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Criteria for prioritization of biomarkers of effect

Deliverable Report

D 14.1

WP 14 Effect Biomarkers

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2 Introduction

Both exposure and effect biomarkers are critical to explore the effect of environmental contaminants on health. The former allow us to fill current data gaps in exposure assessment and provide clear evidence of aggregate population exposure to chemicals of concern. Exposure biomarker measurements directly reflect the total body burden (or biological effect) resulting from all routes of exposure, and inter-individual variability in exposure levels, metabolism, and excretion rates. These data are often the most relevant metric for health impact assessment, especially for bioaccumulative or persistent chemicals that are stored in the body for a long period of time. For chemicals that are excreted rapidly, cross-sectional biomonitoring data reflect recent exposure, while characterization of long-term exposure patterns at individual level requires repetitive sampling.

Effect biomarkers reflect and in some cases allow for quantification of the biologic or toxic activity of a contaminant or mixtures of contaminants. They complement exposure biomarkers and provide further evidence of activity, toxicity, or cumulative effects. For example, exposure to atmospheric particles lacks robust exposure biomarkers, and effect biomarkers could serve in such cases. The overlap between biomarkers of exposure and biomarkers of effect can point to important biological mechanisms that mediate the health effect of chemicals. Omics, miRNA, and epigenetic studies have expanded the range of effect biomarker studies, which now represent one of the most active fields in environment and health research. HBM4EU will further develop such studies in support of human biomonitoring (HBM).

The following operational definition of “biomarker of effect” has been adopted**:** Any accurately and reproducibly quantifiable biological change that provides an objective measure of health status or disease after chemical exposure. A biomarker of effect reflects quantifiable changes in biochemical, physiologic or other parameters in the organism that occur as a result of exposure (DoA HUBM4EU definition).

Our aims are to identify validated biomarkers that reflect specific exposures in different matrices and are quantitatively linked to adverse outcomes in studies of human populations. There will be a focus on endocrine disruption, reproductive toxicity, obesity/altered metabolism, neurobehavioral effects, aging, immunity inflammation and carcinogenesis, because these have been associated with some of the selected prioritized substances.

The information gathered will be used to: i) characterize inter-individual variability, ii) help to ensure that sensitive populations are identified and adequately addressed in the assessments, and iii) reduce uncertainty in the extrapolation of *in vitro* or animal data to humans. These data will help to identify links between exposure and epidemiological data in order to explore potential biological effects and lead to improved health outcomes.

The main objective of deliverable 14.1 is to set up relevant criteria for prioritization of biomarkers of effect that will be searched in the scientific literature, related to the 1st set of prioritized substances in the HBM4EU project listed below:

Chemical family/Substances
Phthalates & Hexamoll®DINCH®
Poly/per-fluorinated compounds
Brominated & organophosphate flame retardants
Bisphenol A, S and F
Cadmium and Chromium(VI)
8 carcinogenic PAHs in REACH, 16 USEPA priority PAHs
Aniline derivatives
Mixtures

These criteria will guide the literature search on effect biomarkers. Once the inventory is made, it will be sent to all other partners for verification, addition, and criticism. All collected data will also be inserted into the HBM module of IPCheM.

Validated biomarkers of effect that provide significant added value to HBM4EU by linking exposure data to measures of biological effects will be searched. Research will provide integrated information

that could be used to delineate the mode(s) of action (MOA) of the adverse response or toxicity. HBM4EU will support such studies of HBM, and decisions will be taken on the budget allocation or internal calls. Moreover, because the investigation of chemical impact should not be limited to single agents but should rather consider the combined effects of chemicals, attention will also be paid to existing biomarkers of effect for mixtures, replicating real-life exposure scenarios, and the functionality of mixture effect biomarkers

3 Qualitative and quantitative criteria

The criteria for prioritization of effect biomarkers will be classified in two categories: qualitative criteria (yes/no) and quantitative criteria (a numerical score will be assigned depending on the characteristics of the specific effect biomarker under study). The qualitative criteria will determine whether the scientific articles found in databases should be selected or discarded. The quantitative criteria will allow us to assign a score for each biomarker of effect in order to rank them and prioritize their use.

