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European interlaboratory comparison investigations (ICI) and external quality assurance schemes (EQUAS) for the analysis of bisphenol A, S and F in human urine: Results from the HBM4EU project

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ABSTRACT

The Human Biomonitoring for Europe initiative (HBM4EU) aims to study the exposure of citizens to chemicals and potentially associated health effects. One objective of this project has been to build a network of laboratories able to answer to the requirements of European human biomonitoring studies. Within the HBM4EU quality assurance and quality control scheme (QA/QC), a number of interlaboratory comparison investigations (ICIs) and external quality assurance schemes (EQUASs) were organized to ensure data consistency, comparability and reliability. Bisphenols are among the prioritized substance groups in HBM4EU, including bisphenol A (BPA), bisphenol S (BPS) and bisphenol F (BPF) in human urine. In four rounds of ICI/EQUAS, two target concentration levels were considered, related to around P25 and P95 of the typical exposure distribution observed in the European general population. Special attention was paid to the conjugated phase II metabolites known to be most dominant in samples of environmentally exposed individuals, through the analysis of both native samples and samples fortified with glucuronide forms. For the low level, the average percentage of satisfactory results across the four rounds was 83% for BPA, 71% for BPS and 62% for BPF. For the high level, the percentages of satisfactory results increased to 93% for BPA, 89% for BPS and 86% for BPF. 24 out of 32 participating laboratories (75%) were approved for the analyses of BPA in the HBM4EU project according to the defined criterion of Z-scores for both low and high concentration levels in at least two ICI/EQUAS rounds. For BPS and BPF, the number of qualified laboratories was 18 out of 27 (67%) and 13 out of 28 (46%), respectively. These results demonstrate a strong analytical capability for BPA and BPS in Europe, while improvements may be needed for BPF.

1. Introduction

Bisphenol A (BPA) is a high production volume chemical with a

global production volume of more than three million tons per year (Erlar and Novak, 2010). It has had multiple uses since 1940, predominantly as (1) a monomer in the manufacturing of polymers such as polycarbonate

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(PC) plastics, epoxy resins, polysulfone, or polyacrylate; (2) an antioxidant and inhibitor of end of polymerization in polyvinyl chloride plastics (PVC); and (3) a precursor for the synthesis of the flame retardant tetrabromobisphenol-A (Geens et al., 2011). BPA has also been widely used as a colour-developing agent in thermal paper, which includes a variety of uses such as cash register and credit card receipts, public transport and parking tickets, self-adhesive labels, etc., but use restrictions came into effect in the European Union in January 2020 (EC, 2016; ECHA, 2015; Geens et al., 2011). Due to concerns about the endocrine disrupting potential of BPA (Rubin, 2011), Bisphenol F (BPF) and bisphenol S (BPS) have been used as presumably less problematic BPA substitutes, including epoxy resins used as food contact material. However adverse effects of both BPS and BPF, similar to BPA, have been reported (Ivry Del Moral et al., 2016; Kolla et al., 2018; Park et al., 2018; Skledar et al., 2016).

Human exposure to BPA is widespread. Human biomonitoring of tissue and fluid samples revealed the presence of BPA in over 90% of the human population in Europe (Covaci et al., 2015), the USA (Calafat et al., 2007) and Canada (Haines et al., 2012). Food is expected to contribute by more than 90% to the general BPA exposure for all age groups (Rudel et al., 2011; Christensen et al., 2012), including both canned and non-canned food categories (Kommission Human-Biomonitoring, 2012; EFSA CEF Panel, 2015). Additional, non-dietary sources include medical devices, dental sealants, thermal paper, toys and cosmetics (EFSA CEF Panel, 2015; Bernier and Vandenberg, 2017). The increased use of BPF and BPS in consumer products has been followed by the detection of their residues and metabolites in human biological matrices (Lehmler et al., 2018; Liu et al., 2018; Deceuninck et al., 2019; Filloz et al., 2021).

