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The European human biomonitoring platform - Design and implementation of a laboratory quality assurance/quality control (QA/QC) programme for selected priority chemicals

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ABSTRACT

A fundamental objective of the human biomonitoring for Europe initiative (HBM4EU) is to progress toward comparable and robust exposure data for a wide variety of prioritized chemicals in human samples. A programme for Quality Assurance/Quality Control (QA/QC) was designed in HBM4EU with the purpose of creating a network of European laboratories providing comparable analytical data of high quality. Two approaches were chosen for two sets of prioritized chemicals with different timelines: (i) Scheme 1, where interested candidate laboratories participated in multiple rounds of proficiency tests (ii) Scheme 2, where selected expert laboratories participated in three rounds of interlaboratory comparison investigations. In both cases, the results were used to identify laboratories capable of generating consistent and comparable results for sample analysis in the frame of HBM4EU. In total, 84 laboratories from 26 countries were invited to participate in Scheme 1 that covered up to 73 biomarkers from Hexamoll® DINCH, phthalates, bisphenols, per- and polyfluoroalkyl substances, halogenated flame retardants (HFRs), organophosphorous flame retardants (OPFRs), polycyclic aromatic hydrocarbons (PAH), cadmium, chromium and aromatic amines. 74 of the participants were successful for at least one biomarker in Scheme 1. Scheme 2 involved 22 biomarkers and successful results were obtained by 2 expert laboratories for arsenic, 5 for acrylamide, 4 for mycotoxins, 2 for pesticides and 2 for UV-filters in skin care products. The QA/QC programme allowed the identification of major difficulties and needs in HBM analysis as well of gaining insight in the analytical capacities of European laboratories. Furthermore, it is the first step towards the establishment of a sustainable European network of HBM laboratories.

1. Introduction

Human biomonitoring (HBM) is the gold standard for assessing the

actual, overall exposure to chemicals in individuals or populations, irrespective of the detailed knowledge of contributing exposure sources or pathways. Differences in body burdens are mainly reflective of diet,

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consumer goods and lifestyles as the main exposure determinants for many environmental or product-use related chemicals (Scherer, 2005; Ginsberg and Balk, 2016; Pacyga et al., 2019). These differences are generally small, whereas occupational exposure to chemicals could result in relatively high body burdens. The chemical analysis of HBM samples is facing a number of challenges, related to the environmental exposure at low levels, to the complexity of mixtures of different chemicals (ubiquitous in some cases) and the complex biological matrices. Being a relatively young discipline, with a rapidly growing number of exposure biomarkers in various human tissues and an increasing number of laboratories venturing into the field of HBM, there is a growing demand for establishing a common platform for HBM laboratories, facilitating knowledge exchange, cross-validations, and an international standardization/harmonization of appropriate biomarkers and analytical methods. In this context, it is crucial to have the confidence that the observed differences in multicentre HBM studies are due to variations in exposure and not due to variability or artefacts in the analytical or pre-analytical phase.

In the majority of the EU countries, HBM has been applied as a tool in research projects, often focusing on specific populations, with the exception of some countries such as Germany, Belgium or France, which have established full-scale HBM programmes (Kolossa-Gehring et al., 2012; Schoeters et al., 2012; Dereumeaux et al., 2017). Although significant HBM data have been generated in European countries in the last few decades, the available information appears somewhat fragmented and not always fully comparable. The differences range from the study design (e.g. target population, selection of biological matrices, etc.) to the pre – analytical phase (e.g. sampling procedures, type of samples, etc.) and to the applied analytical methods. With regard to the latter, unlike in some other fields, no standard reference methods do exist for HBM surveillance purposes yet, as there is currently no structure/network of European and/or National Reference Laboratories as it exists in other fields, such chemical food safety (von Holst et al., 2016; Parvaneh et al., 2017; Broothaerts et al., 2020). 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, OSE-QAS), offering a limited (though increasing) range of biomarker/matrix combinations and relevant environmental and product-use related exposure levels (Göen et al., 2012).

This lack of harmonization was already addressed during the preparation of the EU Environment and Health Action Plan 2004–2010 (COM 416, 2004) and as a consequence, efforts were made to harmonize HBM in Europe. The first steps were implemented by ES BIO (Expert Team to Support Biomonitoring in Europe), followed by COPHES (Consortium to Perform Human Biomonitoring on a European Scale) (Becker et al., 2014; Schindler et al., 2014; Esteban López et al., 2015) and DEMOCOPHES (DEMONstration of a study to COordinate and Perform Human biomonitoring on a European Scale) (Den Hond et al., 2015) and most recently, by the Human Biomonitoring for Europe Initiative (HBM4EU, www.hbm4eu.eu).

HBM4EU is an EU Joint Programme that has developed its research programme for priority substances as defined by EU services and partner countries' policy makers to answer open policy relevant questions. HBM4EU aims to harmonize and use HBM to understand human exposure to chemicals, in occupational settings, through the use of consumer products or behavioural choices and the related health risks to improve the chemical risk management and to support policy-making (Ganzleben et al., 2017). Based on policy-related research needs regarding chemical exposure and potential health effects, two sets of priority chemicals were selected in HBM4EU. First set, including phthalates and their substitute 1,2-cyclohexane dicarboxylic acid diisononyl ester (Hexamoll® DINCH), bisphenols, per- and polyfluoroalkyl substances (PFAS), halogenated flame retardants (HFRs), organophosphorus flame retardants (OPFRs), polycyclic aromatic hydrocarbons (PAHs), cadmium, chromium and aromatic amines and the second, acrylamide, aprotic

solvents, arsenic, diisocyanates, lead, mercury, mycotoxins, pesticides and UV-filters in skin care products.

Because the chemical analyses in HBM4EU and its predecessors have been organized in a decentralized manner, involving multiple laboratories in several countries the need to ensure data comparability has been a central aspect of the project early on. First steps in this direction were undertaken in COPHES/DEMOCOPHES where a programme consisting of Interlaboratory Comparison Investigations (ICIs) and External Quality Assessment Schemes (EQUAS) was implemented, supporting the generation of comparable HBM data in 17 EU countries (Schindler et al., 2014; Esteban López et al., 2015). In HBM4EU the challenge has been even greater since the number of laboratories, countries and chemicals are significantly higher. Also, as the conclusions on exposure differences in HBM4EU will have important public health consequences at policy-making level, the quality and comparability of the analytical results has to be guaranteed by strictest Quality Assurance and Quality Control (QA/QC) measures. Based on the two list of priority substances established along the project and the time frame, two different approaches were applied to ensure the full comparability of the analytical results. The first one comprised 4 rounds of proficiency tests with a high number of participating laboratories while the second approach was an intensive interlaboratory comparison investigation with a reduced number of expert participants.

