Biomarkers of Exposure in Environment-Wide Association Studies: Opportunities to Decode the Exposome Using Human Biomonitoring Data in Environmental Health Research

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Abstract

Background: The European Union's 7th Framework Programme (EU's FP7) project HEALS – Health and Environment-wide Associations based on Large Population Surveys – aims a refinement of the methodology to elucidate the human exposome. Human biomonitoring (HBM) provides a valuable tool for understanding the magnitude of human exposure from all pathways and sources. However, availability of specific biomarkers of exposure (BoE) is limited.

Objectives: The objective was to summarize the availability of BoEs for a broad range of environmental stressors and exposure determinants and corresponding reference and exposure limit values and biomonitoring equivalents useful for unraveling the exposome using the framework of environment-wide association studies (EWAS).

Methods: In a face-to-face group discussion, scope, content, and structure of the HEALS deliverable "Guidelines for appropriate BoE selection for EWAS studies" were determined. An expert-driven, distributed, narrative review process involving around 30 individuals of the HEALS consortium made it possible to include extensive information targeted towards the specific characteristics of various environmental stressors and exposure determinants. From the resulting 265 page report, targeted information about BoE, corresponding reference values (e.g., 95th percentile or measures of central tendency), exposure limit values (e.g., the German HBM I and II values) and biomonitoring equivalents (BEs) were summarized and updated.

Results: 64 individual biological, chemical, physical, psychological and social environmental stressors or exposure determinants were included to fulfil the requirements of EWAS. The list of available BoEs is extensive with a number of 130; however, 11 of the stressors and exposure determinants considered do not leave any measurable specific substance in accessible body specimens. Opportunities to estimate the internal exposure stressors not (yet) detectable in human specimens were discussed.

Conclusions: Data about internal exposures are useful to decode the exposome. The paper provides extensive information for EWAS. Information included serves as a guideline – snapshot in time without any claim to

comprehensiveness – to interpret HBM data and offers opportunities to collect information about the internal exposure of stressors if no specific BoE is available.

Keywords

Human biomonitoring, biomarkers of exposure, environment-wide association studies, reference values, exposure limit values, biomonitoring equivalents

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Abbreviations

µg/l, microgram per liter; µM/l micromolar per liter; Σ, total; 1-HP, 1-hydroxypyrene; 2,3-DHBA, 2,3-dihydroxybenzoic Acid; AAs, alkylating agents; ALARP, as low as reasonably practicable; AM, arithmetic mean; As, arsenic; AUDIT, Alcohol Use Disorders Identification Test; BAC, blood alcohol content; BDCM, bromodichloromethane; BDE 99, 2,2′,4,4′,5-pentabromodiphenyl ether; BE, biomonitoring equivalents; BoE, biomarker of exposure; BPA, bisphenol A; BzBP, benzylbutyl phthalate; CAL REL, California Acute Reference Exposure Levels; CC, critical concentration; Cd, cadmium; CYP1A1, cytochrome P450 1A1; CIT, citrinin; CPK, creatine phosphokinase; Cr, chromium; crea., creatinine; Cu, copper; dB(A), decibel; DBCM, Dibromochloromethane; DBP, di-n-butyl phthalate; dibromochloromethane; DBPs, disinfection by-products; DEDTP, diethyl dithiophosphate; DEHP, Di-2(ethylhexyl) phthalate; DEP, diethyl phthalate; DETP, diethyl thiophosphate; DiNP, Diisononyl phthalate; DMP dimethyl phosphate; DMDTP, dimethyl dithiophosphate; DMTP, dimethyl thiophosphate; DNA, deoxyribonucleic acid; DON, deoxynivalenol; ECO, expired carbon-monoxide; EU's FP7, European Union's 7th Framework Programme; EWAS, environment-wide association studies; FAS, Family Affluence Scale; Fe, iron; GGT, γ-glutamyl transferase; GM, geometric mean; GWAS, genetic-wide association studies; HBCDD, hexabromocyclododecane; HBM, human biomonitoring; HCB, hexachlorbenzene; HEALS, Health and Environment-wide Associations based on Large population Surveys; Hg, mercury; IMD, Index of Multiple Deprivation; IUAPC, International Union of Pure and Applied Chemistry; JEM, Job-Exposure-Matrix; m7Gua, 7-methylguanine; MAA, 2-methoxy acetic acid; MBP, monobutyl phthalate; MBzP, monobenzyl phthalate; MCT, measure of central tendency; MEP, mono-ethyl phthalate; Mn, manganese; mg/kg/day, milligram per kilogram per day; mg/m^3, milligram per cubic meter; MRL, minimal risk level; MVOC, microbial volatile organic compounds; n, sample size; NDMA, N-nitrosodimethylamine; NMTCA, N-nitroso-2-methylthiazolidine-4-carboxylic acid; NNAL, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NOC, N-nitroso compounds; NOx, Nitrogen oxides; NPRO, N-nitrosoproline; NPs, Nanoparticles; NSAR, N-nitrososarcosine; NTCA, N-nitrosothiazolidine-4-carboxylic acid; OCPs, organochlorine pesticides; OPPs, organophosphate pesticides; OTA, ochratoxin A; P_{90}: 90th percentile; P_{95}, 95th percentile; Pb, lead; PBBK, physiology-based biokinetic; PCBs, Polychlorinated biphenyls; PCP, pentachlorphenol; PER, perchlorethylene; pg/ml, pictogram per milliliter; PM, particulate matter; POPs, Persistent Organic Pollutants; PYR, pyrene; RfC, reference concentrations; RfD, reference doses; RI, reference interval for clinical guidance; Rn, radon; RV_{95}, reference value; S-PMA, S-phenyl mercapturic acid; Se, selenium; SES, socioeconomic status; SG, satratoxin G; SHS, second-hand smoke; TCAA,

