Human biomonitoring as a tool for exposure assessment in industrially contaminated sites (ICSs). Lessons learned within the ICS and Health European Network

Biomonitoraggio umano come strumento per valutare l'esposizione nei siti industriali contaminati. Lezioni apprese dal network europeo ICSHNet

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ABSTRACT

BACKGROUND: the mixed and complex nature of industrially contaminated sites (ICSs) leads to heterogeneity in exposure and health risk of residents living nearby. Health, environment, and social aspects are strongly interconnected in ICSs, and local communities are often concerned about potential health impact and needs for remediation. The use of human biomonitoring (HBM) for impact assessment of environmental exposure is increasing in Europe. The COST Action IS1408 on *Industrially Contaminated Sites and Health Network* (ICSHNet) decided to reflect on the potential and limitations of HBM to assess exposure and early health effects associated with living near ICSs.

OBJECTIVES: to discuss challenges and lessons learned for addressing environmental health impact near ICSs with HBM in order to identify needs and priorities for HBM guidelines in European ICSs.

METHODS: based on the experience of the ICSHNet research team, six case studies from different European regions that applied HBM at ICSs were selected. The case studies were systematically compared distinguishing four phases: the preparatory phase; study design; study results; the impact of the results at scientific, societal, and political levels.

RESULTS: all six case studies identified opportunities and challenges for applying HBM in ICS studies. A smart choice of (a combination of) sample matrices for biomarker analysis produced information about relevant time-windows of exposure, which matched with the activities of the ICSs. Combining biomarkers of exposure with biomarkers of (early) biological effects, data from questionnaires or environmental data enabled fine-tuning of the results and allowed for more targeted remediating actions aimed to reduce exposure. Open and transparent communication of study results with contextual information and involvement of local stakehold-

WHAT IS ALREADY KNOWN

- **Residents near industrially contaminated sites (ICSs) may be exposed to a range of different contaminants.**
- **Human biomonitoring (HBM) is often initiated to address public concern on health risks related to residence in ICSs.**
- **HBM aggregates uptake from all exposure routes and allows to measure (early) biological effects.**

WHAT THIS ARTICLE ADDS

- **Based on a geographically balanced selection of six European case studies, challenges and lessons learned on using HBM in ICSs are discussed.**
- **This analysis resulted in specifying advantages and challenges of HBM in ICSs.**
- **Recommendations for preparing HBM guidelines in European ICSs are formulated.**

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ers throughout the study helped to build confidence in the study results, gained support for remediating actions, and facilitated sharing of responsibilities. Using HBM in these ICS studies helped in setting priorities in policy actions and in further research. Limitations were the size of the study population, difficulties in recruiting vulnerable target populations, availability of validated biomarkers, and coping with exposure to mixtures of chemicals.

CONCLUSIONS: based on the identified positive experiences and challenges, the paper concludes with formulating recommendations for a European protocol and guidance document for HBM in ICS. This could advance the use of HBM in local environmental health policy development and evaluation of exposure levels, and promote coordination and collaboration between researchers and risk managers.

Keywords: industrially contaminated sites, human biomonitoring, biomarkers, study design, human exposure

RIASSUNTO

INTRODUZIONE: la natura complessa dei siti industriali contaminati (ICS) comporta un'eterogeneità nell'esposizione e nei rischi per la salute di coloro che risiedono nei pressi di queste aree. Gli aspetti sanitari, ambientali e sociali sono strettamente interconnessi negli ICS e le comunità locali sono spesso preoccupate per il potenziale impatto sulla salute e per la necessità di bonifica delle zone in cui vivono. In Europa, il biomonitoraggio umano è sempre più utilizzato come strumento per valutare l'impatto dell'esposizione ambientale. La COST Action IS1408 relativa ai siti industriali contaminati (ICSHNet) propone una riflessione sulle potenzialità e sui limiti del biomonitoraggio umano per valutare l'esposizione e gli effetti sanitari precoci associati al vivere vicino a un ICS.

OBIETTIVI: discutere le difficoltà e le lezioni apprese dall'affrontare l'impatto dell'ambiente sulla salute di chi vive nei pressi di un ICS utilizzando il biomonitoriaggio umano per identificare bisogni e priorità al fine di stendere linee guida riferite agli ICS in Europa.

METODI: basandosi sull'esperienza dei ricercatori del network ICSHNet, sono stati selezionati sei casi studio svolti in diverse regioni europee che hanno applicato il biomonitoriaggio umano a uno o più ICS. I casi studio sono stati confrontati sistematicamente distinguendo 4 fasi: fase preparatoria; disegno di studio; risultati dello studio; impatto dei risultati a livello scientifico, sociale e politico.