This paper presents the main results and compares the two approaches developed in HBM4EU to obtain high quality and comparable analytical results in multicentre HBM studies for a variety of chemicals and laboratories with different degrees of expertise.

2. Material & methods

2.1. Quality Assurance Unit and QA/QC programme

A Quality Assurance Unit (QAU) was established to discuss and decide all issues related to the QA/QC of the chemical analyses in HBM4EU, including the design of the QA/QC programme. The QAU was formed by experts in the field of HBM and analytical chemistry and included the leaders of the COPHES/DEMOCOPHES QAU, to ensure the continuation of previous successful approaches.

The prime objective of the HBM4EU QA/QC programme was to identify (and in the end certify) analytical laboratories that could analyse the HBM4EU samples accurately, precisely and the most important in a comparable way. For that, the QAU designed two different schemes for each set of prioritized compounds mainly to address the time constraints (Fig. 1). Scheme 1 covered the substances on the 1st priority list and involved four rounds of proficiency tests. Participants were free to decide for which biomarkers they participated. The exercises were organised and evaluated as ICIs or 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. As measure of proficiency, Z-scores were calculated using an assigned value, and a pre-set target standard deviation (e.g. fit-for-purpose standard deviation). In case of ICIs, the assigned value was derived from the participants' results, in case of EQUAS, the assigned value was the mean concentration as established from data generated by designated expert laboratories (ELs).

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 and 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 was in the range commonly observed in the general population (between P25–P90 percentile in available national reference values of

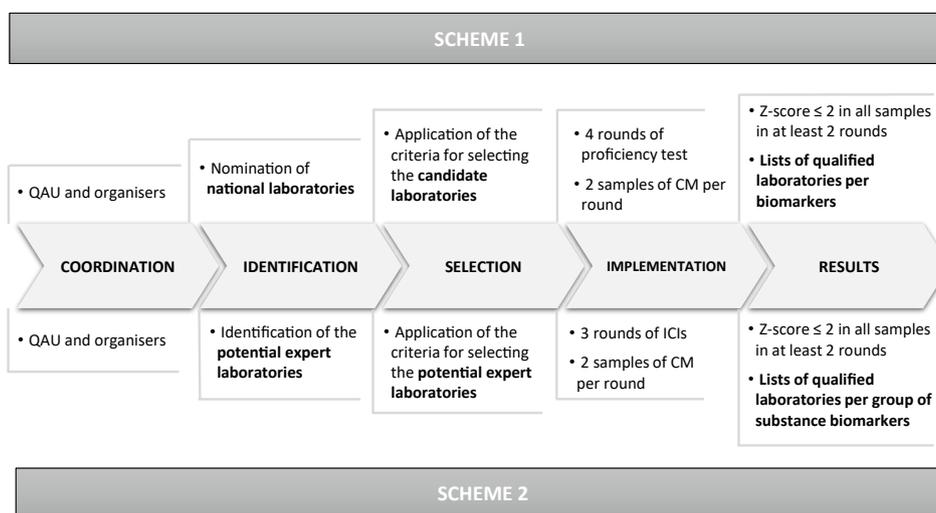


Fig. 1. Steps followed in the two schemes of the HBM4EU QA/QC programme. CM: control materials. ICI: interlaboratory comparison investigation.

EU countries (Den Hond et al., 2015), occupational exposure in the case of Cr) (table S7).

The rounds were spread out over time in such a way that laboratories received feedback on their performance well before 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 analysis of the samples in HBM4EU, participants had to obtain successful results in at least two rounds.

2.2. Identification of the supporting and participating laboratories

The objective of this part of the study was to identify laboratories that could support the QA/QC programme by organising the proficiency tests for a specific group of substances, as well as those laboratories that like to analyse samples in HBM4EU.

The potential candidate laboratories were identified by the HBM4EU National Hub Contact Points (i.e. the contact point for each participating country), who provided information on laboratories performing chemical analyses of the prioritized compounds in human matrices and announcing the activity at country level. Additionally, an announcement was launched on the websites of HBM4EU (www.hbm4eu.eu) and the European Environment Agency (EEA) and in different scientific societies and fora, requesting interested laboratories to sign up as potential candidate laboratories in HBM4EU.

Questionnaires were sent to all nominated laboratories to collect information on their experience in the analysis of the target chemicals in human matrices as well as in organising proficiency tests. The responses were evaluated according to the criteria previously defined by the QAU, having as first criterion the experience in the chemical analysis of the target compound group in human samples (Tables S2 and S3). This process resulted in a list of candidate laboratories who were invited to participate in Scheme 1 or to support the QA/QC programme. Fig. 1 summarises the process.

In Scheme 2, it was agreed to select a reduced number of expert laboratories according to technical and practical criteria defined by the QAU (Table S4). The ELs were invited to join the ICIs and alerted about the tight time frame of these ICIs. All the HBM4EU analyses of the 2nd set of priority substances would be performed only in the ELs obtaining satisfactory results (Fig. 1).

The QA/QC programme was coordinated by the QAU and the supporting selected laboratories were responsible for preparing and sending the control materials to the participants, establishing the communication with the participants, evaluating the results and preparing the

reports.

2.3. Selection of the biomarker/matrix combinations in the QA/QC programme

Specific exposure biomarkers and most suitable matrices to be included in the QA/QC programme were selected for the first group of prioritized chemicals as described in Vorkamp et al. (2021). Briefly, compound-independent criteria were developed for the selection of most suitable biomarkers and matrices for HBM, for example considering the specificity, biological sensitivity and stability of a certain biomarker/matrix combination. These criteria were then applied to review the scientific literature of the last ten years approx., with a view to identify the most suitable biomarkers and matrices for each of the prioritized chemical. This evaluation resulted in a first list of pairs of biomarker/matrix (typically serum or urine) for each compound group (Vorkamp et al., 2021). In the next step, the list of exposure biomarkers was further reduced based on technical feasibility, expected body burdens and policy-related research needs, as evaluated by the QAU. This shortlist was used in the QA/QC programme.

The same procedure was applied to define the biomarkers for the 2nd list of priority substances addressed in Scheme 2.

2.4. Organization of the proficiency tests

In order to provide a harmonised approach for the organization and evaluation of the different ICI/EQUAS exercises, protocols were drafted and described in standard operating procedures (SOPs). These SOPs were based on existing protocols originated from ISO17043 accredited organisations, and included detailed instructions for all aspects of the QA/QC programme, such as the description of the roles and responsibilities of the organisers, timeline of the exercises, definitions of different terms or templates for communication with the participants and reporting of the results. Additional SOPs were drafted for the preparation and characterisation of control materials and for the evaluation of participants' results. Details for the preparation of the various control materials are available in the online library of the HBM4EU website (www.hbm4eu.eu). The characterization of the control materials included homogeneity and stability testing.

