RESEARCH BRIEF

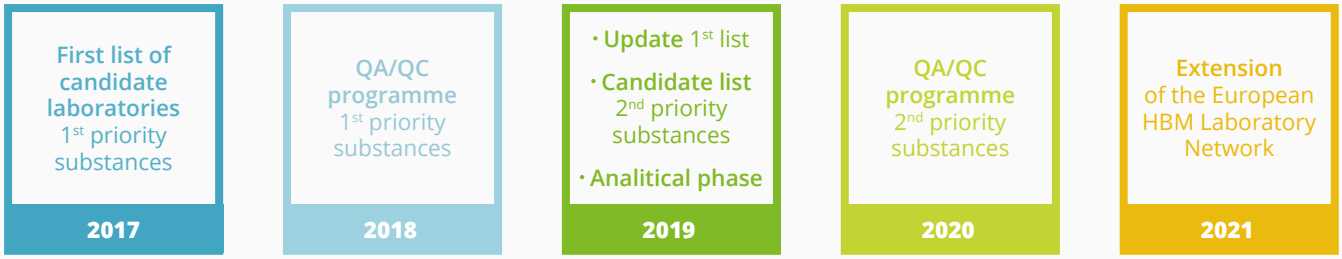
The European Human Biomonitoring (HBM) Laboratory Network: a key for improving chemical analysis applied in human biomonitoring

KEY MESSAGES

- The European Human Biomonitoring (HBM) Laboratory Network has been a **key** to the progress towards obtaining **comparable and robust exposure data** in Europe.
- This network has allowed for the identification of the **EU capacities in HBM chemical analysis,** as well as the **needs and challenges** for the future.
- The establishment of the network has contributed to the **development of new analytical methods** and to the improvement of existing ones.
- It has supported national EU laboratories in improving their analytical skills and will continue to work for the improvement of HBM in Europe.

THE STRATEGY TO GUARANTEE THE QUALITY AND COMPARABILITY OF ANALYTICAL RESULTS IN HBM4EU

The establishment of an European HBM Laboratory Network has come a long way from the beginning of HBM4EU, with the elaboration of a list of candidate laboratories, which in turn was the starting point for the HBM4EU Quality Assurance/Quality Control (QA/QC) programme.



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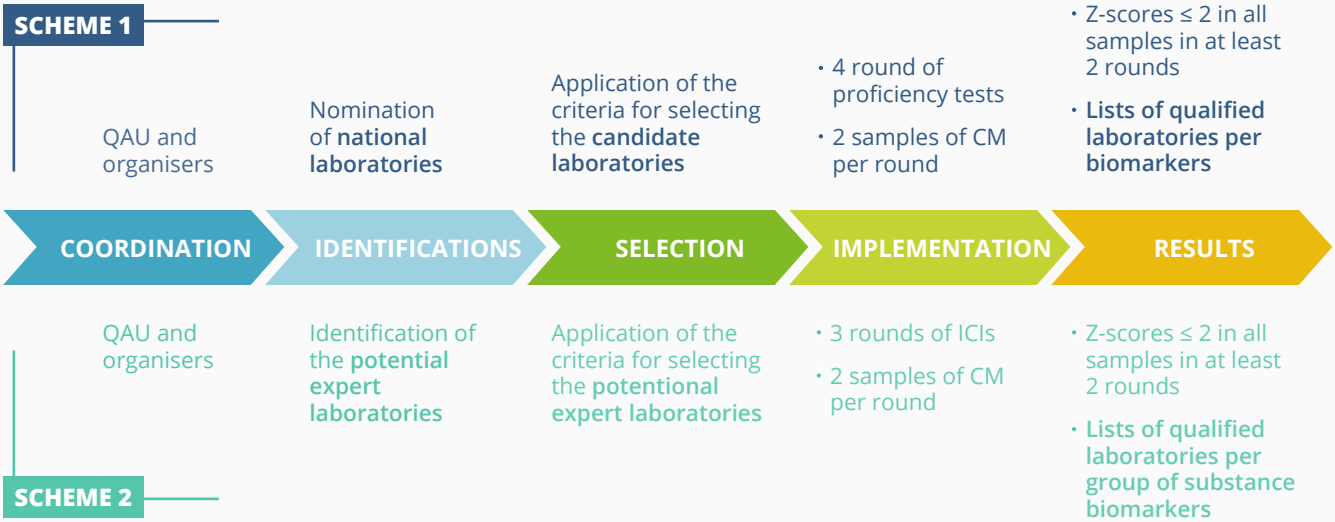
The HBM4EU QA/QC programme applied two different schemes for the two prioritized sets of chemicals.