RISULTATI: tutti e sei i casi hanno identificato opportunità e problematiche nell'applicare il biomonitoraggio umano a studi sugli ICS. Una scelta oculata di (una combinazione di) matrici di campioni per l'analisi dei biomarcatori ha prodotto informazioni importanti sulle finestre temporali di esposizione, che coincidevano perfettamente con il periodo delle attività industriali negli ICS. Combinando i biomarcatori di esposizione con quelli degli effetti biologici (precoci) e i dati dei questionari o dati ambientali ha permesso di perfezionare i risultati e giungere ad azioni di risanamento più mirate volte a ridurre l'esposizione. La comunicazione aperta e trasparente dei risultati dello studio e la contestuale informazione e il coinvolgimento degli stakeholder locali hanno permesso di rafforzare la fiducia nei risultati dello studio, ottenendo anche sostegno verso le azioni di risanamento e facilitando la condivisione delle responsabilità. Utilizzare il biomonitoraggio umano in questo tipo di studi ha aiutato a stabilire priorità per le azioni di politica e per ricerche future. I limiti sono stati la scarsa numerosità della popolazione in studio, le difficoltà nel reclutare le popolazioni più vulnerabili, la disponibilità di biomarcatori validati e l'affrontare l'esposizione a miscele di composti chimici.

CONCLUSIONI: sulla base delle esperienze positive e sulle difficoltà incontrate, il presente studio si chiude con la formulazione di alcune raccomandazioni per la stesura di un protocollo europeo e un documento di orientamento per gli studi di biomonitoraggio umano da utilizzare nei siti industriali contaminati. Ciò permetterà di promuovere l'uso di questo strumento all'interno delle politiche locali relative ad ambiente e salute e nella valutazione dei livelli di esposizione; in più, sarà utile per la promozione del coordinamento e della collaborazione fra i ricercatori e coloro che gestiscono le situazioni di rischio ambientale.

Parole chiave: siti industriali contaminati, biomonitoraggio umano, biomarcatori, disegno dello studio, esposizione umana

INTRODUCTION

Industrially contaminated sites (ICSs) include a wide diversity of settings[1] and are diverse with respect to the origin of contamination, the nature of the contaminants, the duration of exposure, the exposure pathways, the number of residents in these areas, and the sociodemographic characteristics of the residents.[2] In recent years, ICSs represent a major public health concern in most European Countries,[1,3-5] with studies indicating an increased incidence and prevalence of a broad range of several health conditions.[6,7] Contaminated sites and waste have been included in 2017 for the first time as one priority area in the final Declaration of the 6th Ministerial Conference on Environment and Health signed by the 53 Member States of the European Region of the World Health Organisation (WHO).[8]

In ICSs, health, environment, and social aspects are strongly interconnected, and local communities are often concerned about potential health impact and needs for remediation. This public concern is however mostly addressed by studying single aspects, such as collection of emission data and chemical analysis of environmental samples and locally grown food, which can be used for exposure modelling. Environmental data are usually fragmented, often taken on ad hoc basis and as such not representative for the daily exposure situation.[9] Moreover, this assessment procedure may not be able to address the real health impact experienced by populations living in a contaminated area.

Human biomonitoring (HBM) measures concentrations of environmental contaminants, their metabolites, or markers of (early) biological changes, in easily accessible body fluids (e.g., blood, urine or saliva) or body tissues (such as hair, nails) on an individual level. HBM data reflect the total body burden or biological effect resulting from all routes of exposure and take into account inter-individual variability in exposure levels, metabolism, and excretion rates. Hence, HBM allows direct and more precise assessment of the distribution of exposure in the population incorporating individual variability in exposure.[10,11] In addition, personal information of health and lifestyle may be collected.

The use of HBM as a tool for environmental policy and research in general is developing quickly in Europe, with national or regional programmes[12,13] in, e.g., France, Germany, Sweden, the Czech Republic, Slovenia, and the Flanders region of Belgium. In 2017, the European Joint Programme HBM4EU was launched to support national and European policy making based on evidence on actual exposure of the general population.[14] It represents a joint effort of 28 Countries and the European Commission and is co-funded by Horizon 2020. However, there is not yet a focus on residents near ICSs.

In a recently published special issue on the environmental health challenges arising from industrial contamination,[5] different contributions address the issue of HBM in ICSs. These papers showed HBM is used in ICSs in surveillance studies,[15] exposure assessment for epidemiological studies,[16] mechanistic process studies,[17] and health risk assessment,[18] although the use of HBM in ICS studies is still limited to a small fraction of the available published studies.[7] These studies are often characterised by a fragmentation of objectives, methods, and approaches, but are rarely designed with an integrative multidisciplinary approach. It is, therefore, urgent to promote coordination and collaboration between researchers and risk managers to identify common strategies at European level to deal more systematically with the impact of ICSs.