Homogeneity testing was based on ISO13528:2015 and Fearn and Thompson (2001). This involved duplicate analysis of 10 randomly selected test samples of a control material. The control material was considered sufficiently homogeneous if the between-sample standard deviation did not exceed a critical value ($0.3 \times$ target standard

deviation).

The stability of the control materials during the period from shipment to the deadline for submission of the participants' results was assessed in line with ISO 13528:2015 and the international harmonised protocol for the proficiency testing of analytical laboratories (Thompson et al., 2006). For this, the organiser stored test samples under the conditions recommended to the participants (typically freezer < -18 °C), and optionally an additional set at -80 °C. Stability was assessed by comparison of the mean of six stored samples (-18 °C, t = after receiving all participants' results), with the mean of a reference set of six samples. The reference was either the mean as obtained at/before shipment of the samples, or the mean obtained for samples stored at -80 °C (assumed stable) analysed concurrently with the stored samples.

2.5. Evaluation of laboratory performance

The laboratory performance was assessed by calculation of z-scores for each biomarker in the test samples according to the following formula:

$$Z = \frac{x - A}{\sigma_T} \quad (1)$$

with Z = z-score

x = participant's result

A = assigned value

σ_T = standard deviation for proficiency, with $\sigma_T = 0.25 \cdot A$

A z-score of $|Z| \leq 2$ was interpreted as satisfactory, $2 < |Z| < 3$ as questionable, and $|Z| \geq 3$ as unsatisfactory performance. The assigned value was either the consensus value derived from the participants (used in ICI) or a value derived from analysis by selected expert laboratories (used in EQUAS). The parameters from equation (1) are briefly explained below.

- **Standard deviation for proficiency**, or target standard deviation (σ_T), determines the performance boundaries of the ICI/EQUAS. The performance boundaries should be fit-for-purpose and take into account the interlaboratory variability (reproducibility relative standard deviation, RSD_R) currently considered achievable in HBM analysis. The available data on the latter is scarce and variable. Schindler et al. (2014) reported RSD_{RS} ranging from 6% to 32% for cadmium in urine, and 31%–45% (even higher in some cases) for phthalate biomarkers in urine. For selected highly experienced reference laboratories, Göen et al. (2012) reported RSD_{RS} in the range 7%–19% for cadmium in urine. Outside the HBM domain, a generic relationship between expected RSD_R and concentration was originally proposed by Horwitz et al. (1980) and later modified by Thompson (2000), and has often been used as a fitness-for-purpose criterion in proficiency testing. The modified Horwitz equation suggests a constant RSD_R of 22% for concentrations below 120 $\mu\text{g}/\text{kg}$, and a decrease of RSD_R for higher concentrations. The validity of the modified Horwitz function has been a matter of debate (Linsinger and Josephs 2006). A constant RSD_R of 25% over a range of 1 $\mu\text{g}/\text{kg}$ to 10 mg/kg has been suggested as more appropriate (Alder et al., 2001). Based on the literature it appears that, as long as concentrations are (well) above the method limit of detection (LOD), there is no consistent relationship between concentration and RSD_R . It may depend on the analyte and technique, but at this stage, in lack of exhaustive data for achievable RSD_{RS} in HBM analysis and based on the data and discussions from the literature, it was decided to apply a fixed RSD_R of 25% as fit-for-purpose criterion to be used as target relative standard deviation in the ICI/EQUAS programme.

- **Assigned value = consensus value (ICI)**. For determination of the consensus value, robust statistics was performed in accordance with Thompson et al. (2006), the guidelines from (Analytical Methods

Committee, 1989a&b), and ISO 13528. The robust mean was taken as consensus value when the following requirements were met: the number of results submitted for a biomarkers had to be at least seven, the uncertainty (u) of the consensus value should be negligible (not exceed $0.3 \cdot \sigma_T$), with u being 1.25 times the standard deviation of the participants' results, divided by the square root of the number of participants. When the uncertainty of the consensus was not negligible, but not exceeding $0.7 \cdot \sigma_T$, the consensus value was still used for calculation of z-scores, but the uncertainty of the consensus value was taken into account for calculation of the z-scores using the following formula:

$$Z' = \frac{x - A}{\sqrt{\sigma_T^2 + u^2}} \quad (2)$$

with Z' = z-score ($0.3 \cdot \sigma_T < u \leq 0.7 \cdot \sigma_T$)

u = uncertainty of consensus value

In case the uncertainty of the consensus exceeded $0.7 \cdot \sigma_T$, the variability of results was considered too high to derive a meaningful consensus, and, consequently, also z-scores using such consensus value were considered unfit for evaluating individual participants' performance.

- **Assigned value = Expert value (EQUAS)**. Establishment of the expert value to be used as assigned value involved the analysis of six replicates of the control material by at least three selected laboratories with a high level of expertise in the determination of the biomarker. For each expert laboratory, the mean value was calculated. Based on these means, the mean of the expert laboratories was calculated, the RSD, and the relative uncertainty (RSD divided by the square root of the number of expert laboratories). The expert value was considered suitable for use as assigned value as long as the uncertainty did not exceed $0.7 \cdot \sigma_T$ (i.e. 17.5%). For EQUAS, equation (1) was used for calculation of z-scores.

2.6. Programme coordination and follow – up

Monthly web conferences were organised among the QAU, the programme coordinator and the organisers of the different exercises to discuss the problems encountered and to exchange experiences. A help desk was available for the participating laboratories during the whole duration of the scheme and web conferences were offered to the participants when necessary in order to solve the main analytical problems faced during the exercise. Furthermore, participants also received recommendations after each round in the result reports. Nevertheless, some groups (i.e. Hexamoll® DINCH and phthalates) required a more intense and continuous support and a specific training school was organised.

3. Results

3.1. Details of the QA/QC programme

Both approaches were designed under common premises, but there were some important differences, for example in the number of biomarkers and matrices included (Fig. 2). Scheme 1 covered 73 biomarkers in 3 different human matrices while Scheme 2 involved 22 biomarkers in urine. The duration of Scheme 1 was 18–20 months depending on the substance group. The time for implementing Scheme 2 was shorter, from 3 up to 7 months depending on the group of substances.

3.2. Identification of the supporting and participating laboratories

For Scheme 1, a total of 183 questionnaires were sent to identify candidate laboratories for supporting the QA/QC programme and for

SCHEME 1	SCHEME 2
<ul style="list-style-type: none"> - Phthalates, Hexamoll® DINCH, bisphenols, PFAS, BFRs, PFRs, PAHs, Cd, Cr, aromatic amines - 73 biomarkers in total - 4 rounds of proficiency tests - Urine, blood, serum - Participation per biomarker 	<ul style="list-style-type: none"> - As, acrylamide, mycotoxins, pesticides, UV-filters - 22 biomarkers in total - 3 rounds of ICI - Urine - Participation per group of substance

Fig. 2. Main characteristics of the two approaches followed in the HBM4EU QA/QC programme.

participating in the proficiency tests. 115 replies were received (63% response rate), some of them from the same laboratory to participate for different substance groups. Fig. 3 presents the number of candidate laboratories for Scheme 1 per group of substance. Approximately one year later, and after the 1st round of the proficiency test, the list of candidate laboratories was updated (Fig. 3). Questionnaires were sent to 229 potentially interested laboratories, including those already registered as candidate laboratories. The response rate was lower (37%) since some of the laboratories already included on the list of candidate laboratories did not update their data. In order to increase the participation, the selection process was simplified and the only criteria applied for participating in Scheme 1 was the exclusive criterion in table S2 (“have experience in analysing human samples for the given chemical”).

