.* (2013) Stability of selected volatile breath constituents in Tedlar, Kynar and Flexfilm sampling bags, *The Analyst*, **138**, 1405-1418.

Mutti, A. and Corradi, M. (2006) Recent developments in human biomonitoring: non-invasive assessment of target tissue dose and effects of pneumotoxic metals, *La Medicina del lavoro*, **97**, 199-206.

NASEM (2006) *Human biomonitoring for environmental chemicals*. THE NATIONAL ACADEMIES PRESS, Washington, DC.

ECHO/SUB/2015-713844-PREV11 - PEC: Post-Emergency, multi-hazard health risk assessment in Chemical disasters
Project co-funded by ECHO - Humanitarian Aid and Civil Protection

NASEM (2012) *Acute Exposure Guideline Levels for Selected Airborne Chemicals: Volume 13*. The National Academies Press, Washington, DC.

NCCLS (2001) *Approved Guideline GP16-A2: Urinalysis and Collection, Transportation, and Preservation of Urine Specimens; Approved Guideline*. CLSI, formerly NCCLS, Wayne, Pa.

Nethery, E., *et al.* (2012) Urinary polycyclic aromatic hydrocarbons as a biomarker of exposure to PAHs in air: a pilot study among pregnant women, *Journal of exposure science & environmental epidemiology*, **22**, 70-81.

NIOSH (1984) FLUORIDES, aerosol and gas by ISE. NIOSH.

NIOSH METHOD 6015. (1994) Ammonia - EPA.

Ogawa, S., *et al.* (2005) Venepuncture is preferable to heel lance for blood sampling in term neonates, *Archives of disease in childhood. Fetal and neonatal edition*, **90**, F432-436.

Oil Industry Environmental Working Group. (1999) Sampling Protocols and Analytical Methods for Determining Petroleum Products in Soil and Water Ministry for the Environment, Wellington.

Organization, W.H. (1997) Obesity: preventing and managing the global epidemic. Report of a WHO Consultation presented at the World Health Organization, June 3–5, 1997, Geneva, Switzerland, *Geneva, Switzerland: WHO*.

OSHA (1980) Method ORG-18. Diisocyanates 2,4-TDI and MDI. Occupational Safety and Health Administration.

OSHA (1990) The Use of Combination Oxygen and Combustible Gas Detectors. Occupational Safety and Health Administration.

OSHA (1991) Method 91 METHANOL. Occupational Safety and Health Administration.

OSHA (2004) Method PV2139 Kerosene. Chromatography Team Industrial Hygiene Chemistry Division OSHA Salt Lake Technical Center Sandy.

OSHA Method ID-101. (1991) CHLORINE IN WORKPLACE ATMOSPHERES. Occupational Safety and Health Administration.

OSHA., M., ID216SG., (1989) Boron Trifluoride (BF3) Related Information: Chemical Sampling

OSHA., M.I.-. (1990) Bromine In Workplace Atomspheres. Occupational Safety and Health Administration.

Paustenbach, D. and Galbraith, D. (2006) Biomonitoring and Biomarkers: Exposure Assessment Will Never Be the Same, *Environmental health perspectives*, **114**, 1143-1149.

Pfeffermann, D. (2013) *Sample Surveys: Inference and Analysis*. Handbook of statistics (Book 29). North Holland.

Plebani, C., *et al.* (1999) An optimized sampling and GC–MS analysis method for benzene in exhaled breath, as a biomarker for occupational exposure, *Talanta*, **50**, 409-412.

Plebani, M. and Carraro, P. (1997) Mistakes in a stat laboratory: types and frequency, *Clinical Chemistry*, **43**, 1348-1351.

Poulsen, M.H., T, E.; Chistensen, J. M. (1997) CALCULATION AND APPLICATION OF

COVERAGE INTERVALS FOR BIOLOGICAL

REFERENCE VALUES, *Pure & Appl. Chem*, **69**, 11.

Puskas, K., *et al.* (1995) Remediation of oil-contaminated sandy soil in a slurry reactor, *Environment International*, **21**, 413-421.

Reid, A.M., *et al.* (2007) An investigation into possible sources of phthalate contamination in the environmental analytical laboratory, *International Journal of Environmental Analytical Chemistry*, **87**, 125-133.

Richter, E. and Branner, B. (2002) Biomonitoring of exposure to aromatic amines: haemoglobin adducts in humans, *Journal of chromatography. B, Analytical technologies in the biomedical and life sciences*, **778**, 49-62.

Rotter, M., *et al.* (2017) Stability of targeted metabolite profiles of urine samples under different storage conditions, *Metabolomics*, **13**, 4.