4 Criteria for prioritization of biomarkers of effect

Although only the first criterion (1st priority substances list) will be strictly followed, representing a *condition sine qua non*, the search for biomarkers will be prioritized according to:

Qualitative criteria

1. **First set of prioritized substances**

All biomarkers of effect will be related to at least one of the compound/s (mixtures) included in the first set of priority substances.

2. **Selected health endpoints**

The following health outcomes will be particularly searched for:

- Neurodevelopment
- Reproductive diseases
- Endocrine diseases
- Obesity and metabolic disorders
- Cardiovascular diseases
- Allergies and immunological diseases
- Cancer

Quantitative criteria

3. **Mechanisms of Action (MOA) and Adverse Outcome Pathways (AOPs)**

According to the OECD and as studied in WP13, an adverse outcome pathway (AOP) "is a conceptual framework that portrays existing knowledge concerning the linkage between the two anchor points Molecular Initiating Event (MIE) and Adverse Outcome (AO) at a level of biological organization relevant to risk assessment".

WP14 will interact with WP13 in the attempt to delineate possible AOPs that might underlie exposure-disease associations. Therefore, priority will be given to the biomarkers of effects that reflect Key Events (KE) in AOPs of priority chemicals as explored in synergy with WP13. Because the real-life scenario is that humans are exposed to complex mixtures of environmental compounds with multiple mechanisms of action, understanding the AOP induced by a chemical (as well as a mixture) will help to improve assessment of the health risk of a mixture of these chemicals.

4. **Validated *vs.* not validated and implementation in epidemiologic studies**

Biomarkers implemented in epidemiologic studies will be prioritized over those that have not been validated in human populations.

5. **Cost and/or feasibility of biomarkers**

The benefit-cost ratio (BCR) will be the result of the cost per analysis in relation to the efficacy/innovative potential of the biomarkers under study. These criteria will be of especial importance for extremely expensive biomarkers of effect whose utility is not clear.

6. **Clinical measures and/or measures in biological matrices and human sample availability and quantity/volume (blood volume as an issue)**

Biomarkers of effect that can be applied in both clinical and epidemiological settings will be of major interest. Sampling methods for human matrices that are not excessively invasive will be prioritized. Human matrices with high available quantity/volumes (i.e., urine, placenta) and those available across populations (i.e., urine, blood, saliva), are of priority interest. In this line, although blood samples represent a very interesting exposure matrix, their limited volume appears as a methodological issue. Moreover, given the special concern about environmental exposures that

occur during human development, biological matrices such as amniotic fluid, meconium or breast milk would also be relevant in order to monitor prenatal and neonatal exposure.

7. **Specificity, *sensitivity, and reliability* of biological changes in relation to the exposure and interpretation of the biological change**

Biomarkers of effect with a higher specificity for exposure are of major interest for risk assessment and will therefore be prioritized. The sensitivity will affect the volume/mass of matrix needed, and the greater the sensitivity the better. In addition, a clear or "intuitive" interpretation of the biological change assessed by the biomarker under study will be of high interest. In this regard, it is important to distinguish between natural variability (noise) and contaminant-induced effects and to take account of the impact of confounding factors in the biomarker response.

5 Categorization of effect biomarkers

Selected effect biomarkers will be categorized according to their biological level of complexity:

Genetic testing and molecular biomarkers

This category will cover all molecular biomarkers that measure gene mutations and polymorphisms, quantitative gene expression, peptides, proteins, lipid metabolites and other small molecules. These biomarkers may include, as examples, oxidative stress markers, DNA damage, mitochondrial DNA content, and telomere length as well as "omics" and epigenetic markers. This group of biomarkers is complementary to the groups of effect biomarkers specified above in order to add value to the AOP concept as evaluated in WP13.

Biochemical / physiological biomarkers

This category will cover all biomarkers related to biochemical measures normally assessed in (but not limited to) serum, such as endogenous hormones (testosterone, estradiol, cortisol, thyroid hormones, etc.), metabolic parameters (cholesterol, triglycerides, glucose, etc.), and inflammatory markers, among others.