With regard to the origin of the candidate laboratories, almost half of them (48%) were from universities or university hospitals, followed by governmental laboratories with 39% of the total number. Private laboratories accounted for 9%.

Of the laboratories completing the questionnaire for supporting the HBM4EU QA/QC programme, 19% reported experience in organising proficiency tests and 18 laboratories were selected in the first call, based on the criteria in Table S3. In the update, 11 participants out of 48 (23%) provided a positive answer and only three new laboratories were added to the list of potential supporting laboratories. Finally, five laboratories were involved as organisers (Table 1). In case of Scheme 2, the organisers were selected from those supporting the previous scheme based on their proven expertise (Table 1). The proficiency test for aromatic amines required a different organization as so, it will not be addressed in this publication.

Table 2 shows the biomarkers covered in the programme (except those for aromatic amines) as a result of the selection process, grouped by substances classes. While Scheme 1 offered a broad range of biomarkers and the participants decided for which they reported results, Scheme 2 offered a more limited number of biomarkers and the ELs had to participate for all biomarkers included in the programme. However, while the biomarkers were pre-defined, the laboratories were free to choose their own analytical method.

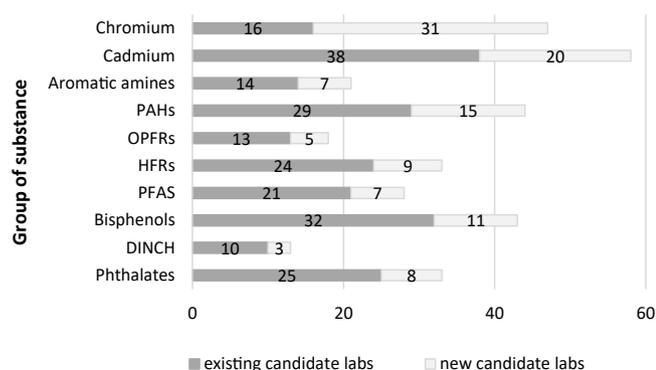


Fig. 3. Number of candidate laboratories in the two calls.

Table 1
Laboratories supporting the HBM4EU QA/QC programme. (Acronyms are defined in Table S1).

Substance group	Organiser	Laboratory preparing & testing control material
1st list of prioritization – Scheme 1		
Phthalates	RIKILT	RIKILT, IPA
Hexamoll® DINCH	RIKILT	RIKILT, IPA
Bisphenols	INRAE	INRAE
PFAS	IPASUM	IPASUM
HFRs	UCT	UCT
OPFRs	UCT	IPASUM
PAHs	IPASUM	IPASUM, UCT ^a , ABF ^b
Cadmium	IPASUM	IPASUM
Chromium	IPASUM, JSI ^d	IPASUM, JSI ^d
2 nd list of prioritization – Scheme 2		
Acrylamide	IPASUM	IPASUM
Arsenic	IPASUM	IPASUM
Mycotoxins	RIKILT	RIKILT
Pesticides	RIKILT	RIKILT, IPA
UV-filters	IPASUM	IPA, Region H

RIKILT, current name: Wageningen Food Safety Research, part of Wageningen University & Research, The Netherlands.

IPA, Institute for Prevention and Occupational Medicine of the German Social Accident Insurance - Institute of the Ruhr-University Bochum, Germany.

INRAE, Laboratoire d'Etude des Résidus et Contaminants dans les Aliments, LABERCA, Oniris-INRAE, France.

IPASUM, Institute and Outpatient Clinic of Occupational, Social and Environmental Medicine, Friedrich-Alexander-Universität Erlangen-Nürnberg, Germany.

UCT, University of Chemistry and Technology, Czech Republic.

ABF, ABF GmbH Analytisch-Biologisches Labor, Planegg, Germany.

JSI, Jozef Stefan Institute, Slovenia.

Region H, Dep. of Growth and Reproduction, Rigshospitalet, University of Copenhagen.

Selection of the parameters in the programme.

^a Only the 1st round.

^b Only 3-BaP in the 1st and 2nd round.

3.3. Participation

A total of 84 laboratories from 26 countries were invited to participate in Scheme 1 but not all of them confirmed their participation. The percentage of invited laboratories that registered in the course of the complete scheme per group of substance varied from 85% for Hexamoll® DINCH to 35% for OPFRs (considering the highest number of registrations in each group). In the case of Hexamoll® DINCH the registration was constant in the four rounds while for the rest of substances, in general, the number of registered laboratories increased after the first two rounds, up to twice or more in case of cadmium and chromium, and decreased after the 3rd round. In total 9 laboratories from Canada, Japan and United States collaborated as reference laboratories in the rounds that were organised as EQUAS (from the 2nd to the 4th round, except for Cr in which all rounds were ICIs) (Table S5).

Looking at the participation of the laboratories in Scheme 1 for different groups of chemicals, more than the half (61%) of the

Table 2
Biomarkers covered in the HBM4EU QA/QC programme.

Substance group	Matrix	Biomarkers
1st list of prioritization – Scheme 1		
Phthalates	urine	MEP, MBzP, MiBP, MnBP, MCHP, MnPeP, MEHP, 5OH-MEHP, 5oxo-MEHP, 5cx-MEPP, MnOP, OH-MiNP, cx-MiNP, OH-MiDP, cx-MiDP
Hexamoll® DINCH	urine	OH-MINCH, cx-MINCH
Bisphenols	urine	BPA, BPF, BPS
PFAS	serum	PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDODA, PFBS, PFHxS, PFHpS, PFOS (sum of all isomers)
HFRs	serum	BDE-47, BDE-153, BDE-209, α -HBCD, γ -HBCD, TBBPA, Syn-DP, Anti-DP, DBDPE, 2,4,6-TBP
OPFRs	urine	DPHP, BDCIPP, BCEP, BCIPP
PAHs	urine	1-naphthol, 2-naphthol, 1,2-DHN ^a , 2-FLUO, 3-FLUO, 9-FLUO, 1-PHEN, 2-PHEN, 3-PHEN, 4-PHEN, 9-PHEN, 1-PYR, 3-BaP ^a
Cadmium	urine blood	Cd
Chromium	urine blood serum	Cr
2 nd list of prioritization – Scheme 2		
Acrylamide	urine	AAMA, GAMA
Arsenic	urine	As total, As (III), As (V), MMA, DMA, AsB
Mycotoxins	urine	DON (total)
Pesticides	urine	TCPy, glyphosate, AMPA, cis-DBCA, cis-DCCA, trans-DCCA, 3-PBA, 4-F-3-PBA, ClF3CA
UV-filters	urine	BP1, BP2 ^b , BP3, BP7 ^b

^a Only in the 1st and 2nd round.