After oral intake, BPA is rapidly absorbed by the gastrointestinal (GI) tract. Human studies have suggested a complete absorption of a relatively low oral BPA dose, based on the urinary recovery of labelled BPA-glucuronide (BPA-G) (EU-RAR, 2003; EFSA, 2008; CEF EFSA CEF Panel, 2015). BPA is primarily metabolized to BPA-G (as monoglucuronide (BPA-MG) and diglucuronide (BPA-DG)), through uridine diphosphate-glucuronosyltransferase (UGT) isoforms. BPA may also be converted by sulfotransferases (SULTs) to BPA-sulfate (BPA-S) forms (as monosulfate (BPA-MS) and disulfate (BPA-DS)), especially during the neonatal period (EFSA, 2008; Ginsberg and Rice, 2009). The BPA-conjugates (BPA-G and BPA-S) formed in the GI tract and in the liver are delivered to the human blood to reach the kidney, being finally excreted in the urine. Within 24 h after oral administration, BPA is almost completely eliminated as glucuronide or sulfate conjugates via urine, with 84–97% of the absorbed BPA being excreted within the first 5–7 h (Völkel et al., 2002; Thayer et al., 2015), and delayed and slower excretion after dermal exposure (Sasso et al., 2020).

Free, unconjugated BPA is regarded the toxicologically most relevant chemical form, as it is available for interaction with the estrogen receptor (ER). However, blood and urine concentrations of free BPA are generally much lower than of conjugated BPA. The trace level determination of free BPA remains challenging not only due to analytical sensitivity issues (Dekant and Völkel, 2008) but also due to interferences by contamination through omnipresent BPA and/or degradation of conjugated BPA. These issues have been recognized during handling in the field (Ye et al., 2013), sample storage and processing (Dekant et al., 2008; Longnecker et al., 2013), and/or short periods at ambient temperatures (Schöringhumer and Cichna-Markl, 2007; Ye et al., 2007). Due to the ubiquitous nature of free BPA, it is difficult to completely avoid external contamination from sample collection (e.g. environment, sample containers) to laboratory analysis (e.g. laboratory environment, consumables and instrumentation) (Buscher et al., 2015), and would require appropriate quality assurance provisions to starting in the pre-analytical phase to evaluate and manage this background.

Thus, the analyses of total bisphenol in urine has been shown to be a sensitive and robust approach for a quantitative assessment of exposure to BPA and related compounds (Dekant and Völkel, 2008; Kommission

Human-Biomonitoring, 2012; Vorkamp et al., 2021).

Bisphenols have been selected as priority substances in the Human Biomonitoring for Europe (HBM4EU) project, a joint effort of 30 countries, the European Environment Agency and the European Commission. This initiative aims to coordinate and advance human biomonitoring in Europe, including evidence of the actual exposure of European citizens to chemicals and the associated impacts in order to support the development of policies to protect human health and design measures to reduce the exposure (Ganzleben et al., 2017; Gilles et al., 2021).

In 2016, the first list of HBM4EU priority substances identified three bisphenols: BPA, BPF and BPS (Vorkamp et al., 2021). From the end of 2018 until the beginning of 2020, four rounds of Interlaboratory Comparison Investigations (ICI) and External Quality Assurance Schemes (EQUAS) were organized as part of a comprehensive QA/QC programme in HBM4EU, with the purpose of assessing the analytical performance of different laboratories and to ensure the comparability of data generated by different laboratories in HBM4EU (Esteban Lopez et al., 2021). The QA/QC programme included the analysis of these three exposure markers (as total BPA, BPS, and BPF) in human urine.

This article presents the results, performance assessment, main difficulties and conclusions from these four European ICI/EQUAS rounds organized within the framework of the HBM4EU project for BPA, BPF and BPS.

2. Experimental design and procedures

ICI/EQUAS are tools to assess the proficiency of laboratories, and the comparability and reliability of analytical methods. The conceptual and organizational design of the HBM4EU ICI/EQUAS was described in detail in Esteban López et al. (2021).

In 2018–2020, one ICI and three EQUASs were organized for BPA, BPS, and BPF in human urine. For each round, participants were given an average period of four weeks to analyze control materials (CMs) and report the results. The rounds were scheduled in such a way that laboratories received their results well in advance of the next round. This gave laboratories the opportunity to evaluate their results and, if required, take corrective action if needed before the upcoming round. Requirements as outlined in ISO/IEC 17043:2010, “Conformity assessment – General requirements for proficiency testing” and in ISO/IEC 13528:2015, “Statistical methods for use in proficiency testing by interlaboratory comparison” were taken into account.