Rusch, G.M., *et al.* (1986) Inhalation toxicity studies with boron trifluoride, *Toxicology and applied pharmacology*, **83**, 69-78.

Samarth, R.M., Gandhi, P. and Maudar, K.K. (2013) A retrospective review of cytogenetic studies on methyl isocyanate with special reference to the Bhopal gas tragedy: is the next generation also at risk?, *International journal of occupational medicine and environmental health*, **26**, 324-336.

Sarah Fidler, H.L., Jodi Meyerowitz, Kristin Kuldanek, John Thornhill, David Muir, Alice Bonnissent, Georgina Timson & John Frater (2017) A pilot evaluation of whole blood finger-prick sampling for point-of-care HIV viral load measurement: the UNICORN study, *Scientific Reports*.

Schatzkin, A., *et al.* (1990) Validation of Intermediate End Points in Cancer Research, *JNCI: Journal of the National Cancer Institute*, **82**, 1746-1752.

Schulte, P.A. and Perera, F.P. (1998) *Molecular Epidemiology: Principles and Practices*. Academic Press.

Shah, V.S., *et al.* (2008) Topical amethocaine gel 4% for intramuscular injection in term neonates: a double-blind, placebo-controlled, randomized trial, *Clinical therapeutics*, **30**, 166-174.

Tang, Z., Liu, Y. and Duan, Y. (2015) Breath analysis: technical developments and challenges in the monitoring of human exposure to volatile organic compounds, *Journal of Chromatography B*, **1002**, 285-299.

TNO (2005) Man-Made Chemicals in Maternal and Cord Blood. TNO-report R2005/129. TNO Built Environment and Geosciences.

TOXNET (2015) BROMINE.

USEPA (2016) Ambient Air Sampling. Environmental Protection Agency Science and Ecosysttem Support Division.

Viegas, S., *et al.* (2010) Genotoxic effects in occupational exposure to formaldehyde: A study in anatomy and pathology laboratories and formaldehyde-resins production, *Journal of occupational medicine and toxicology (London, England)*, **5**, 25.

Vijayan, V.K. (2010) Methyl isocyanate (MIC) exposure and its consequences on human health at Bhopal, *International Journal of Environmental Studies*, **67**, 637-653.

WHI (1997) Blood and urine collection, processing and shipment. Women's Health Initiative. Volume 2, Section 11. pp11.1-11.40.

WHO (2003) HYDROGEN SULFIDE: HUMAN HEALTH ASPECTS. Geneva, pp. 41.

ECHO/SUB/2015-713844-PREV11 - PEC: Post-Emergency, multi-hazard health risk assessment in Chemical disasters
Project co-funded by ECHO - Humanitarian Aid and Civil Protection

WHO (2009) Risk Assessment: Manual for Public Health Management of Chemical Incidents. World Health Organization.

WHO (2010) WHO Guidelines on Drawing Blood: Best Practices in Phlebotomy. World Health Organization, Geneva.

WHO (2015) Human Biomonitoring: Facts and figures. WHO Regional Office for Europe, Copenhagen.

Woodrow, J.E., *et al.* (2014) Determination of Methyl Isocyanate in Outdoor Residential Air near Metam-Sodium Soil Fumigations, *Journal of Agricultural and Food Chemistry*, **62**, 8921-8927.

Yuille, M., *et al.* (2010) Laboratory management of samples in biobanks: European consensus expert group report, *Biopreservation and biobanking*, **8**, 65-69.

ECHO/SUB/2015-713844-PREV11 - PEC: Post-Emergency, multi-hazard health risk assessment in Chemical disasters
Project co-funded by ECHO - Humanitarian Aid and Civil Protection

ANNEX I

Table 9 : Summary of the sampling equipment, the volume air, the preservation, the holding time and the analytical method for the quantification of chemiclas to air samples