^b Only in the 1st round.

laboratories participated in the proficiency test for one or two substance groups (36% and 25% respectively) while a very limited number of laboratories (3%) participated for six or more substance groups.

The registration of the laboratories in the biomarkers offered in Scheme 1 varied considerably and showed a high intra-group variation for some substances. The low number of participants sometimes led to insufficient data for results evaluation and a detailed revision was required, including different statistical evaluations in order to obtain reliable results and conclusions. The specific difficulties encountered in each group of substances and the solutions applied will be addressed elsewhere. Table S6 shows the number of laboratories that registered in the four rounds as well as those reporting results and those consistently achieving satisfactory performance in Scheme 1.

Considering the global results, 74 participants reported successful results for at least one biomarker. The maximum number of biomarkers for which a laboratory reported successful results was 47. The average and P90 were 11 and 30 biomarkers, respectively.

Regarding participation in Scheme 2, five expert laboratories participated for acrylamides, three for arsenic, six for mycotoxins, four for pesticides and three for UV-filters, however, this was reduced to two in the second and third round (Table 3). At least two thirds of the participating laboratories returned satisfactory results (Table 3). All participants in the acrylamide exercise obtained satisfactory results.

Table 3
Participants and results in Scheme 2.

	ROUND 1	ROUND 2	ROUND 3	no.reporting satisfactory results ^a (%)
	no. registered/no.reporting	no. registered/no.reporting	no. registered/no.reporting	
Arsenic and compounds	3/3	3/3	3/3	2 (67%)
Acrylamide	5/5	5/5	5/5	5 (100%)
Mycotoxins	6/6	6/5	5/5	4 (67%)
Pesticides	4/4	4/4	4/4	2 (50%)
UV-filters	3/3	2/2	2/2	2 (100%)

^a Achieving satisfactory z-scores for the biomarker in both control materials from a round, in at least two rounds from the QA/QC programme.

4. Discussion

Although QA/QC is an essential component in any analytical laboratory, robust results that are comparable between laboratories can still be a challenge, in particular in the context of human biomonitoring of the general population, including low concentrations and the co-occurrence of a multitude of chemicals. For the first time, two different QA/QC approaches were implemented to ensure the quality and comparability of the analytical results in a multicentre EU-wide HBM project.

The design of the HBM4EU QA/QC programme had to be adapted to certain predefined characteristics of the project, mainly the time constraints and the support of capacity building in the participating countries. Scheme 1 offered the possibility of including a high number of laboratories (including less HBM experienced laboratories) and improving their analytical performance while Scheme 2, had to be done in a shorter time period and focused on assessing comparability of results for a small pre-selected group of expert laboratories. Thus, the two approaches were designed according to different priorities.

This work has allowed the identification of a high number of EU laboratories with experience in human biomonitoring and created the first HBM laboratory network in Europe. Nevertheless, despite the two calls and different communication channels employed to reach the laboratories, the authors are aware that a number of analytical laboratories from the different participating countries were not involved in the programme. This could be due to the information not reaching the laboratories or to a lack of interest in participating in the programme (e.g. not aligned with the laboratory interests or because it was a non-funded activity). However, the number of participants allowed to achieve the objectives of HBM4EU, i.e. obtain high quality and comparable HBM results and to provide the capacities for a Europe wide HBM study.

The process of identifying the candidate laboratories revealed interesting information. Significant differences were observed in the number of candidate laboratories for the different groups of substances, primarily reflecting expertise in the analysis of the substances involved. For example, the analysis of cadmium in human samples has been established for years, and validated analytical methods are available. As a consequence, the highest number of candidate laboratories to participate in the QA/QC programme was found for cadmium analysis. However, for biomarkers related to chemicals of more emerging concern the number of laboratories with experience is lower and therefore the number of candidate laboratories was reduced e.g. for Hexamoll® DINCH or OPFRs. A kind of specialization or interest in certain substances was observed for the majority of the laboratories since in general the participation was restricted to 1–3 groups of substances while a reduced number of them covered a wider spectrum. Nevertheless, this could be influenced by other factors such as individual interests and therefore not reflect the real situation in terms of expertise and capacities of the laboratories. Independently of that, there was a clear difference between the participation and results of the inorganic and organic chemicals selected in the project.

A great challenge during the first stages of the programme was the identification of the laboratories to support the QA/QC programme (proficiency tests organisers) since only a limited number of laboratories

meet the HBM4EU criteria to support the QA/QC programme. In addition the short timeframe did not help neither since proficiency test had to be organized in parallel for 73 biomarkers in 3 matrices for more than 80 laboratories across 30 countries. In some cases, the laboratories did not have experience in organising proficiency tests for the target compounds. In others, the laboratories had wide experience even in the target chemicals but in other research areas, including in non-human matrices. In addition, although some laboratories had experience in organising these exercises, they could not prepare and test the homogeneity and stability of the control materials employed in the programme and, for some substances (phthalates, Hexamoll® DINCH, OPFRs and PAHs in Scheme 1 and for pesticides and UV – filters in Scheme 2), it was necessary to involve both an organiser and an expert laboratory able to prepare and test an adequate and reliable control material to use in the QA/QC programme. This was indeed another great challenge since, due to the lack of reference materials (i.e. target matrix and biomarkers in the concentrations expected in the general population) the preparation (and test the homogeneity and stability) of the control material for all the exercises increased the time period of the programme. This process was done under strict QA/QC measures and precisely described in the corresponding SOP, to ensure that organisation and evaluation by the different parties involved were done in a harmonised way.

In general, the adherence to the programme was good, although in the first rounds of phthalates, HFRs, OPFRs and PAHs the percentage of registered laboratories reporting results was low for certain biomarkers. In case of phthalates, this occurred for OH-MiNP, OH-MiDP and cx-MiDP, with reporting percentages below 90%. This could probably be explained by initial difficulties in the laboratories that were solved after the first round. The same tendency was observed for BPS and PFPeA. The HFRs also showed an increase in the reporting percentage after the first round except for TBBPA, DBDPE and 2,4,6-TBP. For these compounds, the potential analytical problems were not solved as the number of laboratories with satisfactory results remained low. The situation was similar for OPFRs with a low number of participants reporting results (and high variability among them) for the four biomarkers, making the evaluation of the results difficult. The highest variability in the participation per biomarker and the percentage of registered laboratories reporting results was found in the PAHs group, not only due to the technical difficulties but also due to the specialization of the participants in specific biomarkers.