trichloroacetic acid; TCEQ ReV, Reference Value of the Texas Commission on Environmental Quality; TDI, tolerable daily intakes; THMs, trihalomethanes; THS, third-hand smoke; U/L, units per litre; UK, United Kingdom; US, United States; UVR, ultraviolet radiation; WHO, World Health Organization; Zn, Zinc

Declaration of interest

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1 Introduction

The European Union's 7th Framework Programme (EU's FP7) project HEALS – Health and Environment-wide Associations based on Large Population Surveys – started in 2013 with a term of 5 years. The objective of HEALS is the refinement of an integrated methodology and the application of analytical and computational tools for elucidating human exposome through the integrated use of advanced statistical tools for environment-wide association studies (EWAS) in support of EU-wide environment and health assessments (www.heals-eu.eu).

Important determinants for the development of diseases are genetic influences and the interaction of environmental stressors (Schwartz and Collins, 2007). Described with the complementary approach of nature and nurture, the term "environment" includes everything that is not genetic (Smith et al., 1999). Consequently, the genome needs to be complemented by the exposome (Wild, 2005; Wild, 2012). While the human "genome is fixed at conception" (but changed by mutagenic influences) (Rappaport, 2011), "the exposome encompasses life-course environmental exposures […], from the prenatal period onwards" (Wild, 2005). Based on the above, genome-wide association studies (GWAS) attempt to describe the influence of genetic factors for the development of diseases (Hirschhorn and Daly, 2005), while EWAS investigate the associations between a wide range of environmental factors and diseases (Patel et al., 2010). In this context, human biomonitoring (HBM) provides a valuable tool for understanding the magnitude of exposure from all pathways and sources. Biomarkers include either stressors themselves (e.g. the parent compounds), or their metabolites (reaction products), identified in a variety of human specimens such as blood, urine, deciduous teeth or hair (CDC, 2005).

HEALS encompasses a more integrative approach for associating environmental exposures and disease mechanisms and outcomes. Data from the external environment, e.g., measurements of chemicals in different media (e.g. air, water, soil and food), are combined with data regarding internal exposure, e.g., measurements of chemicals in urine or blood, to build the exposome and to derive environment-wide associations between exposure and disease. Starting from HBM samples, quantification of exposure biomarkers, together with identification of markers of effect and susceptibility (mainly-omics), builds the analytical exposure biology framework for unraveling the human exposome using multi-omics technologies according to the HEALS paradigm.

To evaluate HBM data, reference and exposure limit values as well as biomonitoring equivalents are useful and receive particular attention in the HEALS project. Reference values describe the upper level of the populations' background concentration (Angerer et al., 2007; Schulz et al., 2011). The HBM Commission of the German Environment Agency defines reference values as the 95th percentile of the concentration level of the respective