2.1. Participants and expert laboratories

A total of 33 laboratories, having expressed interest in bisphenol analyses in HBM4EU, were contacted for the first ICI round. This number increased to 46 laboratories for the three following EQUAS rounds. Within HBM4EU, a successful participation in ICI/EQUAS exercises was mandatory for laboratories that would be further involved in the chemical analysis of the HBM4EU samples.

For EQUAS, international expert laboratories were identified to act as reference laboratories, based on their experience in bisphenols analysis as documented in peer-reviewed publications (Table A1, SM). Other selection criteria, such as years of experience, appropriate limit of detection (LOD) and limit of quantification (LOQ), application of isotopically labelled standards for quantification, ISO17025 accreditation, success in previous ICI/EQUAS, were taken into account. Based on their results, the expert assigned values were then established by the organizer. Participants' identities were only known to the organizer and all data handling was strictly anonymous.

2.2. Test materials

For each ICI/EQUAS round, two CMs were systematically prepared from new human urine pools. These CMs were different for each ICI/EQUAS round. The two CMs per round consisted of one low level sample

(L) and one high level sample (H), corresponding to the P25 and the P95 value, respectively, of a typical exposure distribution in the general population of Europe. Results obtained within the French national biomonitoring program (ESTEBAN study) were considered for that purpose (Fillol et al., 2021; Santé Publique France, 2019), which included the analysis of BPA, BPS and BPF in 900 adult urine samples collected between April 2014 and March 2016. Based on these results, P25 values of 1.12 µg/L, 0.14 µg/L and 0.13 µg/L and P95 values of 8.10 µg/L, 6.33 µg/L and 1.01 µg/L were determined for BPA, BPS and BPF respectively. The L-CMs were then pools of background exposed urine samples selected to approximate these targeted P25 values but with a certain variability across the four successive ICI/EQUAS rounds. The L-CM levels, as well as the spiking concentrations used to prepare H-CMs are shown in Table A2 (SM). The L-sample was prepared from a pool of native individual human urine samples and the H-sample pool was fortified with individual solutions of glucuronide-BPA, glucuronide-BPS and glucuronide-BPF (Toronto Research Chemicals, Toronto, Canada). The sample preparation and supplementation protocol is presented in the Supplementary Materials. Aliquots of 10 mL were transferred to propylene tubes (15 mL, Falcon) and were stored in the freezer (≤ -18 °C) until shipment.

Packages, containing one L-sample and one H-sample, were shipped under frozen conditions (polystyrene containers with dry ice) to the participants' laboratories whereas the selected expert laboratories received six samples of each CM. A letter with instructions related to sample handling, a form for acknowledgment of receipt, as well as a result submission/method information form were sent to all participants by e-mail at the same time.

Homogeneity and stability of the CMs (L and H) were systematically tested for each ICI/EQUAS based on ISO 13528:2015, Fearn and Thompson (2001) and Thompson et al. (2006), and according to the Standard Operating Procedures elaborated for the HBM4EU QA/QC programme (Esteban López et al., 2021). Prior to shipment, homogeneity was tested by analysing ten aliquots in duplicate for the three bisphenol biomarkers (Table A3, SM). For evaluation of the stability, the samples were stored at conditions representative for transport to and storage at the participant's laboratory until analysis (frozen). Stability was tested by analysing six aliquots after the ICI/EQUAS deadline and comparing the mean with the mean obtained during the homogeneity assessment before sample shipment (Table A4, SM). All measurements were carried out using a validated method based on gas chromatography coupled to tandem mass spectrometry (GC-MS/MS) in the laboratory organizing the bisphenols ICI/EQUAS (Deceuninck et al., 2015). A brief description of the analytical method is available in the Supplementary Material (Table A5, SM).

Participants were asked to determine BPA, BPF and BPS concentrations (µg/L) by single analysis in each CM using the same procedure they would routinely use in the forthcoming HBM4EU analyses. Expert laboratories were requested to provide individual results for each of the six aliquots per control material. Participants had approximately four weeks to analyze the samples analysis and to report the results in the adapted spreadsheet.