***Ex vivo cell-based (in vitro)* biomarkers**

This category will cover all biomarkers that use *in vitro* models. In these assays, an extract of human matrices (containing chemical mixtures) is added *ex vivo* to a cellular system and the outcome (i.e., gene expression/ receptor activity/ cell proliferation) is measured. These assays have the potential to elucidate real combined effects, reflecting the integrated dose addition, independent action, synergism, or antagonism of the actual chemical mixture. Such assays may serve to mimic the final

in vivo effect. These biomarkers, which directly reflect specific modes of action (MoA), will help to identify KEs in the priority AOPs and track these within assessed cohort studies.

***In vivo* biomarkers**

This category will cover all biomarkers derived from *in vivo* models. Examples include the *Xenopus Laevis* larval assay, zebrafish embryo assay, and rodent uterotrophic assays, among others.

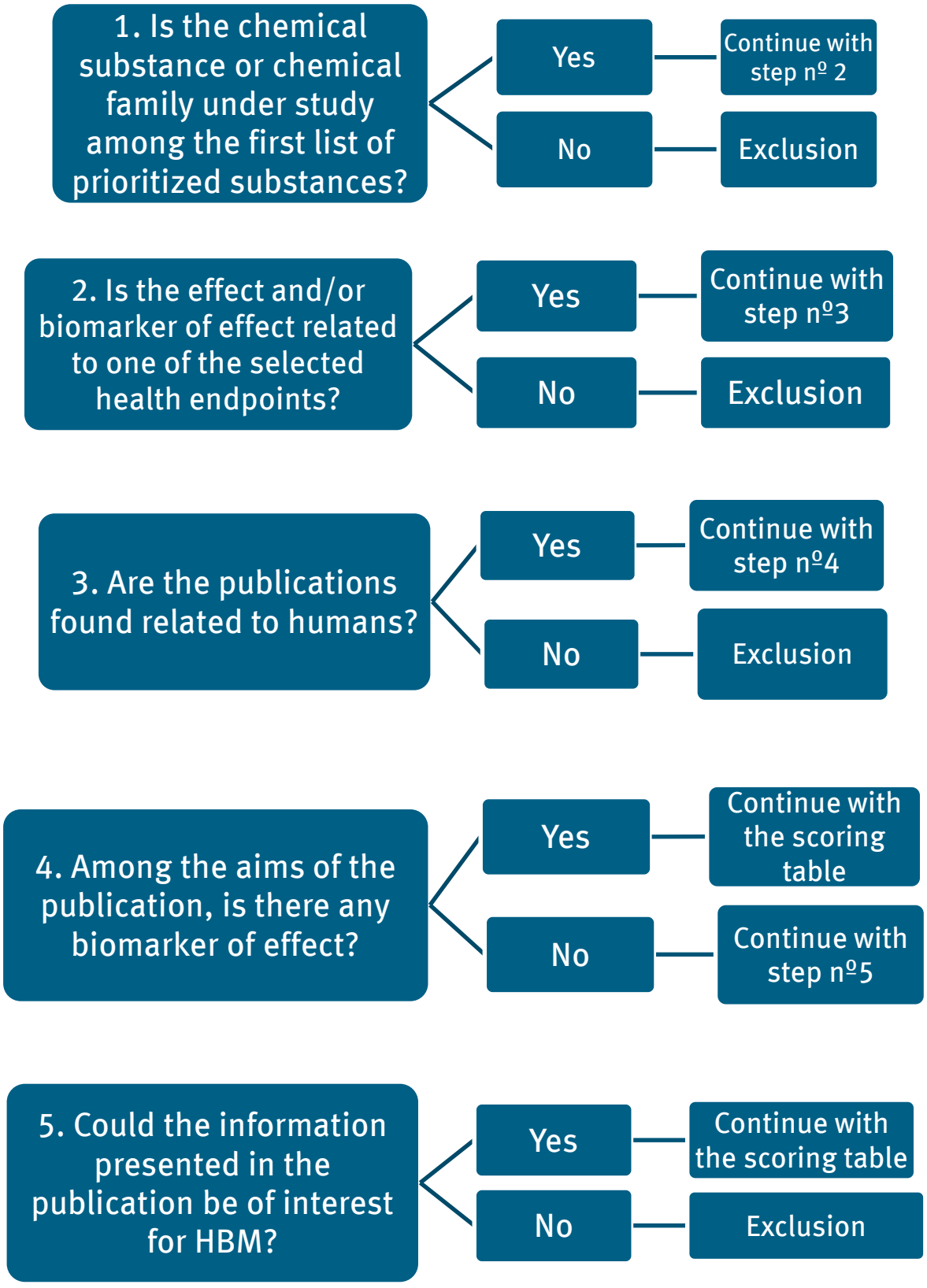
Anthropometric biomarkers

This category will cover all biomarkers related to measures of the entire body or its parts, including all anthropometric measures such as body weight and height, waist and hip measures, tricipital skin-fold thickness and other skin-fold measures, anogenital distance (AGP), and testicular volume, etc.

6 Decision tree-like graph and scoring table

Because the scope of the literature search that will be reported in D14.2 ("List of effect biomarkers for the 1st set of prioritized substances, M12") is inherently wide, the best approach will be to conduct a **comprehensive review**. Thus, we should not adopt highly restrictive criteria, but it is also necessary to systematize the literature search in order to provide a non-biased search that can be replicated. For this reason, the following schemes will contribute to maximize systematization of the literature search, capturing the most relevant biomarkers of effect for human biomonitoring (HBM) purposes while minimizing the loss of information, which is always a concern with this type of wide search. The following decision tree-like graph and scoring table are reported as the protocol for the literature search and prioritization of effect biomarkers.

1. Decision tree-like for the inclusion or exclusion of PubMed/Medline references based on qualitative criteria (Steps):



2. Scoring table for the ranking of biomarkers of effect based on quantitative criteria previously defined.