In response to challenges related to ICSs, the COST Action IS1408 on Industrially Contaminated Sites and Health Network (ICSHNet) was launched in 2015 (supported by the European Cooperation in Science and Technology – COST). The ICSHNet Action aims at consolidating a European Network of experts and relevant institutions, and developing a common framework for research and response with the production of information for decision makers who have to deal with ICS issues.[5] As part of the action, the potential and limitations of HBM to assess exposure and early health effects associated with living near ICSs was considered as another research strategy.

The purpose of this paper is to describe experiences on exposure assessment and detection of early biological effects related to ICS with HBM, based on a selection of HBM case studies conducted in different Countries in Europe. This analysis intends to identify challenges and lessons learned for addressing environmental health impact and for promoting public health interventions in ICS based on HBM results. We conclude with formulating recommendations for a European protocol and guidance document for HBM in ICSs.

MATERIALS AND METHODS

Based on the goals of ICSHNet COST Action (https://www.icshnet.eu/) and on discussions during the Third Plenary Conference of this Action, held on the Aristotle University of Thessaloniki (Greece), 6-10 February 2017, one of the targets of the 'Exposure assessment' working group (WG2) was to reflect on the use of HBM to assess exposure and early health effects related to ICSs and formulate advice and lessons learned from the HBM experts of WG2. With the ambition to present a balanced distribution from different European geographical areas, covering also some cultural and socioeconomic diversity of Europe, a selection of case studies distributed across different European Countries was conducted and discussed during the Fourth Plenary Conference of this Action, held on WHO European Centre for Environment and Health (ECEH), UN Campus, Bonn, Germany, 21-22 February 2018. This strategy resulted in six case studies:

1. ICS Genk-Zuid in Belgium;
2. the community of Mammari in Cyprus;
3. the red mud disaster in Hungary;
4. Modena in Italy;
5. the Piekary Śląskie area in Poland;
6. the Panasqueira mine area in Portugal.

All these case studies concerned the residential exposure of people living within the vicinity of one or more ICSs. We requested information from the centres that conducted the studies and asked for both published and unpublished information on the case studies. The analysis was conducted to identify differences in the process pursued in each case study to set up and run an HBM study using a four-stages HBM framework from our own experiences (table 1):

1. the pre-phase;
2. the study design and fieldwork;
3. the results and interpretation;
4. the impact of the study.

The so-called **pre-phase** is conducted prior to the start of the HBM study. Following, five key elements were checked: • availability of data on environmental pollu-

STAGE	KEY CRITERIA
Pre-phase	Data available on environmental pollution and residents' health conditions
	Public concern
	Involvement of stakeholders and local actors
	Key decision to set-up HBM
	Funding
Study design and fieldwork	Type of study
	Biomarkers of exposure and selection criteria
	Biomarkers of effect and selection criteria
	Information on determinants of exposure
	Selection of the study population
	Selection of the study area
Results	Participation rate
	Exposure assessment
	Health assessment
	Identified determinants of exposure or health effects
	Identified vulnerable populations
	Communication of the results: which audiences targeted
Impact of the study	Short-term impact
	Long-term impact
	Levels: scientific, societal, policy making
	Involvement of stakeholders and local actors

Table 1. Four stages of a HBM study and key criteria.
Tabella 1. Le 4 fasi e i criteri fondamentali di uno studio di biomonitoraggio umano.

tion and health conditions of the residents; • public concern and awareness; • involvement of stakeholders and other actors in early stages of the process; • key decisions that facilitated the instigation the HBM study; • how the study was funded.

In the **second stage** (study design and fieldwork), the information was checked that related to: • type of study and the research question; • selection of biomarkers of exposure and • selection of health outcomes; • determinants of exposure studied; • selection of the study population; • definition of the study area.

The **third stage** is on study results and included information on: • participation rates; • exposure levels; • health assessment; • identified determinants of exposure or effects; • indications for vulnerable sub-populations. Each case study was evaluated also on the way these results were communicated and which audiences were reached.

The **fourth stage** (impact) of evaluation concerned the available information on: • the short-term impact; • the long-term impact of the study results; • the level of these impacts (scientific, societal and policy-making levels); • whether stakeholders were involved in this process.

The main characteristics of the six case studies are summarised in table 2. A short description of each of the case studies is also given in the supplementary material (available on-line).

RESULTS

For the six case studies, the available information on the different study stages are summarised in tables S1-S4 (see on-line supplementary material). These results were used to document the lessons learned, formulated in the discussion section.