parameter in the matrix obtained from the reference population (Schulz et al., 2011). Reference values contain no information about health-related biological exposure limits (Angerer et al., 2007). An example of popular health-related biological exposure limit values are the German HBM I and II values. There is no health risk assumable if the concentration of a substance in urine or blood is below the HBM I level. A health risk cannot be excluded if the concentration of a substance in urine or blood is between HBM I and HBM II. An increased risk for adverse health effects is given if the concentration is above HBM II (Schulz et al., 2011). Besides reference values and exposure limit values, biomonitoring equivalents (BEs) are of importance, because they are a first screening method to evaluate potential risk from exposure to environmental stressors using HBM data. BEs are defined as the concentration of a chemical or metabolite in a biological matrix (blood, urine, human milk, etc.), consistent with defined exposure guidance values or toxicity criteria. These include reference doses (RfD) and reference concentrations (RfC), minimal risk levels (MRLs) and tolerable daily intakes (TDIs), which have been defined using the knowledge available regarding the toxicokinetic properties of the chemical (Boogaard et al., 2011). The application of BEs is based on the assumption that intake and excretion are at equilibrium. This ensures coherence between the guidance values for chronic exposure and the estimated BE (Angerer et al., 2011). Use of reliable physiology-based biokinetic (PBBK) models is the most convenient way to translate external exposure reference values into BEs.

In the framework of HEALS, BoEs of a large number of environmental stressors were reviewed and used for supporting environment-wide associations. The main objective of this work was to summarize the availability of BoEs for the broad range of environmental stressors and exposure determinants of interest in HEALS (including heavy metals, persistent and non-persistent organic compounds, particulate matter and biologicals) and corresponding reference and exposure limit values and biomonitoring equivalents useful for unraveling the exposome using the EWAS framework. Additionally, environmental stressors and exposure determinants without known BoEs were discussed.

2 Methodology

This review was based on a face-to-face group discussion to determine scope, content, and structure of the HEALS deliverable “Guidelines for appropriate BoE selection for EWAS studies”. An extensive list of the most important environmental stressor categories as well as selected stressors relevant to human health of the population in the EU was created based on the joint expert opinion. An expert-driven, distributed, narrative review process involving around 30 individuals of the HEALS consortium made it possible to include extensive information targeted towards the specific characteristics of the individual stressor.

The review process was organized on the basis of stressor-specific fact sheets including information about chemical properties, effects on biological systems, exposure routes, absorption, elimination, specimens for analysis, and eventually reference and exposure limit values. While most fact sheets were created for specific environmental stressors (e.g., mercury), in some cases it was necessary to summarize a group of stressors in one fact sheet (e.g., psychological occupational hazards). This was an essential, yet feasible approach in some cases, so as to represent a wide range of stressors important to determine the exposome of the EU population.

Information was obtained from comprehensive reports of international organizations (e.g., WHO's Environmental Health Criteria) and other mainstream scientific literature supplemented by the latest research results published in PubMed listed journal papers. Overall, more than 800 references were reviewed.

For quality assurance, all contributors were involved in an internal review process. Each fact sheet was reviewed by at least two project partners, while one of them was the project coordinator, co-coordinator, or leader of the HEALS HBM work package. The leading question for the review process was: "Is the quality, content, and extent of the fact sheet as well as the literature selection suitable and is the information included up to date?"

The literature review process described above resulted in a dedicated technical report available for download on the HEALS website: http://www.heals-eu.eu/wp-content/uploads/2013/08/HEALS_D4.2.pdf. A concise selection of information was extracted, updated, and key conclusions are summarized in this paper. The paper focuses on the availability of BoE in body fluids (blood/serum/plasma, breast milk, urine) as well as hair. Presented are reference values, exposure limit values and biomonitoring equivalents (BEs). If available, the reference value (RV_{95}) as defined by the Human Biomonitoring Commission of the German Environment Agency (Schulz et al., 2007) on the basis of a guideline from the International Union of Pure and Applied Chemistry (IUPAC) (Poulsen et al., 1997) is presented. If not available, the 95th percentile (P_{95}) was included as reference value. Otherwise, the third choice was the 90th percentile and the fourth choice was (the range of) measures of central tendency (MCT) like mean or median presented in combination with the maximum value, if available. Condensed values for a population (distinguished in children and adults) were preferred (e.g., P_{95} for adults aged 18 to 69 years) instead of values separated by subgroup (e.g., P_{95} for 18 to 19 years old, P_{95} for 20 to 29 years old, etc.). If a condensed value is not given in the original publication, the range of youngest to oldest is presented in this paper. Values based on the general population are preferred instead of subgroups with special exposures (e.g. like smokers, people with amalgam fillings or high fish consumption). Latest values are presented. Non-creatinine-corrected values are preferred, if available. For reference values, the main – but not exclusive – focus lay on populations in the EU.