Chemical family	Chemical compound	Sampling	Condition for storage/transport	Preservation procedure	Minimum Volume Required	Analytical method	Detection limit	Reference
Heavy metals	Arsenic compounds	Sampling with a high/low-volume sampler using glass-fiber filters	Weezers, labels, Petri dishes	Cool to 4°C	No data	ICP MS (a mixture of 3% HNO3 / 8% HCl is the best extraction matrix for total extraction of metals from)	0.9 µg/L	(EPA, 1999)
	Nickel compounds						0.02 µg/L	
	Lead compounds						0.08 µg/L	
Inorganic compounds	Phosphine	Tedlar bags sample bag in the Vacu-Case vacuum pump	No data	No data	1 litre	Thermal desorption	1 µL/m3	(Buszewski, et al., 2012)
	Sulphur dichloride	No data			No data	Ultraviolet Fluorescence (Measuring absorbance at 560 nm)	No data	(CPCB, 2011)
	Hydrogen sulphide	No data			No data	Gold Film Analyzer	0.02 ppb	(EPA, 2008)
	Boron trifluoride	A known volume of air drawn through a midget glass bubbler containing 10 mL of 0.1 M NH4F			30 liters	Orion Model 93-05 Fluoroborate electrode	0.5 µg/mL	(OSHA., 1989)
	Anhydrous Ammonia	Solid sorben tube (sulfuric acid-treated silica gel) A 0.8-micron MCE prefilter may be used toremove particulate interferences.	Cap the sampling tubes with plastic (not rubber) caps immediately after sampling	One day after the ammonia is desorbed	30 liters	Visible Absortion Spectrophotometry (630 or 660 nm)	0.5 µg per sample	(NIOSH METHOD 6015., 1994)
	Bromine	A known volume of air through a midget fritted glass bubbler containing a sodium bicarbonate/sodium carbonate buffer solution	Ship the samples to the laboratory using appropriate packing materials to prevent breakage	No data	30 liters	In the buffer collection solution, bromine disproportionates to bromide and bromate ions. Samples are analyzed with minimum sample preparation as these anions by ion chromatography	0.001 ppm	(OSHA., 1990)
	Chlorine	A known volume of air through a midget fritted glass bubbler containing a 0.1% sulfamic acid solution	No data	Special precautions are not necessary during storage	15 liters	Ion selective electrode [ISE]	0.014 ppm	(OSHA Method ID-101., 1991)

	Fluorine	Sample at 1–2 L/minute using cellulose ester membrane filter and alkaline-impregnated backup pad to collect particulate and gaseous fluorides;				Extract hydrogen fluoride and soluble fluorides with 50 mL 1:1 TISAB: water; insoluble fluorides require NaOH fusion Ion selective electrode [ISE]	3 µg/sample	(NIOSH, 1984) 55
Chemical family	**Chemical compound**	**Sampling**	**Condition for storage/transport**	**Preservation procedure**	**Minimum Volume Required**	**Analytical method**	**Detection limit**	**Reference**
Nitrates	Ammonium nitrate	Chroma membrane cells preextract and preconcentrate sample				Ion chromatography with conductivity detection	6 µg NH_3/sample	(EPA, 1999)
Amines	Piperidine	Charcoal Tube	Sealed with silicone septa and placed on dry ice	No data	10 liters	Gas Chromatography; GC/FID / SPB-5 Column	No data	
	Bis(2-dimethylaminoethyl) (methyl)amin			No data			No data	
	3-(2 Ethylhexyloxy)propylamin			No data			No data	
Aliphatic organic compounds	Formaldehyde	DNPH-Coated Sep-PAK Cartridges	The sample cartridges are capped and placed in borosilicate glass tubes with polypropylene caps	Cool to 4°C		High Performance Liquid Chromatography (HPLC), Method 8315, with an ultraviolet (UV/Vis) detector at 360 nm	0.01 ug/ml	(EPA, 1996)
	Acetylene Ethylene oxide Propylene oxide	Column of silica gel	No data	No data	No data	No data	No data	
	Methylisocyanate	XAD-7 cartridge	The cartridges wrapped in foil and transported on ice packs to the laboratory	Stored under refrigeration (1–7 °C)	0.12 m^3	Liquid chromatography–mass spectrometry	No data	(Woodrow, et al., 2014)
Aromatic organic compounds	4′-Methylene bis (2-chloraniline)	Collection in a bubbler containing nitro reagent in toluene	Stored at 2°C or at room temperature in the dark (23°C).	No data	Sampling rate 20 L at 1 L/min	High pressure liquid chromatography	0.10 ppb (1 µg/m^3)	(Buszewski, et al., 2012; OSHA, 1980)
	Toluene diisocyanate			No data	Sampling rate 20 L at 1 L/min		0.15 ppb (1 µg/m^3)	(OSHA, 1980)
	Polychlorodibenzofurans and polychlorodibenzodioxins	Polyurethane foam (PUF) downstream of a teflon-coated glass fibre particulate filter.	Filter storage container - Typically a glass petri dish sealed with Teflon® - PUF cartridges sealed with nylon end cap - Samples shipped in coolers with freeze packs	No data	Between 1400 m^3 and 3800 m^3	High resolution gas chromatographic column coupled to a high resolution mass spectrometer	No data	
Petroleum products	gasolines and naphthas	collection on Tenax GC cartridge / thermal		No data	No data	Gas Chromatography Mass Spectometry	No data	

		desorpion						
	kerosenes (including jet fuels),	A known volume of air through glass sampling tubes containing coconut shell charcoal	Charcoal tubes were allowed to equilibrate for 6 h	No data	20-L	GC using a flame ionization detector (GC/FID)	4.79 mg/m^3	(OSHA, 2004)