Although an HBM study consists of several stages, the information published in peer-review papers is mostly restricted to a description of the study design, fieldwork, and the results of the HBM study. In the analysed case studies, information on the pre-phase (e.g., public concern, decision point to use HBM), selection criteria for biomarkers, stakeholder involvement, communication of the results or on the impact of the study on societal and policy level are often scarcely available from the study description or are only available through other sources, such as reports in the local language or personal communication. The first three stages are present in each of the discussed case studies. The fourth stage (impact) is sometimes missing or less well documented compared to the other stages.

The decision point to organise an HBM study was comparable in all six case studies: to what extent can the environmental pollution that is associated with the ICS enter the human body.

Governmental funding for the HBM studies was received in the case studies of Belgium, Cyprus, Hungary, Italy,

COUNTRY	INFORMATION FOR SITE	MAIN CONTAMINANTS	NUMBEROF PARTICIPANTS IN THE STUDY	INCLUSION CRITERIA	BIOMARKERS OF EXPOSURE	BIOMARKERS OF EFFECT	INFLUENCING FACTORS
Belgium	The Eastern part of Flanders, Genk-Zuid industrial area	Heavy metals, dioxins, PCBs, and PAHs	200 adolescents of Genk-Zuid and 200 adolescents Flemish reference group	Age:14-15 yeras old; social inequalities; the vicinity of the site	Heavy metals (Cd, Pb, Ni, Cr, Mn, Tl, Sb, As, Hg) POPs (PCBs, HCB, DDE, dioxins, PBDEs) PAH-marker, benzene marker	Oxidative stress, DNA-damage, asthma, allergy, hormone levels, puberty, neurological tests	Sex, age, BMI, diet, smoking, socioeconomic status, locally produced food, pesticide use, stoves and wood/waste burning, distance to industrial site, concentrations in ambient air
Cyprus	Mammari (a village located in the Nicosia District of Cyprus, North of Kokkinotrimithia)	Arsenic	56 nail specimens from Mammari non-smokers and 48 matched controls	The vicinity of the site	As	Increased incidence of cancer	Exposure to higher concentrations of arsenic in drinking water
Hungary	Kolontár, Devecser, and Somlóvásárhely, an area of about 1,000 hectares	Heavy metals	351 children (176 from the affected area and 175 from the control area)	Age: 6- 14 years (most girls); the vicinity of the site	Toxic metals (As, Cd, Co,Cr, Ni, Pb, Sr, Mn, V), particulate matter (PM10)	Respiratory diseases	Sex, age, air, drinking water
Italy	The industrial/rural area of Modena, a medium sized town located in the middle of the Emilia-Romagna region, in the Po Valley (a circular area with radius of 4 km)	Ten metabolised PAHs Heavy metals PM10	488 participants	Age: 18-69 years old, living within 4 km from the incinerator, randomly selected from the population register	Particulate matter PM, PAHs, from naphthalene to chrysene, 1-hydroxypyrene and twelve metals (Cd, Cr, Cu, Hg, Ni, Pb, Ni, Zn, V, Tl, As, Sn)		Sex, age, diet, smoking, traffic, occupation and personal characteristics, the proximity of SWI
Poland	Silesian voivodeship, located in the Southern part of Poland (smelter heap, gasoline station, car workshop, industrial plants, busy roads)	Heavy metals, such as lead and cadmium	678 pre-school children: 341 girls and 337 boys	Age: 3-6 years	Pb, Cd		The vicinity of the sites (smelter heap, gasoline station, car workshop, industrial plants, busy roads), time spent outdoor parental level of education, smoking at home, age and sex of children
Portugal	The Panasqueira mine is located in Castelo Branco district, Central Portugal	Ag, As, Bi, Cu, Cd, Sb, Sn, W, and Zn	122 subjects: 41 living in villages located in the vicinity of the mine (16 males and 25 females), 41 male workers from the mine (occupationally exposed), 40 additional subjects	The vicinity of the mine	As, Cr, Mg, Mn, Mo, Ni, Pb, S, Se, Si, Zn	Immunotoxicity biomarkers (lymphocytes), DNA-damage, genotoxicity biomarkers	Sex, age, smoking habits, lifestyle factors, health conditions, medical history, medication, diagnostic tests, water and fish consumption, agriculture practice

Table 2. Summary of the characteristics of the selected case studies.
Tabella 2. Caratteristiche dei casi studio selezionati.

and Poland. In Portugal, the study received funding from scientific grants. In the Belgian case study, the organised follow-up study using HBM was co-funded by industrial partners, the local government and the Flemish government.

HBM results in ICS studies can have impacts on scientific (valorisation in scientific publications and PhD thesis, or additional research), societal (awareness rising and empowerment of participants and general public), and political (drawing political attention or initiating remediating actions or leverage for political decisions) levels.