To achieve satisfactory results in the programme and take part in the analysis of samples in HBM4EU, participants had to obtain successful results in at least two rounds of the proficiency tests and this could explain the general decrease in the number of participants in the 4th round, especially for Cd and Cr. However, this reduction was not so clear in the PAHs group.

Looking at the laboratories that obtained satisfactory results in the programme, the overall goal of analysing the samples in HBM4EU in a comparable way was achieved for all the target chemicals with a high improvement in the number of biomarkers with satisfactory results per laboratory (Figs. 4 and 5). For Hexamoll® DINCH, around 70% of participating laboratories obtained successful results for the two biomarkers (OH-MINCH and cx-MINCH). The phthalates group had higher variability with 31–95% of the participants with satisfactory results depending on the biomarker. There was a set of phthalate metabolites (MEP, MBzP, MnBP, 5OH-MEHP, 5oxo-MEHP, 5cx-MEPP and cx-MiDP) with a satisfactory percentage above 75% while a second set had fewer participants and poorer results (MCHP, MnPeP, MnOP, OH-MiNP, cx-MiNP and OH-MiDP). Issues encountered for the phthalates group included the diversity in coverage of biomarkers (ranging from 3 to all prioritized 15) and limits of quantification (LOQs) (0.02–3.5 ng/ml), as well as background contamination for some of the biomarkers. For biomarkers of the long-chain phthalates (OH-MiNP, cx-MiNP, OH-MiDP, cx-MiDP) and Hexamoll® DINCH (OH-MINCH, cx-MINCH) initially a very high variability of results was observed. The reason for this was that

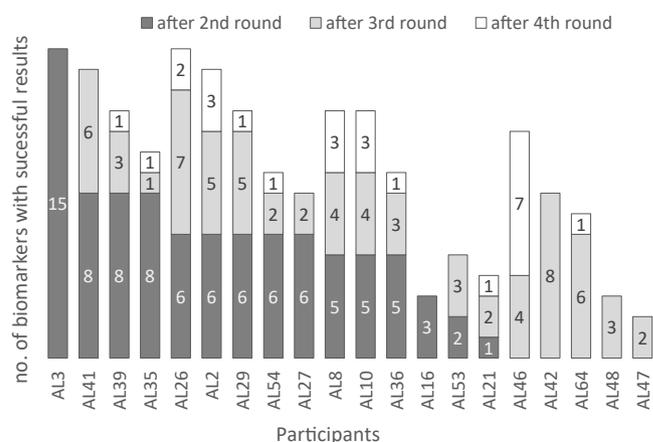


Fig. 4. Progress in the satisfactory results of phthalate biomarkers.

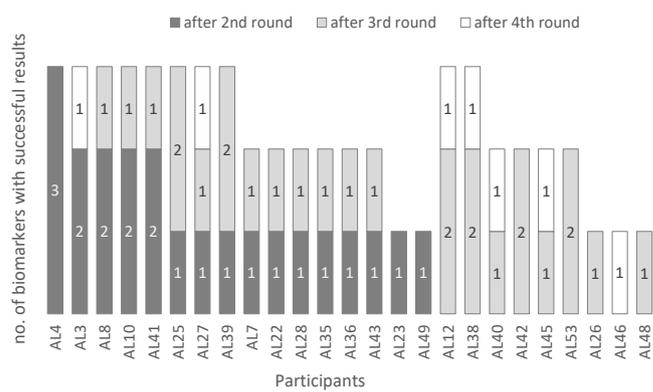


Fig. 5. Progress in the satisfactory results of bisphenols.

the parent compounds are mixtures of isomers resulting in multiple and/or broad peaks in real samples, and because the transition used for quantification in LC-MS/MS analysis affected the results. Standardizing to prescribed mass-transitions for quantification and recommendations regarding the acquisition window to ensure all relevant isomer peaks were included in the measurement reduced the variability. The inter-laboratory variability (RSD_R) derived from the participants' results improved during the programme. Details will be presented in a future paper.

For bisphenols while no major differences were found in terms of participation, the laboratories with satisfactory results varied with the highest number for BPA (83%) and lowest for BPF (50%). Some issues were encountered for this group, especially during the 1st round. Firstly, BPA results appeared overestimated for some participants, probably impacted by an external contamination source. Secondly, BPS and especially BPF were more rarely included and reported by participants, leading to a non-achievable performance assessment especially for BPF during the 1st round, together with a high variability of the results reported by this limited number of laboratories. However, a significant improvement of the results for both BPA, BPS and especially BPF was observed between the 1st and 4th round, demonstrating a good capacity building and methodological consolidation after considering lessons learnt from each round (detailed results and discussion will be presented in a future paper). Globally, the whole exercise for bisphenols finally permitted to attest the existence of a core network of competent HBM laboratories for BPA, BPS and BPF.

For the PFAS group, in general, laboratories showed a high reporting rate for all the biomarkers and the percentage of successful results was above 70% (except for PFHxA) and up to 100% for PFHpA, PFOA, PFNA, PFDA, PFHxs and PFOS. While laboratory performance in the 1st round

(ICI) varied considerably for the individual PFAS biomarkers (PFPeA: 54%; PFOA and PFHxS: 94% each, of laboratories with successful results), the switch to EQUAS from the 2nd round on led to an overall improvement and better homogeneity of results. However, despite this general improvement, especially the analysis of PFHxA at low levels and PFDoDA at high levels proved to be challenging for some laboratories. Detailed results and discussion will be presented in a future paper.

For HFRs, BDE-47, BDE-153 and BDE-209 showed the highest registration in all rounds, thus the calculation of consensus value in the 1st round was possible unlike the other HFR biomarkers. From the 2nd round, organized as EQUAS, an expert assigned value was established for BDE-47, BDE-153, BDE-209, anti-DP and syn-DP. For others (α -HBCD, γ -HBCD, DBDPE, TBBPA and 2,4,6-TBP) the calculation of an assigned value was not possible because of the limited scope of reported results by experts or too high uncertainty of the assigned value. In this case, the calculation of the consensus value from the results submitted by experts and candidates was successful only for α -HBCD and γ -HBCD. Due to the low number of candidates and expert results the calculation of consensus or assigned value was not possible for DBDPE, TBBPA and 2,4,6-TBP (the laboratory in [table S6](#) correspond to the laboratory preparing and testing the CM for this exercise). The highest number of satisfactory results was achieved for BDE-47 and BDE-153. For BDE-209 the success rate was not as high, because of a higher number of results assessed as questionable or unsatisfactory.