Scheme 1 covered the substances on the **1st priority list**[1] and involved four rounds of proficiency tests and participants could decide for which biomarkers they would participate. The exercises were organised and evaluated as Interlaboratory Comparison Investigations (ICI) or External Quality Assurance Schemes (EQUAS), depending on the needs and situation for each substance group. In both cases, the exercises involved the assessment of the comparability of analytical results for the same control material analysed in parallel by multiple laboratories, with their own analytical method.

Scheme 2 addressed a reduced list of chemicals, compared with the original **2nd list of prioritization**, to match the studies planned in HBM4EU: acrylamide, arsenic, mycotoxins, pesticides and UV-filters. Scheme 2 included three rounds of ICIs. The laboratories should participate for all the biomarkers within a substance group. For both schemes, two control materials were sent to the participants in each round.
The target concentrations of the biomarkers in the control materials were in the range commonly observed in the general population (between P25–P90 percentile in available national reference values of EU countries, or occupational exposure in the case of Cr). The ICI rounds were spread out over time in such a way that laboratories received feedback on their performance well in advance of the next round, allowing them to perform corrective actions, if needed, before participation in the next round. To achieve satisfactory results in the schemes and take part in the final analysis of the samples in HBM4EU, participants had to qualify in minimally two rounds.



QAU: Quality Assurance Unit
CM: control materials
Steps followed in the two schemes of the HBM4EU QA/QC programme.

SCHEME 1

- Phthalates, DINCH, bisphenols, per- and polyfluoroalkyl substances (PFAS), halogenated flame retardants (HFRs), organophosphorus flame retardants (OPFRs), polycyclic aromatic hydrocarbons (PAHs), cadmium, chromium and aromatic amines.
- 73 biomakers in total
- 4 rounds of proficiency tests
- Urine, blood, serum
- Participation per biomarker

SCHEME 2

- Arsenic, acrylamide, mycotoxins, pesticides, UV-filters
- 22 biomarkers in total
- 3 rounds of ICI
- Urine
- Participation per group of substances

1 Hexamoll® DINCH, phthalates, bisphenols, per- and polyfluoroalkyl substances, flame retardants (halogenated and organophosphorus), polycyclic aromatic hydrocarbons, cadmium, chromium, and aromatic amines.

PROFICIENCY TESTS

Interlaboratory Comparison Investigations (ICI) and External Quality Assurance Schemes (EQUAS) are tools to assess the proficiency of laboratories, and the comparability and reliability of analytical methods. Participation in ICI / EQUAS forms an integral part of quality control, in addition to initial and on-going in-house validation of methodology.

The aim of the ICIs is to measure the comparability, i.e. the degree of variation in analytical results, of participating laboratories. For that, a "consensus value" is calculated as the mean of the results of the participating laboratories after exclusion of outliers. Results from the ICIs are valid if they are within the defined range, normally two-fold standard deviation of the consensus value. External quality assessment schemes are used to improve the accuracy, i.e. the ability to quantify the actual analyte concentration in the sample. The comparison value, in this case the "assigned value", is derived from the results of the Reference Laboratories (RLs). Normally, RLs are laboratories with a worldwide reputation and proven excellence through peer-reviewed publications and/or experience in organization of established international inter-laboratory exercises.

ICI

Interlaboratory Comparison Investigation

Consensus value

COMPARABILITY

EQUAS

External Quality Assessment Scheme

Assigned value and tolerance ranges

ACCURACY

The design of a QA/QC programme requires a meticulous planning to define the basic characteristics of the programme, such as the type of proficiency test (ICI, EQUAS, other) or the number of rounds and samples per round. In this regard, it is very helpful to set up a Quality Assurance Unit with experts in different fields (analytical chemists, toxicologists, experts in human biomonitoring and statisticians). The selection of the biomarker(s), as well as the biological matrix, is crucial as some technical factors should be considered and ensured for the success of the programme. The biomarkers should be stable in the conditions in which the proficiency test will be performed and analytical methods should be available to test the control materials (CM) sent to the participants. The concentration of the target chemical in the CM should be defined according to the needs, e.g. occupational settings or levels in the general population.

After the discussions to design the QA/QC programme, a set of Standard Operating Procedures (SOPs) should be elaborated (and updated if required along the programme). These SOPs should describe the scope of the programme, the detailed process for the preparation and testing of the control material to be sent to the participants, the statistical analysis to assess the results (both CM testing and the participants results), as well as the criteria to consider the results acceptable. The SOPs must define also how to ensure a fluid and continuous communication between the organiser and the participants in order to solve any problem or difficulty encountered appearing during the programme.