DISCUSSION: LEARNING FROM EXPERIENCES

HBM studies are used to assess human exposure to environmental pollutants and their health implications, since internal levels of a pollutant typically reflect the overall exposure from different sources of exposure and exposure pathways.[19,20] The studies are also a key aspect of promoting surveillance and prevention measures from potentially harmful exposures to chemicals in the population, and for tracking progress in reducing public exposure to environmental chemicals.[21] The use of HBM in environmental health policy and research has advanced rapidly in Europe, but there are major challenges ahead that should be addressed.

COST Action IS1408 provided support for collaboration between international HBM experts and academic researchers to strengthen HBM capacity in Europe and improve data comparability. The network also involved all management, governing, and administrative functions necessary for managing remediation and response.

The positive experiences, as well as the challenges described by the six European case studies detailed in this paper, have potential to help promote HBM as a tool for exposure assessment in ICSs and design efficient HBM studies to answer research question and/or policy questions.

In the long-term, preparing a European Human Biomonitoring Plan for ICS, which will advance the use of HBM in environmental health policy development and evaluation of exposure levels, would be advantageous.

POSITIVE EXPERIENCES

USE LOCAL DATA FOR OPTIMISATION OF THE STUDY DESIGN

For all six case studies, environmental exposure data were available before the commencement of HBM activities (table S1). The data consisted of measurements of contaminants in environmental media (all cases), toxicity profiles of particulate matter (PM) (Belgium and Hungary)[22] and modelled PM concentrations (Italy).[23,24] These data were used for the delineation of the study area (Belgium, Italy) and for the selection of the biomarkers (all six cases). Before setting up an HBM study as part of monitoring potential exposures associated with an ICS, collecting available information on industrial activities, emissions, contaminant levels in environmental media, or using validated computer models to calculate dispersion of pollutants and collecting available health data proved to be very useful when defining different aspects of the study design. Since documented data are often fragmented or unavailable and researchers performing the study are not always familiar with the local area and history, involvement of local stakeholders from the beginning of the study can add valuable local knowledge to the project and improve the study design and the success of the study. Stakeholders advised on the relevance of organizing an HBM study in changing economic conditions leading to reduced industrial activities (Belgium), on the emergency response (Hungary), on possible sources of exposure (Belgium and Cyprus) and on the concerns of the target population (Belgium, Cyprus, Hungary, Poland).

A WELL-CONSIDERED CHOICE OF MATRIX OR A COMBINATION OF MATRICES DEPENDING ON THE RESEARCH QUESTIONS TO ANSWER

Biological matrices of interest strongly depend on the physico-chemical properties of the pollutants, but also on the type and duration of the exposure. For a number of pollutants, biomarkers can be measured in several different human matrices, often giving information about a different exposure time-window. In the ICS studies of Portugal[25] and Belgium,[26,27] choice of sampling matrix enabled differentiating between past and recent exposure and associating ICS activity patterns with measured levels of biomarkers of exposure (see tables S2 and S3, on-line supplementary materials). Blood and urine are the most frequently used biological matrices to measure internal doses of pollutants in human subjects. In the Portuguese case study, biomarkers of exposure were additionally measured in fingernails and toenails and in hair samples to reflect on long-term exposure. Urine, nails, and hair can be sampled in a non-invasive manner, whereas blood requires invasive sampling. Compared to hair, nails are less easily contaminated, especially toenails, since they are less frequently exposed to ambient air, metallic objects, and other contaminating sources, such as dyes.[28] Two additional human sampling matrices which are interesting for measuring long-term exposure to persistent organic pollutants (POPs), but non-used in the case studies selected in this paper, are adipose tissue and human milk. Adipose tissue is considered the main reservoir of POPs and, therefore, an adequate estimator for the evaluation of long-term exposure[29] as well as an important biological matrix in the development of chronic non-infectious diseases, e.g., cancer and metabolic syndrome.[30-32] However, it is clearly a non-accessible matrix for most HBM studies, especially in ICS areas, where population size is often limited. Human milk is used by the WHO to monitor levels of POPs in the population, because the high lipid content of human milk makes it very suitable for POPs measurements.[33,34] Using human milk as a sampling matrix limits the eligible participants to breastfeeding mothers, which is not recommended in ICS settings with limited population sizes.

CHILDREN AS STUDY POPULATION REFLECT LOCAL POLLUTION

Selection of the study population depends on whether the research question requires information on the potentially most exposed population, the most vulnerable population or a representative sample of the study population. In Belgium,[26] Hungary,[35] and Poland,[36] biomarkers were measured in children (see Table S2), because of their known vulnerability to environmental exposure and their good representation of the local environmental pollution. Choosing children and/or pregnant/breastfeeding women as a study population means choosing vulnerable sub-groups whose bodies and organs are still developing. In addition, children are not occupationally exposed, usually have a negligible impact of active smoking and spend most of their time in the neighbourhood of their residence. Children are also often more exposed (at least in terms of dose) to environmental pollution, because they eat, drink, and breathe relatively more per unit body weight compared to adults.