ECHO/SUB/2015-713844-PREV11 - PEC: Post-Emergency, multi-hazard health risk assessment in Chemical disasters
Project co-funded by ECHO - Humanitarian Aid and Civil Protection

Table 10 : Summary of the sampling equipment, the volume air, the preservation, the holding time and the analytical method for the quantification of chemicals to water samples

Chemical family	Chemical compound	Condition for storage/transport	Preservation procedure	Minimum Volume Required	Max.holding time	Analytical method	Reference
Heavy metals	Arsenic compounds	Plastic acid washed or Glass acid washed	Acidify with nitric acid to pH 1–2	500 ml	1 month	ICP-MS	(EPA, 1997)
	Nickel compounds						
	Lead compounds						
Inorganic compounds	Phosphine	Air-tight polyethylene plastic bottles without introducing air bubbles. Sampling during early morning to minimise sun-light induced phosphine oxidation.	Samples were stored in a dark refrigerator (temperature 4 oC)	100 ml	No data	Gas chromatograph / Nitrogen-phosphorus-detector (NPD)	(Environmental Protection Authority., 2005)
	Hydrogen sulphide	No data	No data	No data	No data	Hydrogen sulphide, measured as dissolved sulphide	
	Boron trifluoride	Plastic - Fill container to exclude air	None required	100ml	5 min		
	Anhydrous Ammonia	Plastic or glass (Strict protocol required to reduce effects of contamination. Store in area free of contamination (ammonia vapor may permeate the walls of even high density polyethylene containers. Pressure filtering is required.)	Refrigerate	500ml	6h	Colorimetric determination of indophenol blue following reaction of any ammonia (Method 1690)	(EPA, 2001)
	Bromine	Plastic and Glass	No data	500ml	No data	ICP-MS	(Larson T. E. and Sollo P. W., 1970)
	Chlorine		Keep sample out of direct sunlight	500ml			
	Fluorine		No data	500ml			
	Oxygen	Analyse in the field using an oxygen electrode and follow user manual instructions carefully		A minimum of 5 samples is required		Measurements should be taken 0-0.5 m above the sediment surface. Measurements should be taken in daylight hours at the same time (preferably) for each sampling run, as oxygen levels vary diurnally.	(Environmental Protection Authority., 2005)
Nitrates	Ammonium nitrate	Plastic or glass (Strict protocol required to reduce effects of contamination. Store in area free of contamination (ammonia vapor may permeate the walls of even high density polyethylene containers. Pressure filtering is required.)	Refrigerate	500ml	6h	Ultraviolet Spectrophotometry	(EPA, 2007)

Chemical family	Chemical compound	Condition for storage/transport	Preservation procedure	Minimum Volume Required	Max.holding time	Analytical method	Reference
Aliphatic organic compounds	Formaldehyde	Glass containers without air bubbles	Samples are maintained at 0-4°C	20 mL	Samples must be derivatized within five days of collection	Collected using a DNPH cartridge. / High-performance liquid chromatography	(ASTM D6303-98., 1998)
	Methanol	Glass containers	No data	No data	No data	Gas chromatography, using a Flame Ionized Detector	(OSHA, 1991)
Aromatic organic compounds	4′-Methylene bis (2-chloraniline)	Glass vialw with PTFE Lined septum	Fill container completely exclude air. Acidify with hydrochloric acid to ph 1 to 2 and refrigerate	500ml	1 week	Gas chromatography/mass spectrometry (GC/MS)	(EPA, 1995)
	Toluene diisocyanate						
	Polychlorodibenzofurans and polychlorodibenzodioxins						
	Aminobiphenyl and/or its salts, Benzotrichloride, Benzidine and/or salts, Bis (chloromethyl) ether, Chloro methyl methyl ether, 1,2-Dibromoethane, Diethyl sulphate, Dimethyl sulphate, Dimethylcarbamoyl chloride, 1,2-Dibromo-3-chloropropane, 1,2-Dimethylhydrazine, Dimethylnitrosamine, Hexamethylphosphoric triamide, Hydrazine, 2- Naphthylamine and/or salts, 4-Nitrodip henyl, and 1,3 Propanesultone						
Petroleum products	gasolines and naphthas	Glass solvent washed	Do not pre-rinse container with sample. Refrigerate	1000ml	24 hours	Extract on site where practical. Extract sample container as part of the sample extraction procedure	(Oil Industry Environmental Working Group., 1999)
	kerosenes (including jet fuels),						
	gas oils (including diesel fuels, home heating oils and gas oil blending streams)						
	heavy fuel oils						
	alternative fuels serving the same purposes and with similar properties as regards flammability and environ mental hazards						
	Liquefied flammable gases, Category LPG) and natural gas						

Table 11 : Summarizes the equipment of sampling, the volume air, the preservation, the holding time and the analytical method for the quantification of chemicals to soil samples.