2.3. Assessment of performance

The procedures for evaluation of the results submitted by laboratories participating in the HBM4EU QA/QC programme were described in detail by Esteban López et al. (2021).

In brief, for ICI, the consensus value (C) was calculated from the results submitted by the participants and corresponded to the robust mean (ISO 13528:2015). The uncertainty (u) of the consensus value was calculated as follows:

$$u = 1.25 * \alpha / \sqrt{n} \quad (1)$$

With: u = uncertainty of the consensus value;

σ = standard deviation of the participants' results (ICI standard deviation);

n = number of results used for calculation of the consensus value.

(U) was considered as negligible when $u \leq 0.3 * \sigma_T$ (with σ_T = predicted by Horwitz/Thompson i.e. 22%)

For EQUAS, each expert laboratory analysed and reported six replicate results per CM. Using the individual means of each expert laboratory's results, the mean of the means was calculated, its relative standard deviation (RSD), and the relative uncertainty (u) of the mean of the means which is given by:

$$u = \text{RSD}_{\text{mean-of-mean}} / \sqrt{n} \quad (2)$$

With u = relative uncertainty of the mean of the mean concentrations from the expert laboratories;

RSD = relative standard deviation of the mean of the mean concentrations;

n = the number of expert laboratories (after exclusion of outliers if applicable).

The mean of the means was considered suitable for use as expert-assigned value (A) in EQUAS studies when u did not exceed a value of 17.5% derived from the following equation:

$$u \leq 0.7 * \sigma_T \quad (3)$$

With σ_T = a pre-set relative target standard deviation for proficiency of 25%.

The target relative standard deviation (σ_T) reflected the maximum variability that was considered acceptable for a certain biomarker concentration in a given matrix. The value of σ_T (25%) was set based on expert opinion, taking into account what is technically feasible and realistic in current routine practices. When $u > 0.7 * \sigma_T$, the individual means were checked for outliers by using the Grubbs' outlier test and discarded if identified as such. If the condition $u \leq 0.7 * \sigma_T$ was still not met, then the expert-derived mean could not be used as assigned value. If the expert-derived mean could not be established as A, C was used as an alternative to assess the laboratories performances. That was applied in the case of BPF in round 2 for the low control material (L-CM). The switch from ICI to EQUAS from the 2nd round aimed to harmonise the exercises for all substances groups in the HBM4EU QA/QC programme (Nübler et al., 2021; Esteban López et al., 2021; Dvorakova et al., 2021).

The laboratory performance was assessed by calculation of the Z-scores for each bisphenol biomarker in each CM according to the following equation:

$$Z = (x - X) / (\sigma_T * X) \quad (4)$$

With x = participant's result;

X = C for ICI or A for EQUAS;

σ_T = a pre-set relative target standard deviation for proficiency of 25%.

A resulting z-score of $|Z| \leq 2$ was interpreted as satisfactory, $2 < |Z| < 3$ as questionable, and $|Z| \geq 3$ as unsatisfactory performances. If the uncertainty of C or A could not be considered negligible, the uncertainty was taken into account in the Z'-score calculation (**calculation procedure available in SM**). This applied to round 1 for BPA and BPS in the L-CM and to round 2 for BPA in the L-CM.

If no numerical value for a CM was reported by a participant, the indicated LOQ was used for the Z-score calculation according to equation (4) where x was replaced by the LOQ. These LOQ-Z-scores (LOQ-Z) were not included in the final evaluation, but were only used to assess the performance of the laboratory with respect to its reported LOQ. LOQ-Z below -2 were then considered as false negative results.

3. Results and discussion

3.1. Target concentrations, homogeneity and stability assessment of the CMs

The average concentrations obtained during the homogeneity analyses for each ICI/EQUAS round in the two CMs (L and H) and their associated RSD are presented in Table 1, and the associated conclusions are available in Table A3 (SM).