COMBINING EXPOSURE MARKERS WITH BIOMARKERS OF EARLY EFFECTS CAN HELP IN ASSESSING EARLY HEALTH DAMAGE

Observing elevated levels of exposure in populations residing near ICSs compared to control groups or reference populations does not de facto mean these exposures will pose a health risk. One way to assess health risks of observed exposure levels is to compare them with available (health-based) guidance values in human matrices, such as HBM-I and HBM-II values derived by the German HBM commission[37] or published Biomonitoring Equivalents (BE-values)[38] derived by other groups (e.g., in the case studies of Belgium[27] and Poland[36]) (table S3). These guidance values are only available for a limited set of biomarkers, and thus often lacking. When data both on exposure levels and on health effects of the same subjects are available, studying associations between exposure and health outcomes can be another approach. In the case studies of Belgium[39] and Portugal,[40,41] these data allowed to observe statistically significant associations between the elevated exposures and the elevated biomarkers of effect. Secondly, measuring biomarkers of early biological effects allows to develop preventive actions to improve residents' health and quality of life before people get sick. Using these biomarkers of early biological effects also helps in avoiding long follow-up times waiting for severe health outcomes.[42] A side note on this is that many early markers are not clinical markers, which can complicate interpretation at the level of the individual.

QUESTIONNAIRE DATA ARE USEFUL TO FINE TUNE RESULTS

Since HBM data reflect aggregated sources and routes of exposure, five of the six case studies (see table S3) used well-designed questionnaires to get more details on additional sources at the ICSs (other than the targeted industry: e.g., heavy traffic because of the ICS activities), on exposure routes relevant for the ICSs (e.g., locally grown food), and on other non-ICS related sources (e.g., smoking). Using well-designed questionnaires offered opportunities to control sources of variability (e.g., season, time spent outdoors), to identify other determinants of exposure (e.g., diet, smoking, traffic) and vulnerable sub-populations, such as specific age, gender, and socioeconomic groups. This additional information will improve the study results and allow more targeted remediating actions to reduce exposure and differentiate towards more vulnerable populations.

HBM IN ICSS HELPS IN SETTING PRIORITIES

The case studies discussed in this paper illustrate that using HBM in ICSs helped in setting priorities in multiple topics, enhancing delineation of the concerns and remediation possibilities specific for the studied ICSs. The HBM results refined the knowledge about the pollutants of concern by identifying those exposure biomarkers that are elevated in the target population (Belgium,[27] Italy,[24] and Portugal[25]) (see table S3) compared to control populations, reference values or health-based guidance values (priorities in exposure of concern). Combining these biomarkers of exposure with health data, preferably from the same study subjects, also enabled identification of the main health concerns associated with the measured exposure levels (e.g., Belgium, Portugal[40,41]) (see table S3) (priorities in health effects of concern for, e.g., monitoring purposes or provision of specialist healthcare resources), supporting the prioritisation of remediating actions (e.g., Belgium,[43]) (table S4) (priorities for policy actions), and/or providing guidance to future research and development activities (e.g., Belgium, Cyprus) (table S4) (priorities for further research). Combining the biomarkers of exposure with (early) biomarkers of health effects also supported development of preventative health policy. Questionnaire data helped to identify sub-populations within the residents near the ICSs that are likely to experience relatively greater exposure or to identify sub-populations that are more vulnerable to this exposure, such as socially vulnerable groups (case studies of Belgium,[26] Italy,[24] and Poland[36]) (table S3) or subgroups related to age and sex of the participants.

COMMUNICATING RESULTS SERVES AWARENESS RAISING AND AGENDA SETTING

When communicating modelled exposure risks, messages can become impersonal with a tendency to talk about theoretical constructs of (sub)populations. In this regard, there is a need for studies assessing the perception and attitudes of the general population towards the exposure,[44] which can be of help inform, e.g., HBM studies and public health interventions.[45] HBM measures indicators of exposure at individual level within identified population groups and therefore makes the pollution personal.[43,46] Personal results were provided to the participants in the Belgian and the Italian case studies (table S3). Study results were available in public reports in the Belgian, Cypriot, Polish, Hungarian case studies. A dialogical risk communication and reporting back study results to participants or the general population might result in larger attention and understanding by society of environmental health topics, creating opportunities for empowerment and awareness raising on personal choices. This strategy should be carefully designed to meet the population requirements,[47] particularly vulnerable groups, which frequently need tailored approaches to risk communication and remediation actions.[48,49]