Chemical family	Chemical compound	Condition for storage/transport	Preservation procedure	Minimum Volume Required	Max.holding time	Analytical method	References
Heavy metals	Arsenic compounds	Glass ar with Teflonlined cap	Cool to 4°C (ice in cooler)	10 g	6 months from date sampled	Inductively Coupled Plasma Optical Emission Spectrometry	(EPA, 1997)
	Nickel compounds						(Bettinelli, et al., 2000)
	Lead compounds						
Inorganic compounds	Phosphine	Sealed plastic bag	Cool to 4°C (ice in cooler)	25g	No data	Extract with organic sovent / Gas Chromatography – Flame Photometric Detector (GC-FPD)	(Idler et al. 1981)
	Boron trifluoride	Poly-propylene tubes	No data	10g		Spectrophotometre - (420nm)	
	Anhydrous Ammonia	No data	No data				
	Bromine	Glass ar with Teflonlined cap	Cool to 4°C (ice in cooler)	100 g		Total X-ray fluorescence spectroscopy (TXRF)	(Gallardo, et al., 2016)
	Chlorine						
	Fluorine						(Tarsoly et al., 2010)
Nitrates	Ammonium nitrate	Containers with an appropriate closure material such as PTFE	-20 °C (deep-frozen)	No data	As soon as possible	Flow injection analysis (FIA, reference method) and spectrometric detection	(Force, 2005)
Amines	Piperidine	No data	No data	No data	No data	High-performance liquid chromatography (HPLC) method	
	Bis(2-dimethylaminoethyl) (methyl)amin	No data	No data	No data	No data		
	3-(2-Ethylhexyloxy)propylamin	No data	No data	No data	No data		
Aliphatic organic compounds	Formaldehyde	Glass art with teflonlined cap	Cool to 4°C (ice in cooler)	30 g	10 days to extract from date received	High-Performance Liquid Chromatography (UV Detector)	(EPA, 1996)
	Acetylene Ethylene oxide Propylene oxide						
	Methanol						
	Methylisocyanate						
Chemical	**Chemical compound**	**Condition for storage/transport**	**Preservation**	**Minimum**	**Max.holding**	**Analytical method**	**References**

family			procedure	Volume Required	time		
Aromatic organic compounds	4′-Methylene bis (2-chloraniline)	Two 40-mL vials; no air space	Cool to 4°C (ice in cooler)	15 g	10 days from date received	High-Performance Liquid Chromatograph	(EPA, 1996)
	Toluene diisocyanate						
	Polychlorodibenzofurans and polychlorodibenzodioxins						
	Aminobiphenyl and/or its salts, Benzotrichloride, Benzidine and/or salts, Bis (chloromethyl) ether, Chloro methyl methyl ether, 1,2-Dibromoethane, Diethyl sulphate, Dimethyl sulphate, Dimethylcarbamoyl chloride, 1,2-Dibromo-3-chloropropane, 1,2-Dimethylhydrazine, Dimethylnitrosamine, Hexamethylphosphoric triamide, Hydrazine, 2-Naphthylamine and/or salts, 4-Nitrodip henyl, and 1,3 Propanesultone						
Petroleum products	gasolines and naphthas	Glass ar with Teflonlined cap	Cool to -20°C	50 g	28 days from date sampled	Gas chromatography/mass spectrometry (GC/MS)	(Barman N. B., 2000)
	kerosenes (including jet fuels),					Gas chromatography/mass spectrometry (GC/MS)	
	heavy fuel oils					Liquid extraction with CS2 and by direct injection into the Gas chromatography	

ANNEX II

Table 12 : Chemical compounds biomonitoring according to Seveso III – Directive (Directive. 2012/18/EU).