The variability observed during homogeneity testing appeared satisfyingly low overall, ranging from 2.8% to 12% for the four rounds at low and high levels for all biomarkers except for BPS in L-CM of round 1. These RSDs are in the same range as the repeatability values of the method as determined during its within-laboratory-validation, ranging from 8.1% to 28.2% for concentrations close to low level and from 7.8% to 15.8% for concentrations close to high level. For BPS in L-CM of round 1, a higher variability was observed (61%). Nevertheless, based on the satisfactory results obtained for BPS in H CM and for BPA, BPF in L-CM by extrapolation the L-CM was considered homogeneous for BPS.

The results of the stability testing for L and H CMs within four rounds of ICI/EQUAS are presented in Table 2 and the associated conclusions are available in Table A4 (SM).

In most cases, for the four rounds, no statistically significant instability was revealed for any of the three considered bisphenol biomarkers. Nevertheless, the statistical stability criteria (Table A4, SM) were not met in few cases including BPA, BPS, BPF in the L control sample during the 1st round, and BPS in the L control sample during the 4th round. This potential non-stability factor was included in the score calculation (Z_i -score). The procedure to calculate Z_i -score is available in SM.

3.2. Participation, LOQ range and method characteristics

Table 3 presents the number of registered laboratories and the number of laboratories submitting results for the target biomarkers. For the 1st ICI round, 33 laboratories were contacted of which 24 sent results for at least one of the three biomarkers. A total of 46 laboratories were invited to the three following EQUAS rounds and the number of participants increased to 34. For these three last ICI/EQUAS rounds, this number of participating laboratories included four expert laboratories (two European and two non-European laboratories) (see Table A1, SM). Across all rounds, the highest participation rate was observed for BPA (57–73%), followed by BPS (46–64%) and BPF (48–61%). Similar international proficiency tests have also been proposed for food, spirit drinks or waste water (organized by FAPAS and BIPEA for example). To our knowledge, only one program, called OSEQAS (Organic Substances in urine Quality Assessment Scheme) initiated by the Centre de Toxicologie du Quebec, offers the possibility to participate in a proficiency test dedicated to BPA, BPS, BPF and bisphenol Z (BPZ) in human urine. However, the number of participants in this program is typically low (below eight laboratories).

The range of LOQs reported by the participants and by the expert laboratories in the four ICI/EQUAS rounds is presented in Table 4. The

observed interlaboratory variability in terms of LOQ of participating laboratories appears elevated, ranging from 62% to 311% depending on the considered biomarker and ICI/EQUAS round. This high variability can be explained by differences in analytical sensitivity between the methods and instrumentation used by the participants, and also by different calculation methods for the LOQs. However, information of LOQ calculation methods had not been requested from the participants. It is noteworthy that LOQs reported by some participating laboratories in the 1st round were nearly 2–3 times higher than LOQs in the following ICI/EQUAS rounds, which could be the result of an improvement over the course of the QA/QC programme. In general, it should be noted that the LOQ values from all participants tightened over the rounds. Mean LOQ values for external expert laboratories were systematically lower than those reported by participants. Table A6 (SM) presents the LOQ values obtained by all participants according to the instrumental method (LC versus GC) and compares these values with previous biomonitoring studies on bisphenols (Gys et al., 2020; Karrer et al., 2020; Sanchis et al., 2020). For BPA, and more significantly for BPS, LOQ values associated with the LC methods were systematically lower than those obtained by the GC method. For BPF, LOQ values were in the same range for both techniques in rounds 2 and 3, and lower for GC-based methods in round 4.

A summary of the analytical methods used by candidates and expert laboratories for the analysis of BPA, BPS and BPF is presented in Table 5. Half of the participants used less than 1 mL of urine to perform the analysis whereas the maximum sample amount used was 5 mL. A deconjugation step was applied by 97% of the laboratories and a glucuronidase/arylsulfatase enzyme was used by most participants. One laboratory did not apply a deconjugation step and measured the three bisphenols through their conjugated form using ^{13}C -labelled glucuronide reference standards. It should be noted that this participant obtained satisfactory results, indicating the consistency of both measurement approaches (i.e. detection of deconjugated or conjugated forms) as well as the efficiency of the deconjugation process (enzymatic hydrolysis) performed by laboratories employing this approach. Offline solid phase extraction (SPE), liquid/liquid extraction and online SPE purification steps were carried out by 38%, 38% and 28% of the participants, respectively. A derivatisation step was performed by 44% of the participants mainly using N-methyl-N-(trimethylsilyl)-trifluoroacetamide (MSTFA), N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) or trifluoroacetic anhydride (TFAA) as derivatisation agents for the GC methods and dansyl-chloride for liquid chromatography (LC) methods. BPA, BPS and BPF were quantified by experts and participants using three different analytical techniques. LC coupled to tandem mass spectrometry (MS/MS) was applied by 75% of the participants whereas GC-MS/MS and GC-MS were used by 22% and 6% of the laboratories, respectively. An isotope dilution approach was applied by most participants (91%) using mainly ^{13}C -labelled or deuterated internal standards, added prior to extraction.