INVOLVING LOCAL STAKEHOLDERS BUILDS KNOWLEDGE, CONFIDENCE, AND SHARED RESPONSIBILITIES

Local stakeholders can also be very important for motivating residents within the vicinity of an ICS to participate in HBM studies and other research activities. This early involvement of stakeholders also helps to build confidence in the study results, to get support for remediating actions, and to take on responsibilities for implementing these remediating actions when the study is completed. Local stakeholder participation should be incorporated in policy planning irrespective of the source encouraging this engagement, which could be due to state actions or bottom-up mobilization of communities.[50]

CHALLENGES IN PERFORMING HBM IN ICSs

In addition to sharing positive experiences, the WG2 discussions also revealed several encountered difficulties in using HBM for assessment of ICSs. The potential on successful development of the study can significantly be enhanced by taking these challenges into account before planning an HBM study in an ICS. In this section, the challenges that most frequently emerged in the analysed case studies are identified and provided with solutions to tackle them.

STUDY POPULATIONS OF LIMITED SIZE OBSTRUCT CONVINCING RESULTS

One of the major challenges when performing HBM in ICSs is the size of the study population. Residential areas near an ICS can be very limited in population size, making it difficult to recruit a sufficient number of participants for robust statistical analysis. For reliable biomarker reference values that characterise a population between at least 100 and 200 participants are needed.[51] This issue also hampers the evaluation of exposure of vulnerable population sub-groups such as children, patients with chronic diseases, or pregnant women. This challenge might not always be possible to overcome. In case of limited number of residents near an ICS, attention should be paid to avoid strong triage in the eligible population to participate and research questions to be answered should not be overambitious.

SOME SUB-GROUPS ARE DIFFICULT TO RECRUIT

Some population sub-groups, such as people with a low attained level of education, low family income or belonging to specific ethnic groups are often underrepresented in HBM studies because of difficulties in recruiting them. Although this is a general challenge for HBM studies and other types of research, ICS studies are even more challenged, as low income and socially deprived population sub-groups are likely to be a significant proportion of the population living in the vicinity of ICSs. This can be an obstruction to obtaining a representative study population or limits analytical capabilities such as exploration of sociodemographic determinants. A better knowledge of the specific barriers that limit the participation of the targeted populations in HBM studies, together with the possibility to get relevant advise and/or practical assistance from the local community, is very important to increase involvement of population subgroups.[52] This would also allow to better assess the potential unequal distribution of health impacts related to ICS within the population. In the Belgian case study, local stakeholders were actively engaged in door-to-door visits to help recruit participants. Some of the discussed case studies experienced working with children as study population as an advantage, because they better reflect local environmental pollution compared to adults. However, recruiting children can also be challenging, e.g., with respects to getting ethical permission. A less invasive study design (e.g., urine, hair or nails as matrix, sampling in a comfortable and trusted environment, short questionnaires) can offer a solution.

AVAILABILITY OF VALIDATED AND REPRESENTATIVE BIOMARKERS

For all discussed case studies, validated and well-known biomarkers were at the disposal of the researchers, representative of the known environmental pollution associated with the ICS. However, progress of industry and technology generates new emerging chemicals, for some no validated biomarkers are yet available.

THE MOST SUITABLE MATRIX OF BIOMARKER MEASUREMENTS CAN LIMIT ACCESS OF PART OF THE RESIDENTS TO PARTICIPATE THE STUDY

The choice of matrix can also be a weakness, in case the ideal matrix for the temporal variation in exposure does not match with the choice of matrix to facilitate subject recruitment. Blood and adipose matrix require an invasive sampling approach, which is not easily accepted by participant subjects in an HBM study, especially when children are involved. Choosing cord blood as a matrix restricts the eligible participants to only pregnant women. This restriction is even stronger when human milk is used as matrix, because only women who gave birth and opt for breastfeeding their baby can participate. The limiting effect of these matrices on recruitment is lower, if the population size of residents living in an ICS is large. When difficulties in recruitment can be expected, special attention should be paid to involvement of stakeholders, who can help with communicating the planned study and motivating residents for participation.

RELEVANCE OF THE OBSERVED CONCENTRATIONS FOR HEALTH RISKS

Interpretation of the HBM results in terms of health risks is not always straight forward. For some biomarkers of exposure, HBM health-based guidance values for biological matrices are available such as the HBM-I and HBM-II values from the German Human Biomonitoring Commission[37] or Biomonitoring equivalents.[38] For many other compounds, these guidance values do not yet exist or are still under discussion, due to multiple or changing threshold values. Deciding whether the measured levels of biomarkers of exposure indicate the necessity of some form of public health intervention is often fraught with difficulty and uncertainty. Also, when personal results are communicated to the participants, not being able to inform for sure about implications for people's health can make the message very complex and difficult to understand. Linking biomarkers of exposure with measured biomarkers of effect by statistical analysis of exposure-effect associations can help to inform the messaging around project findings.