Chemical Compound		Analyte	Medium	Sample pretreatment	Dectection method	LOD	Reference
Metals	Arsenic compounds	Inorganic arsenic, monomethylated arsenic, and dimethylated arsenic	Urine for recent or chronic exposure Blood for high-level exposure	These samples are placed in polyethylene bottles. Samples stored at-20 °C and thawed at room temperature before analysis. All samples are filtered through 0.2 mm membrane filters before injection onto the HPLC-ICPMS column for analysis.	ICP-MS	0.5-1 µg/l	(IEHIAS, 2009)
	Nickel compounds	Nickel	Whole blood or serum	Alkali dilution method, were aliquots (0.2 mL) of the whole blood or serum samples are diluted 1:25 with an alkali solution consisting of 2% (w/v) 1-butanol, 0.05% (w/v) EDTA, 0.05% (w/v) Triton X-100, 1% (w/v) NH4OH and internal standards (20 µg/L), is preferable. The mixture is sonicated for 5 min and centrifuged at 1000 rpm for 2 min before ICP-MS analysis.		0.01	(Lu, et al., 2015)
	Cadmium compounds	Cadmium	Whole Blood/Plasma/Urine	Dilution with nitric acid, Triton X-100, and butanol		0.011 µg/L (blood), 0.008 µg/L (plasma), 0.007 µg/L (urine)	(Klotz, et al., 2013)

Chemical Compound		Analyte	Medium	Sample pretreatment	Dectection method	LOD	Reference
	Lead compounds	Lead	whole blood	A quantity of 1.6 ml 5% HNO3 in ultrapure quality is poured into a polypropylene tube (volume 4 ml) and then a 0.4 ml venous blood sample is added to avoid premature precipitation. Immediately after blood sampling, the tube is vigorously shaken using a mixer. The samples should be left for at least 1 h at room temperature and next centrifuged for 15 min /speed 11500 at 4°C. After centrifugation, the supernatant is transferred to autosampler tubes and Pb and Cd are analyzed by ICP-MS. All vessels used during the analysis should be pre-washed with 20% nitric acid and deionized water.		0.05-0.1 µg/l	(IEHIAS, 2009; Malgorzata Trzcinka-Ochocka, 2016)
Inorganic Compounds	Phosphine	phosphate	urine	Urine samples are being collected in quality certified precleaned 125 mL amber glass jars with Teflon lids.	LC-MS/MS-GC-MS/MS	0.3ppm	(Dodson, et al., 2014)
	Hydrogen sulfide	free sulfate or thiosulfate	Urine/Blood	no data	GC-FID/LC-MS	3 and 20 mg/m^3	(Lu, et al., 2015; WHO, 2003)
	Boron trifluoride	Free fluoride in serum	Serum/urine	An exposure-related depression of total protein concentrations (up to 16%) accompanied by an exposure-related depression of globulin concentrations (up to 38%). Serum fluoride concentration markedly increases. In urines, an exposure-related depression in calcium amounts and an exposure-related increase in urinary fluoride were noted.	LC-MS	4.1 mg/m^3 (1.5 ppm)	(NASEM, 2012; Rusch, et al., 1986)

Chemical Compound		Analyte	Medium	Sample pretreatment	Dectection method	LOD	Reference
	Anhydrous Ammonia	Ammonia	Urine/Blood/Breath /Saliva	A free-flowing venous (or arterial) blood sa mple should be collected into a specimen tube (preferably pre-chilled) con taining either lithium heparin or EDTA as an anticoagulant and w hich has been determined to be free of amm onia contamination. The sample should be t ransported on ice to the laboratory, separate d within 15 minutes of collection and analys ed immediately. Once separated, plasma [a mmonia] is stable for 4 h at 4 °C and 24 h at -20 °C.	1. Use of a micro-diffusion apparatu s is an indirect met hod, which is ofte n used in point-of-care and dry-slide analysers. 2. The enzymatic method is a direct method and is the most commonly us ed method in the UK. 3. The ammonium electrode is a dire ct method that use s an NH4+-selecti ve membrane, whi ch is typically base d on a mixture of t he antibiotics nonactin and mon o action.		(Hawke, 2012)
	Bromine Chlorine Fluorine Hydrogen Oxygen	Bromide Chloride, Fluoride		No data	ICP-MS/Ion Chromatography	Br_2: 14 mg/kg	(TOXNET, 2015)
Nitrates	Ammonium	Nitrate	Urine	Urine samples are collected in bottles	One ml urine is		(Abdel Mohsen, et