QA/QC programme design

Preparation and testing (homogeneity and stability) of the control material

Shipment of the control material and instructions to the participants

Statistical analysis of participants results

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WHY IS ANALYTICAL HARMONIZATION NEEDED?

Human biomonitoring has been applied as a tool in research projects and national programmes, generating a significant amount of HBM data in Europe in the last few decades. However, the available information is somewhat fragmented and is not always fully comparable. Part of this lack of harmonization is established in the analytical phase, because of the limited availability of reference material, standard reference methods or National Reference Laboratories, among others. **An important gap detected in HBM4EU, crucial for HBM harmonization as well as for its improvement, is the limited availability of sustainable procedures and schemes for proficiency testing applied to human matrices.**

The lack of harmonization in HBM was already addressed during the preparation of the EU Environment and Health Action Plan 2004–2010 and consequently, efforts were made to harmonize HBM analytical procedures in Europe. Since then, significant achievements have been reached thanks to the work done by ESBIO (Expert Team to Support Biomonitoring in Europe), followed by COPHES (Consortium to Perform Human Biomonitoring on a European Scale) and DEMOCOPHES (DEMOnstration of a study to COordinate and Perform Human biomonitoring on a European Scale) and most recently, by HBM4EU.

The performance of HBM studies in a harmonised way and applying stringent quality control measures guarantee the quality and comparability of the results and ensure that differences observed in exposure levels are not related to variability in analytical methods and protocols. Therefore, harmonization of the analytical phase is an important component in the whole HBM harmonization. The robustness and reliability of the analytical data are hereby crucial, as a basis to the definition and implementation of protective measures and legislation related to chemicals.

THE IMPORTANCE OF HAVING AN EUROPEAN HBM LABORATORY NETWORK

Chemical analysis in human biomonitoring requires complex methodologies and sophisticated equipment. During the last decades important technical and methodological advances have allowed to increase the number of chemicals that can be analysed as well as identified alternative biological matrices. Therefore, we can today detect and quantify substances that we could not detect some years ago. However, no standard reference methods are available for HBM surveillance purposes. Unlike in other fields, for example such as chemical food safety, there is currently no structure/network of European and/or National Reference Laboratories for HBM. In addition, sustainable procedures and schemes for proficiency testing applied to human matrices have not yet been extensively developed and there are only few suppliers of proficiency tests for HBM biomarkers (e.g. G-EQUAS, QMEQAS, OSEQAS), offering a limited (though increasing) range of biomarker/matrix combinations and relevant environmental and product-use related exposure levels. These issues should be addressed to advance in HBM in Europe and the EU network of HBM laboratories can be the basis for it.

The current network established within HBM4EU, can be the vehicle for implementing the activities and measures to solve the deficiencies identified. For example, it can be the means to develop and define standard analytical methods, to create a stable European QA/QC programme, specific for HBM laboratories. The connexion among HBM laboratories in Europe will increase the possibilities of developing new analytical methods or improving the existing ones (e.g. reducing the limits of quantification for certain substances).

Certainly, the European HBM Laboratory Network would support the increasing human biomonitoring and risk assessment studies providing expertise for new method development and high-quality analytical results.

THE FUTURE OF THE NETWORK

The network of laboratories created in HBM4EU should be considered as one the project's legacy for future human biomonitoring actions in Europe. In the short term, the continuation of this network is ensured by means of the PARC (Partnership for the Assessment of Risk from Chemicals, Horizon 2020). The coordinators of this network, the National Centre for Environmental Health of the Instituto de Salud Carlos III (ISCIII), are already working in the continuation and extension of the network under WP9 in PARC.

Read more:

- Esteban López et al. The European human biomonitoring platform - Design and implementation of a laboratory quality assurance/quality control (QA/QC) programme for selected priority chemicals. Int J Hyg Environ Health 234: 113740. 2021.
- Dvorakova et al. Interlaboratory comparison investigations (ICIs) and external quality assurance schemes (EQUASs) for flame retardant analysis in biological matrices: Results from the HBM4EU project. Environ Res 2002: 111705. 2021.
- Nübler et al. Interlaboratory comparison investigations (ICI) and external quality assurance schemes (EQUAS) for cadmium in urine and blood: Results from the HBM4EU project. Int J Hyg Environ Health 234: 113711. 2021.