The group that presented most difficulties due to the low number of participants and high variability of the results was the OPFRs. The calculation of consensus or assigned values according to standardized ICI/EQUAS approach was not possible at all (BDCIPP, BCIPP and BCEP) or only to a limited extent (DPHP) in the first three rounds. It was necessary to apply a more flexible approach, in order to draw conclusions. It is worth noting, that following discussions of main analytical difficulties after the 1st and 3rd round, the 4th round was very successful. The calculation of assigned values and the evaluation of results using the ICI/EQUAS approach was realized for BDCIPP, BCIPP and DPHP. Finally, from a total of six laboratories, which participated in any or the four rounds, five laboratories were successful for DPHP and BDCIPP and four for BCIPP. Details of the flame retardants results will be presented in a future paper.

The PAHs group had the highest variability in the number of participants per biomarker and also in the laboratories reporting satisfactory results. The main difficulties with PAHs metabolites was that no evaluation was possible for some biomarkers (1,2-DHN, 3-FLUO, 9-FLUO, 9-PHEN, 3-BaP) as the number of participating laboratories was too small (<7). Even after switching to EQUAS from the 2nd round on, no z-scores could be obtained for 9-FLUO, 9-PHEN and 3-BaP, while the only laboratory to analyse 1,2-DHN could not provide quantitative results. From the 3rd round on, the initial scope of 13 biomarkers were reduced to 11 (1,2-DHN and 3-BaP were omitted), but still no z-scores could be provided for 9-FLUO and 9-PHEN in the remaining two rounds. A general improvement in results from the 2nd to the 4th round is not discernible, in fact for some biomarkers even the opposite development is noticeable (especially for 2-FLUO). Details will be presented in a future paper.

The groups involving inorganic biomarkers were those with the highest rate of participation probably due to a more well-established and robust methodology for the analysis of these metals, although the percentage of satisfactory results were in line with those observed in other groups (e.g. PFAS, HFRs). The proficiency tests on cadmium (in blood and urine) were characterized by a large number of participants and satisfactory results from the 1st round on. Thus, there was no noticeable improvement from one round to the next. The main problem encountered was that some laboratories had a too high LOQ for their analytical method and thus failed at low analyte concentrations (Nübler et al., 2021). Unlike the other substance groups, no EQUAS was performed for chromium from the 2nd round onwards, but an ICI was performed in all four rounds. The ICI exercises on chromium (in blood, urine and serum)

were characterized by satisfactory results throughout, with too high LOQs being the only problem encountered for some laboratories. Detailed results and discussion will be presented in a future paper.

Globally, the participation in Scheme 1 improved the capacities of the laboratories since the number of laboratories obtaining satisfactory results and the number of biomarkers with successful results per laboratory increased from the first to the last round.

For Scheme 2, the main difficulty was the selection of a reduced number of laboratories with enough expertise because in addition to the technical capability, practical aspects had to be considered in order to ensure the availability of the analytical results by the deadline defined within HBM4EU. The reduced time for implementing Scheme 2 was challenging for both the organisers and the participating ELs. The unexpected shutdown due to the first wave of covid-19 sanitary crisis put further strain on the scheme. The first round was implemented as planned from January to February 2020. The second round started in February–March, but the shutdown of laboratories and restrictions in the shipment of samples because of the covid-19 caused a significant delay in the ICI for acrylamide, UV-filters, pesticides and mycotoxins (samples for As had been sent before the shutdown). Furthermore, some laboratories withdrew their participation in the ICI as a result of the difficulties derived from this situation. Despite these delays, Scheme 2 was implemented between 3 months (arsenic) and 7 months (mycotoxins), so the objective of reducing the time for identifying laboratories with comparable results that could analyse the HBM4EU samples was achieved (Scheme 1 lasted at least one year and a half). In general, Scheme 2 presented less difficulty related to technical aspects since the laboratories involved had wide experience in the analysis of the target biomarkers and a baseline for their selection was defined (e.g. limit of quantification). Nevertheless, some interesting observations were made, for example the differences in total DON levels reported depending on the enzyme used for deconjugation. In several control materials, significantly lower concentrations of total DON were obtained when using β -glucuronidase/sulfatase from *Helix Pomatia* than when using β -glucuronidase from *E. Coli*. As expected, the pesticide group was the one with more difficulties, with consequences for the results evaluation. While for glyphosate and AMPA results were comparable in general, for chlorpyrifos and pyrethroid biomarkers the relative uncertainty of the mean was too high in several cases for a straightforward statistical evaluation of the reported results.

5. Conclusions

The QA/QC programme designed and implemented in the frame of the HBM4EU initiative can be termed a success and, as in previous studies, the need and utility of this kind of activities in HBM studies was evident. As long as there are no commercial proficiency tests offering a wide of biomarkers of interest in HBM at the concentrations in the range observed in the general population, the proposed approach appears as the best tool to investigate and improve results comparability. However, its implementation in the framework of such research project is complex and sustainability beyond the project is an issue regardless of the approach applied. Apart from the time constraints, questions such as the experience and capacities of the partners for supporting these activities should be considered, as well as the issues related to the funding. The organization of and participation in proficiency tests require a large amount of resources that cannot always be justified within a research project, possibly limiting the participation and thereby, the results achieved.

The main challenges of Scheme 1 approach were the time required for completing the scheme and, for some biomarkers, the rather low number of valid results, which hampered the evaluation of the ICI/EQUAS. Scheme 2 approach permitted to reduce the time required for having a set of laboratories with comparable results and, since it is based on the participation of laboratories fulfilling specific technical criteria (e.g. having a minimum LOQ for all the biomarkers in a group), the

potential problems related to the low experience were avoided. However, there was no opportunity for capacity building and supporting the national hubs in HBM4EU. Therefore, the design of the QA/QC programme has to consider the specific requirements or objectives for each situation.

Although the development and implementation of Scheme 1 required more efforts and time, it is the preferred one in the HBM4EU context since it allows the participation of more laboratories, and provides an opportunity to improve their analytical skills. This approach therefore boosts capacity building in EU laboratories, which in the end contributes to the sustainability of human biomonitoring in Europe. Major milestones and challenges for the future in this field are the definition of standard analytical methods and the establishment of a sustainable and periodical HBM QA/QC programme in Europe to support research activities and analytical laboratories. In line with this, the creation of an institution/network to prepare and provide certified control material in different human matrices would be very helpful for analytical laboratories working in human biomonitoring.

The HBM4EU QA/QC programme has revealed the utility and need in establishing a European network of analytical laboratories for human biomonitoring. This network would support the increasing human biomonitoring and risk assessment studies providing expertise for new method development and high quality analytical results. The network of laboratories created in HBM4EU can be considered as the project's legacy for future human biomonitoring actions in Europe.

Declaration of competing interest

The authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ijheh.2021.113740>.