Chemical Compound		Analyte	Medium	Sample pretreatment	Dectection method	LOD	Reference
	nitrate/Potassium nitrate			containing 15 g solid NaOH added to prevent bacterial growth and nitrite decomposition. After recording the total volume, the urines were well mixed, and 2 × 50 ml specimens were removed, and then they are being stored at −20°C. Immediately before analysis, samples are thawed and centrifuged at 1,000g for 10 min, and the supernatants are analyzed for nitrite.	diluted to 5 ml with ammonium chloride/EDTA solution, poured into a cadmium/copper column (5 cm height × 0.7 cm i.d.) and collected. The flow rate is 0.5 ml/min. Aliquots of the eluate (25–100 µl) were analyzed for nitrite as described above. This analysis gives a measure of nitrate plus nitrite in the samples.		al., 1999)
Amines	Piperidine	Piperidine	Urine/Blood	No data	LC-MS/NMR	4.1 mg/m3 (1.5 ppm)	(NASEM, 2012)
	Bis(2-dimethylaminoethyl) (methyl)amin		Blood	No data	GC-MS		(Richter and Branner, 2002)
	3-(2-Ethylhexyloxy)propylamin						
Organic Compounds	Formaldehyde	Formaldehyde	Whole blood/serum/urine	Blood specimen (10 mL) was collected using heparin as anticoagulant. The samples were kept refrigerated and processed within 6 hours of the blood collection. Lymphocytes were isolated using Ficoll-Paque gradient and placed in RPMI 1640 culture medium with L-glutamine and phenol red added with 10% inactivated fetal calf serum, 50 µg/mL streptomycin + 50 U/mL penicillin and 10 µg/mL phytohaemagglutinin. Duplicate cultures	GC-MS/LC-MS		(Ladeira, et al., 2011; Viegas, et al., 2010)

Chemical Compound		Analyte	Medium	Sample pretreatment	Dectection method	LOD	Reference
				from each subject were incubated at 37°C in a humidified 5% CO2 incubator for 44 hours. Cytochalasin-b 6 µg/mL was added to cultures. After 28 hours of incubation, cells were spun onto microscope slides using a cytocentrifuge. Smears were air-dried and double stained with May Grünwald- Giemsa and mounted with Entellan.			
	Acetylene/ Ethylene /Propylene oxide	Acetylene/ Ethylene oxide /Propylene oxide	Urine/Blood/breath/ saliva	Blood samples of approximately 5 ml were collected from the an-tecubital vein into a Vacutainer tube containing sodium edetate as an anticoagulant using a c-radiation-sterilized syringe and needle. The samples were immediately transported to the laboratory for isolation of the erythrocytes.The cells were isolated by centrifu- gation (10 min, 1000 g, room temperature) and washed twice with gentle suspension in isotonic saline followed by centrifugation. The washed cells were suspended in deionized water and then subjected to four cycles of freezing at)80 °C and thawing at 37 °C. The lysed cell suspension was centrifuged at 6000 g for 90 min at room temperature to remove cell debris and nonlysed red cells. The supernatant was transferred into polypropene tubes and stored frozen at)80 °C until analysis.	GC-MS/LC-MS	0.84 mg m(-3)	(Boogaard, et al., 1999)

Chemical Compound		Analyte	Medium	Sample pretreatment	Dectection method	LOD	Reference
	Methanol	Methanol		To achieve higher analytical sensitivity, exhaled air after the exposure was adsorbed on stainless steel adsorption tubes filled with Carbosieve SIII (60/80 Mesh) (Supelco). Samples were desorbed (Perkin Elmer Automated Thermal Desorption system ATD-400) and analyzed using the same GC systems described previously (Nihle´n et al., 1998a). Venous blood (10 ml) was collected from the brachial vein in heparinised tubes (Venoject VT-100H) prior to exposure. Urine was collected in 500-ml glass bottles, which were immediately capped with polyethylene screw caps. Samples were processed within 5 min and analyzed by head-space GC later the same day, except samples taken at home at 9, 13 and 22 h, which were processed the following morning. Further, pH and ammonia in urine were measured as soon as possible the day of sampling. Fractions of urine samples were acidified and stored in glass vials at –20 C for later analysis of calcium and creatinine. Fractions of urine samples were also stored at –20 C for later analysis of formic acid.		2.6 mg/L	(Ernstgard, et al., 2005)
	Methylisocyanate (MIC)	Methylisocyanate		No data		0.02ppm	(Samarth, et al., 2013; Vijayan, 2010)
	4′-Methylene bis (2-chloraniline) (MOCA)		Urine/Blood	Blood collected was transferred immediately to EDTA-containing Vacutainers. A 3-ml aliquot of the whole blood was mixed at room temperature with 5 ml of phosphate- buffered saline (PBS), pH 7.4 and carefully layered onto 3 ml of Histopaque-1077 (Sigma Chemical Co., St. Louis, MO). The lymphocytes and serum were separated by centrifugation at 400g for 30 min. Following this, lymphocytes were transferred to a clean centrifuge tube, washed, with PBS, and stored at -70°C until	LC-MS / NMR / GC-MS		(Bailey, et al., 1993; Cheever, et al., 1991)