The following scoring table aims to provide an example of a quantitative assessment of the possible biomarkers of effect in order to rank the most relevant biomarkers of effect for HBM purposes. A range of 0-5 points will be assigned to each criterion. For this purpose, UGR will give WP14 partners an Excel file with a template that includes the following scoring table in an extended format.

Family of prioritized substances: Selected health endpoints:									
					Quantitative criteria (Max. 20 points)				
Type of biomarker of effect based on the biological level of complexity					**6. Has the biomarker been assessed in human matrices?**	**7. A) Is there a plausible mechanism of action (MoA)?**	**7. B) Is an AOP reported for this effect biomarker?**	**8. Has the biomarker been implemented in epidemiologic studies?**	**9. How would you define the feasibility, based on cost, efficacy, specificity, sensitivity and reliability of the biomarker?**
Molecular	Biochemical	Cellular	Tissue/Organ	Organism					Unsure: Indicate your concern(s) and add 0 points Low: 0 points Middle: 2 points High: 5 points
<u>Short description of the bio-marker:</u>					**Non-invasive:** Urine: 5 points Saliva: 4 points Placenta: 2 point **Invasive:** Serum: 3 points Others: 1 point	Yes: report it and add 2 points No: 0 points	Yes: report it, provide the email link, and add 3 points No: 0 points	Yes: provide the DOI, and add 5 points No: 0 points	

7 Literature search methodology

All of these criteria will guide the literature search. Thus, all scientific publications up to 2017 that address the relationship between biomarkers of effect and the first set of prioritized substances will be included. The MEDLINE/PubMed database will be searched for publications written in English, using the name of each family of prioritized substances (Step 1) plus key words such as “neurodevelopment” “reproductive”, “cardiovascular”, “obesity”, “metabolic” and “allergy”… (Step 2). Among the PubMed options on the left side of the screen, we will select: full text, 10 years, and humans (Step 3). A screen capture is shown below as an example.

References cited in the retrieved papers will also be examined. Depending on the number of references available for each prioritized substance and the perceived need, a more restrictive and progressive systematic search will be carried out following the above-reported criteria. In all cases, the principles of the systemic literature search will be followed in order to prevent bias. If two or more partners share the same family of prioritized substances, the literature search will be distributed according to their expertise (molecular, *in vitro*, *in vivo*, epidemiology) and/or the selected health endpoints.