3.3. Assessment of laboratory performance

Table 6 presents the comparison between (A) and (C) calculated over

Table 1
Results of the homogeneity testing for the three bisphenol biomarkers based on duplicate analysis of 10 samples of each CM (L and H).

CM	Biomarker	Round 1		Round 2		Round 3		Round 4	
		Mean 1 (µg/L)	RSD (%)	Mean 1 (µg/L)	RSD (%)	Mean 1 (µg/L)	RSD (%)	Mean 1 (µg/L)	RSD (%)
L	BPA	0.655	5.3	0.608	4.0	0.945	5.0	0.397	9.9
	BPS	0.192	61	0.145	5.1	2.44	9.8	0.105	11
	BPF	0.058	5.8	0.056	4.1	0.159	6.5	0.077	11
H	BPA	6.01	4.8	6.70	3.6	7.06	4.1	5.80	12
	BPS	7.08	10	5.81	5.5	7.49	2.8	6.46	8.2
	BPF	1.02	5.5	2.64	2.9	3.15	10	2.42	11

Mean 1: average concentration values obtained during homogeneity analyses ($t = 0$).

Table 2

Results of the stability testing for the three bisphenol biomarkers calculated from duplicate analysis of six samples of CMs (L and H) for the four ICI/EQUAS rounds.

CM	Biomarker	Round 1		Round 2		Round 3		Round 4	
		Mean 2 (µg/L)	Diff to homogeneity (%)	Mean 2 (µg/L)	Diff to homogeneity (%)	Mean 2 (µg/L)	Diff to homogeneity (%)	Mean 2 (µg/L)	Diff to homogeneity (%)
L	BPA	0.548	16	0.641	-5	0.977	-2	0.409	-2
	BPS	0.132	31	0.140	4	2.316	2	0.076	29
	BPF	0.064	-10	0.057	-2	0.159	0	0.081	-6
H	BPA	5.92	2	6.82	-2	7.12	0	5.66	1
	BPS	7.23	-2	5.45	4	7.19	5	5.13	21
	BPF	1.02	1.02	2.64	-4	3.06	6	2.42	5

Mean 1: concentration average values obtained during homogeneity analysis (t = 0).

Mean 2: concentration average values obtained during stability analysis (six samples withdrawn from the freezer and analysed after the ICI/EQUAS deadline of the respective rounds (t = 65 days, 68 days, 59 days, 77 days for rounds 1 to 4, respectively).

Diff to homogeneity: difference of concentration between mean 1 and mean 2.

Table 3

Number of registered laboratories and of laboratories submitting results for BPA, BPS and BPF (including expert laboratories in rounds 2–4).

		Round 1	Round 2	Round 3	Round 4
Invited laboratories		33	46	46	46
BPA	Registration	24	34	30	26
	Participation	24	32	30	26
BPS	Registration	23	32	28	23
	Participation	21	27	26	21
BPF	Registration	22	31	27	23
	Participation	19	28	26	22

Table 4

Range of the limits of quantifications (LOQs) reported by the participants and expert laboratories for BPA, BPS and BPF.