References

- Alder, L., Korth, W., Patey, A., et al., 2001. Estimation of measurement uncertainty in pesticide residue analysis. *J. AOAC Int.* 84, 1569–1578. <https://doi.org/10.1093/jaoac/84.5.1569>.
- Analytical Methods Committee, 1989. Robust statistics - how not to reject outliers Part 1. Basic concepts. *Analyst* 114, 1693–1697. <https://doi.org/10.1039/AN9891401693>.
- Analytical Methods Committee, 1989. Robust statistics - how not to reject outliers Part 2. Interlaboratory trials. *Analyst* 114, 1699–1702. <https://doi.org/10.1039/AN9891401699>.
- Becker, K., Seiwert, M., Casteleyn, L., et al., 2014. A systematic approach for designing a HBM Pilot Study for Europe. *Int J Hyg Environ Health* 214, 321–322. <https://doi.org/10.1016/j.ijheh.2013.07.004>.
- Broothaerts, W., Cordeiro, F., Robouch, P., Emons, H., 2020. Ten years of proficiency testing reveals an improvement in the analytical performance of EU National Reference Laboratories for genetically modified food and feed. *Food Contr.* 114 <https://doi.org/10.1016/j.foodcont.2020.107237>.
- COM, 2004. Communication from the Commission to the Council, the European Parliament, the European Economic and Social Committee - The European Environment & Health Action Plan 2004-2010" (SEC(2004) 729), p. 416.
- Den Hond, E., Govarts, E., Willems, H., et al., 2015. The First steps towards harmonised human biomonitoring in Europe. *Environ Health Perspect* 123, 255–263. <https://doi.org/10.1289/ehp>.
- Dereumeaux, C., Fillol, C., Charles, M.A., et al., 2017. The French human biomonitoring program: first lessons from the perinatal component and future needs. *Int J Hyg Environ Health* 220, 64–70. <https://doi.org/10.1016/j.ijheh.2016.11.005>.
- Esteban López, M., Schindler, B.K., Jimenez, J.A., et al., 2015. Mercury analysis in hair: Comparability and quality assessment within the transnational COPHES/DEMOCOPHES project. *Environ Res* 141, 24–30. <https://doi.org/10.1016/j.envres.2014.11.014>.
- Fearn, T., Thompson, M., 2001. A new test for 'sufficient homogeneity'. *Analyst* 126, 1414–1417. <https://doi.org/10.1039/b103812p>.
- Ganzleben, C., Antignac, J.P., Barouki, R., et al., 2017. Human biomonitoring as a tool to support chemicals regulation in the European Union. *Int J Hyg Environ Health* 220, 94–97. <https://doi.org/10.1016/j.ijheh.2017.01.007>.
- Ginsberg, G.L., Balk, S.J., 2016. Consumer products as sources of chemical exposure to children: case study to triclosan. *Curr Opin Pediatr* 28, 235–242. <https://doi.org/10.1097/MOP.0000000000000329>.
- Göen, T., Schaller, K.H., Drexler, H., 2012. External quality assessment of human biomonitoring in the range of environmental exposure levels. *Int J Hyg Environ Health* 215, 229–232. <https://doi.org/10.1016/j.ijheh.2011.08.012>.
- Horwitz, W., Kamps, L.R., Boyer, K.W., 1980. Quality assurance in the analysis of foods and trace constituents. *JAOAC Int.* 63, 1344. <https://doi.org/10.1093/jaoac/63.6.1344>.
- ISO 13528, 2015. Statistical Methods for Use in Proficiency Testing by Interlaboratory Comparison.
- Kolossa-Gehring, M., Becker, K., Conrad, A., et al., 2012. Environmental surveys, specimen bank and health related environmental monitoring in Germany. *Int J Hyg Environ Health* 215, 120–126. <https://doi.org/10.1016/j.ijheh.2011.10.013>.
- Linsinger, ThP.J., Josephs, R.D., 2006. Limitations of the application of the Horwitz equation. *Trends Anal. Chem.* 25, 1125–1130. <https://doi.org/10.1016/j.trac.2006.11.002>.
- Nübler, S., Esteban López, M., Mol, H., et al., 2021. 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. <https://doi.org/10.1016/j.ijheh.2021.113711>.
- Pacyga, D., Sathyanarayana, S., Strakovsky, R.S., 2019. Dietary predictors of phthalate and bisphenol exposures in pregnant women. *Adv Nutr* 10, 803–815. <https://doi.org/10.1093/advances/nmz029>.
- Parvaneh, H., Herrmann, S.S., Poulsen, M.E., 2017. Role of sample processing strategies at the European union national reference laboratories (NRLs) concerning the analysis of pesticide residues. *J Agric Food Chem* 65, 5759–5767. <https://doi.org/10.1021/acs.jafc.7b00728>.
- Scherer, G., 2005. Biomonitoring of inhaled complex mixtures—ambient air, diesel exhaust and cigarette smoke. *Exp Toxicol Pathol* 57 (Suppl. 1), 75–110. <https://doi.org/10.1016/j.etp.2005.05.007>.
- Schindler, B.K., Esteban, M., Koch, H.M., et al., 2014. The European COPHES/DEMOCOPHES project: towards transnational comparability and reliability of human biomonitoring results. *Int J Hyg Environ Health* 217, 653–661. <https://doi.org/10.1016/j.ijheh.2013.12.002>.
- Schoeter, G., Den Hond, E., Collesa, A., et al., 2012. Concept of the Flemish human biomonitoring programme. *Int J Hyg Environ Health* 215, 102–108. <https://doi.org/10.1016/j.ijheh.2011.11.006>.
- Thompson, M., 2000. Recent trends in inter-laboratory precision at ppb and sub-ppb concentrations in relation to fitness for purpose criteria in proficiency testing. *Analyst* 125, 385–386. <https://doi.org/10.1039/b000282h>.
- Thompson, M., Ellison, R., Wood, R., 2006. The international harmonized protocol for the proficiency testing of analytical chemistry laboratories. *Pure Appl Chem* 78, 145–196. <https://doi.org/10.1351/pac200678010145>.
- Von Holst, C., Robouch, P., Bellorini, S., González de la Huebra, M.J., Ezerkis, Z., 2016. The work of the European Union Reference Laboratory for Food Additives (EURL) and its support for the authorisation process of feed additives in the European Union: a review. *Food Addit Contam* 33, 66–77. <https://doi.org/10.1080/19440049.2015.1116127>.
- Vorkamp, K., Castaño, A., Antignac, J.-P., Boada, L.D., Cequier, E., Covaci, A., Esteban López, M., Haug, L.S., Kasper-Sonnenberg, M., Koch, H.M., Luzardo, O.P., Osite, A., Rambaud, L., Pinorini, M.-T., Sabbioni, G., Thomsen, C., 2021. Biomarkers, matrices and analytical methods targeting human exposure to chemicals selected for a European human biomonitoring initiative. *Environ Int* 146, 106082. <https://doi.org/10.1016/j.envint.2020.106082>.