The first choice of exposure limit values was the German HBM values (HBM I and II). Otherwise, critical concentrations, cut-offs or other values are included. Some examples of occupational exposure limit values (e.g., BAT) were included. Completeness was not intended. Stressors without measurable BoE are explicitly discussed. All content was updated to at least January 2017 or later as appropriate.

3 Results

A total of 64 chemical, biological, physical, social, or psychological stressors organized in 13 broad stressor categories were selected (Table 1) to fulfil the requirements of EWAS, although the BoEs for some exposure determinants/modifiers (e.g., socioeconomic status) were not expected to be available. In total, information of 141 BoE is summarised. If available, reference values (Table 2), exposure limit values (Table 3), and biomonitoring equivalents (Table 4) are presented. From the complete list of individual stressors (Table 1), 11 were identified without a BoE. These stressors (and some summarized groups of stressors like psychological occupational hazards) are included in Table 5 to discuss opportunities other than HBM to collect information about their internal exposure.

Table 1 includes the stressor categories and stressors with available BoEs as well as – if available – an incomplete selection of corresponding reference values. Reference values were found for 97 of the 130 considered BoEs. Table 3 contains exposure limit values and Table 4 biomonitoring equivalents (BEs) by stressor, when available. Exposure limit values are available for 14 of the 130 considered BoEs. BEs are available for not more than 28 of the 130 BoEs considered.

4 Discussion

Specific BoEs are available for several environmental stressors but not for others. While chemicals and their primary metabolites may be measureable in human specimens, it is not possible at this time to identify BoEs for stressors such as electromagnetic fields or for exposure determinants/modifiers such as socioeconomic status using biomonitoring. Although possible ways of representing the aggregate exposure of some stressors without specific BoEs were found (see Table 5), lack of specificity introduces uncertainties in using these to unravel the exposome. As the characteristics of environmental stressors may be very diverse, HBM needs to be complemented by other tools and technologies that will allow effective HBM data assimilation (Sarigiannis et al., 2014). This includes an array of technologies, employing environmental monitoring or food item analysis for chemical residuals, or ancillary exposure information retrieved from questionnaires or exposure related databases.

For EWAS, it is essential to consider a large range of diverse environmental stressors to enable the most complete decoding of the exposome. Relying on only one monitoring method (in this work we refer to biomonitoring) is insufficient. Although analysis of human biosamples for identifying the BoE levels is a good starting point, further elucidation of the individual exposome requires the use of additional molecular analysis such as transcriptomics, metabolomics or adductomics according to the HEALS paradigm; in turn, this requires additional computational tools that have to be used to interpret the biomonitoring and multi-omics results in the frame of a more integrative approach. This is actually one of the key aspects investigated in HEALS.

Limitations and strengths

Despite the amount of information collected in this narrative review, this work has limitations. Information was collected in an expert-driven, distributed, narrative review process which might involve individual researcher decisions. The list of stressors included is not exhaustive but evaluated based on the joint opinion of the participating partners as a list of important stressors for the population in the EU. The lists of reference values, exposure limit values and biomonitoring equivalents were not intended to be complete; rather, examples are listed to provide an inside in the interpretation of data. HBM itself contains limitations such as the use of diverse methods for analyses. Also, the derivation of reference and exposure limit values is based on expert decisions usually on the basis of a consensus process.

Strengths of this work are the broad inclusion of diverse environmental stressors, the extensive list of BoEs and corresponding reference values, exposure limit values and biomonitoring equivalents as well as the inclusion of possibilities to measure the internal exposure of stressors without specific BoE.

5 Conclusions

Given the diversity of environmental stressors that need to be examined to unravel the exposome, current-day human biomonitoring is suitable for determining the internal exposome of several stressors (e.g., metals, PCBs, VOCs) but not for many others (e.g., NO_x, PM, physical activity). Most chemical and biological stressors are measureable in human specimens whereas exposure to the majority of physical, social and psychological stressors needs to be assessed using methods complementary to HBM. The joint and harmonized application of methods and tools to unravel the exposome represents the main task of the HEALS project.

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Table legend

Table 1: Summarizing table, comprising a list of stressor categories, individual stressors and biomarkers of exposure considered and availability of reference values, exposure limit values and biomonitoring equivalence

Table 2: Biomarkers of exposure and reference values

Table 3: Exposure limit values

Table 4. Biomonitoring equivalent (BE) values for selected stressors

Table 5: Opportunities to collect information about the internal exposure of stressors if no specific biomarker of exposure (BoE) is available

Appendices

The complete HEALS report can be downloaded from the HEALS website, http://www.heals-eu.eu/wp-content/uploads/2013/08/HEALS_D4.2.pdf.