		Participating laboratories				Expert laboratories
		Round 1	Round 2	Round 3	Round 4	Round 2 to round 4
BPA	Number of participants	24	28	26	22	4
	Lowest LOQ (µg/L)	0.020	0.006	0.006	0.027	0.031
	Highest LOQ (µg/L)	4.00	0.560	0.560	0.500	0.200
	Median LOQ (µg/L)	0.200	0.200	0.200	0.200	0.080
	Mean LOQ (µg/L)	0.593	0.214	0.214	0.242	0.098
	RSD (%)	185	77	74	62	74
	CV (%)	311	108	122	143	105
BPS	Number of participants	24	24	23	18	4
	Lowest LOQ (µg/L)	0.010	0.030	0.005	0.030	0.010
	Highest LOQ (µg/L)	10	1.15	1.15	1.15	0.190
	Median LOQ (µg/L)	0.060	0.125	0.100	0.095	0.060
	Mean LOQ (µg/L)	0.734	0.229	0.205	0.191	0.080
	RSD (%)	311	108	122	143	105
	CV (%)	311	108	122	143	105
BPF	Number of participants	24	25	22	18	4
	Lowest LOQ (µg/L)	0.020	0.020	0.020	0.020	0.030
	Highest LOQ (µg/L)	5.63	1.00	0.810	0.600	0.200
	Median LOQ (µg/L)	0.150	0.106	0.125	0.125	0.068
	Mean LOQ (µg/L)	0.583	0.275	0.202	0.174	0.091
	RSD (%)	210	106	100	91	83
	CV (%)	210	106	100	91	83

RSD: relative standard deviation.

Table 5

Summary of the analytical methods used by participants in the ICI/EQUAS scheme for bisphenols.

Volume of urine used (mL)	Max: 5; Min: 0.1; Median: 1
Type of deconjugation Enzyme used	Enzymatic deconjugation for 97% of participants
SPE offline	Majority of glucuronidase/arylsulfatase
Type of column used for SPE offline	38% of participants
Liquid/liquid extraction SPE online	HLB, C18
Type of column used for SPE online	38% of participants
Derivatisation	28% of participants
Derivatisation agent	Majority of C8, C18
GC-MS	44% of participants
GC-MS/MS	MSTFA, BSTFA, TFAA (67%, 22% and 22% of GC method respectively)
LC-MS/MS	dansyl-chloride, pyridine-3 sulfonyl chloride (8% and 8% of LC method)
Type of GC column	6% of participants
Type of LC column	22% of participants
Number of ions/transitions	75% of participants
Use of ion ratio	Capillary column - DB5MS and 30 m for almost all laboratories
Response normalised to IS	Reversed phase (majority of C18 column)
Use of internal standards	Max: 9; Min: 1; 59% of participants used 2 transitions
Internal standard for BPA	63% of participants
Internal standard for BPS	91% of participants
Internal standard for BPF	97% for BPA; 77% for BPS; 84% for BPF
Calibration type	BPA ¹³ C for 35%; deuterated BPA for 52%; hydrogenated BPA for 13%
	BPS ¹³ C for 71%; deuterated BPS for 17%; deuterated BPA for 12%
	BPF ¹³ C for 54%; deuterated BPA for 23%; deuterated BPA for 19%; Hydrogenated BPA for 4%
	Isotopic dilution before extraction for 78% of participants

Millilitres (mL), solid phase extraction (SPE), N-methyl-N-(trimethylsilyl)-tri-fluoroacetamide (MSTFA), N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA), gas chromatography (GC); liquid chromatography (LC), mass spectrometry (MS), maximum (Max), minimum (Min), bisphenol A (BPA), bisphenol S (BPS), bisphenol F (BPF).

all rounds in the two CMs for each of BPA, BPS and BPF. The (A) and (C) values were comparable and their differences were within the range of the standard deviation calculated from all participants for each value. This highlights the fact that the assessment of participants' performance generates comparable scores independent of the ICI or EQUAS approach. Moreover, it should be noted that for the 1st ICI, no Z-scores could be calculated for BPF in L- and H-CM as the condition $u \leq 0.7 \cdot \sigma_T$ was still not met. Similarly, due to non-quantified samples for some expert labs (" $< LOQ$ "), no expert assigned value was associated with BPF in the L-CM for the 2nd round. In this last case, a (C) was calculated from the

Table 6
Comparison of (A) and (C) in the bisphenols ICI/EQUAS scheme.