Chemical Compound		Analyte	Medium	Sample pretreatment	Dectection method	LOD	Reference
				time of analysis.			
	Toluene diisocyanate			No data		No data	(Aylward, et al., 2008)
	Polychlorodibenzofurans and polychlorodibenzodioxins			No data		No data	
	Aminobiphenyl and/or its salts, Benzotrichloride, Benzidine and/or salts, Bis (chloromethyl) ether, Chloro methyl methyl ether, 1,2-Dibromoethane, Diethyl sulphate, Dimethyl sulphate, Dimethylcarbamoyl chloride, 1,2-Dibromo-3-chloropropane, 1,2-Dimethylhydrazine, Dimethylnitrosamine, Hexamethylphosphoric triamide, Hydrazine, 2-Naphthylamine and/or salts, 4-Nitrodip phenyl, and 1,3 Propanesultone			No specific data		No specific data	

Chemical Compound		Analyte	Medium	Sample pretreatment	Dectection method	LOD	Reference
Petroleum products	gasoline and naphthas, kerosenes (including jet fuels), gas oils (including diesel fuels, home heating oils and gas oil blending streams), heavy fuel oils, alternative fuels serving the same purposes and with similar properties as regards flammability and environ mental hazards, Liquefied flammable gases, Category LPG) and natural gas	Benzene	Breath	Collection in 3-l Tedlar bag, thermostated at 40°C for at least 30 min, transfer to a 1-l bag passing through a CaCl2 trap, add perdeuterated benzene (C6D6) as an internal standard, injection of sample	HRGC-MS	1.5 ng	(Plebani, et al., 1999)
		Toluene	Breath	Collection in a Perkin Elmer automated thermal desorption (ATD) sampling tubes, thermal absorption, injection to GC-MS	GC-MS	No data	(Dyne, et al., 1997)

Chemical Compound		Analyte	Medium	Sample pretreatment	Dectection method	LOD	Reference
			Blood	Collection in a EDTA-2Na-containing vacuum blood tubes, transfer 10-mL into a headspace of vial, insertion of 5mL into a vial charged with chlorine gas, exposure to direct sunlight for 10 min, add of 3 drops of water, swirl for 3 min, evaporation under a gentle stream of nitrogen at 50oC, add 50µL of BSTFA (1% TMCS), heat at 65oC for 20min, injection into the GC-MS	GC-MS	0.088 µg/L	(Dyne, et al., 1997; El-Haj, et al., 2000)
		Xylene	Breath	Collection into 3-liter Tedlar bags in a CO2 controlled manner using breath sampler, injection into an evacuated SPME vial (20mL in volume sealed with a 1.3 mm butyl/PTFE septum, where the pressure in the vial was balanced with high-purity nitrogen (of quality 6.0, i.e. with a purity of 99.9999%).	GC-MS	0.7 ppb (p-xylene) and 0.3 ppb (o-xylene)	(Mochalski, et al., 2013)
			Blood	Collection into into 2.7 mL blood monovettes, add a small amount of heparin, extraction of volatiles from blood samples in 20 mL headspace vials (Gerstel, Germany) crimped with 1.3 mm butyl/PTFE septa (Macherey-Nagel, Germany) and containing stirring bars, add 0.3 mL of Dulbecco's PBS using a glass syringe, transfer heparinized blood samples from the monovette into the evacuated vial, where pressure in the vials was balanced with high-purity nitrogen (6.0–99.9999%), using an appropriate needle adapter, incubation in a temperature-controlled agitator at 37 °C, inense stirring (1200 rpm), add a 75 µm Carboxen-PDMS SPME fiber (Supelco, Canada) into the vials and exposing to the headspace gas for 50 minutes.	GC-MS	0.7 nmol/L (p-xylene) and 0.08 nmol/L (o-xylene)	(King, et al., 2010)

Chemical Compound		Analyte	Medium	Sample pretreatment	Dectection method	LOD	Reference
		kerosene (Analytes: Aliphatic hydrocarbons with carbon numbers over 9 as well as the aromatics with carbon number 9 group including cumene, mesitylene, pseudocumene and 1,2,3-trimethylbenzene)	Blood	Add internal standard, extraction with n-pentane, centrifugation, freeze, add decant solvent, concentrate, injection to GC-MS	GC-MS	50 pg	(Kimura, et al., 1988)
		polycyclic aromatic hydrocarbons (PAHs) (metabolites: 1-hydroxypyrene (1-OHP), 1-hydroxypyrene-O-glucuronide (1-OHP-gluc), 3-hydroxybenzo[a]pyrene, 7,8,9,10-tetrahydroxy-7,8,9, 10-tetrahydrobenzo[a]pyrene, and a number of other hydroxylated PAHs)	Urine	Collection of first-morning voids (minimum 10 ml), enzymatic deconjugation of the analytes, injection to GC-MS	GC-MS	2.5 ppt	(Agilent, 2012; Nethery, et al., 2012)