POLICY UPTAKE OF THE RESULTS

In some of the case studies, policy uptake of the HBM results by the competent authorities to implement actions to reduce exposure of local residents was also experienced as a difficulty. In the Belgian case study, a participative approach was used, involving local stakeholders as well as representatives of the Flemish government throughout the entire study working together with experts and researchers on policy uptake of the study results.[43,53] To be able to achieve this, efforts are needed in open and transparent communication with all involved parties, as well as willingness and mandates to participate are required from the authorities.

HOW TO DEAL WITH EXPOSURE TO MIXTURES OF POLLUTANTS?

Another challenge for current HBM research is that most of the studies deal with risks related to individual chemicals of interest. However, people (including those individuals residing close to ICS) are usually exposed to complex mixtures of pollutants, which might present synergistic interactions.[54] Furthermore, exposure to chemicals of concern from a particular ICS might also interact with baseline exposures to other pollutants (e.g., organochlorine pesticides, metals), which are frequently found in the general population. In this regard, several statistical[55] and biological approaches have been proposed.[56] There is not yet a good solution to deal with the total chemical's body burden.

FINAL CONCLUSIONS AND RECOMMENDATIONS FOR HBM IN EUROPEAN ICSS

Since HBM is becoming more frequently used for exposure assessment in ICSs all over Europe, a European protocol and guidance document for HBM in ICSs would be useful. This could advance the use of HBM in local environmental health policy development and evaluation of exposure levels.
Based on the experiences of and the discussions with members of the working group 'Exposure assessment' of the Industrially Contaminated Sites and Health Network (ICSHNet) COST Action, the following recommendations should be noted.
1. Study population of sufficient size: the study population should be of sufficient size to be representative for the area and to provide sufficient statistical power to draw conclusions (between 100 and 200 participants).
2. When possible, avoid research triage in the study design: in case of small populations living in the affected area, we must be careful to not limit too much the eligible population to participate in the study by setting strict inclusion criteria (for example, to consider only pregnant women or a narrow age range), unless there is a very specific targeted study population.
3. Study design that correctly evaluates the exposure time-windows of interest: the periods when the industrial emissions occurred/still occur in relation to • the time when the HBM study is carried out; • the validity of

the exposure biomarker as to integrate the relevant exposure routes and signal past or recent exposures; • the capacity of the biomarkers of effects to capture the early effects of relevant exposures; • the time when the different health effects are expected to appear.

4. Smart choice of sampling matrix: a combination of sampling matrices (blood, urine, hair, nails, etc) reflecting short- and long-term exposure should provide a more complete characterisation of the exposure and to allow comparison of measured biomarker levels with activity patterns of the ICS.

5. Use harmonized and validated questionnaires, spatial data, and environmental monitoring/modelling to increase the utility of the study: supplementing the biomarker measurement with questionnaire data widens the number of questions that the study can help inform, many of which are policy relevant. Questionnaire data can allow identification of additional sources in the ICS, of relevant routes of exposure for the ICS and of specific vulnerable sub-groups at the ICS. As stated by David Briggs:[9] «What determines levels of exposure is consequently not just the distribution of pollution within the environment, but also human behaviours and lifestyles, and thus the sorts of exposure environments in which people spend their time. By the same token, exposure is not only an environmental process; it is also a social, demographic and economic one».

6. Combine biomarkers of exposure with biomarkers of (early) effects to get more information on the health relevance of the measured exposure.

7. There is a strong **need for a harmonized approach on advanced biomarkers and/or statistical techniques** to deal with exposure to multiple compounds or mixtures.

8. Use a participatory approach: it is advised to actively involve authorities and local stakeholders from the beginning of the study to include local knowledge, gain local support, and obtain commitments for policy uptake of the results.

9. Invest in communication of the study results: communication of the study results to participants, public, and stakeholders is a crucial part of an HBM project. When done well it promotes trust and mutual understanding. So, besides publishing the results in scientific articles, it is important to present the results to people involved and offer them opportunities to discuss their results and their significance at an individual and collective level.

10. HBM also offers opportunities for monitoring exposure of residents near an ICSs over time and contribute to evaluation of efficacy of exposure management efforts.

Depending on the type of biomarker measurements and the choice of study design, HBM studies can become expensive. In situations where limited resources are available, like in many low-income Countries, other assessment methods might be more feasible.

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