Biomarker	Round	CM	Results from four expert laboratories		Results from all participants		Difference of C from A (% relative to A)
			Expert assigned value (A) (µg/L)	SD (µg/L)	Consensus value (C) (µg/L)	SD (µg/L)	
BPA	1	L	nc	nc	0.922	0.378	nc
		H	nc	nc	7.09	0.355	nc
	2	L	0.763	0.153	0.778	0.244	2
		H	7.21	0.361	7.02	1.31	-3
	3	L	1.10	0.187	1.07	0.311	-3
		H	8.40	1.18	7.17	1.55	-15
	4	L	0.578	0.121	0.592	0.186	2
		H	7.54	0.980	7.41	1.14	-2
BPS	1	L	nc	nc	0.187	0.073	nc
		H	nc	nc	5.38	0.646	nc
	2	L	0.143	0.023	0.174	0.075	22
		H	5.47	0.821	5.54	1.06	1
	3	L	3.12	0.780	2.99	0.723	-4
		H	8.51	1.53	7.72	1.38	-9
	4	L	0.101	0.015	0.145	0.087	44
		H	6.02	0.903	5.76	1.13	-4
BPF	1	L	nc	nc	nc	nc	Nc
		H	nc	nc	nc	nc	Nc
	2	L	nc	nc	0.134	0.071	Nc
		H	3.40	0.272	3.25	1.11	-4
	3	L	0.182	0.027	0.224	0.097	23
		H	3.35	0.402	3.21	0.755	-4
	4	L	0.100	0.008	0.104	0.021	4
		H	3.42	0.513	3.13	0.72	-8

nc: not calculated; L: Low; H: High; CM: Control Material; SD: Standard Deviation.

participants' results.

BPA and BPS levels in the L-CMs were consistently above the participants' LOQ median (for BPA, (C): 0.922/0.778/1.07/0.592 µg/L; participant's LOQ median: 0.200 µg/L) (for BPS, (C): 0.187/0.174/2.99/0.145 µg/L; participant's LOQ median: 0.060/0.125/0.100/0.095 µg/L) contrary to BPF ((C): not calculated/0.071/0.097/0.021 µg/L; participant's LOQ median: 0.150/0.106/0.125/0.125 µg/L). This means that for BPF, half of the participating laboratories were unable to quantify BPF at the low level. As a pool of native urine samples was used,

this indicates sensitivity issues in some laboratories for quantitative determinations at commonly occurring levels in the European population.

A summary of the participants' results and their assessment in each of the four rounds of the QA/QC programme is available in [Table 7](#).

Graphical representations of the participant's Z-scores for the respective CMs in the respective rounds are provided in [Fig A1 \(SM\)](#) and the LOQ-Z-scores achieved by the candidates are presented in [Table A7 \(SM\)](#).

Table 7
Summary of participants' results and their assessment for BPA, BPS and BPF in each round of the HBM4EU QA/QC programme.

	Round	CM	(C) value or (A) value (µg/L)	Uncertainty of ICI/EQUAS value (%)	Study RSD _R (%)	Expert RSD (%)	No of labs reporting quantitative results (<LOQ)	Satisfactory (%)	Questionable (%)	Unsatisfactory (%)	
BPA	1	L	0.922	11	41	nc	21 (3)	76	0	24	
		H	7.09	7	5	nc	24	88	5	7	
	2	L	0.763	10	31	20	31 (1)	88	3	9	
		H	7.21	2	19	5	32	94	4	3	
	3	L	1.10	9	29	17	29 (1)	83	10	7	
		H	8.40	7	22	14	30	97	0	3	
	4	L	0.578	11	31	21	23 (3)	85	11	4	
		H	7.54	7	15	13	26	92	8	0	
	BPS	1	L	0.187	12	39	nc	16 (3)	69	6	25
			H	5.38	4	12	nc	19	68	6	26
		2	L	0.143	8	43	16	20 (8)	61	3	36
			H	5.47	8	19	15	27 (1)	89	0	11
3		L	3.12	13	24	25	26	96	0	4	
		H	8.51	9	18	18	26	100	0	0	
4		L	0.101	9	60	15	16 (5)	57	14	29	
		H	6.02	8	20	15	21	100	0	0	
BPF	1	L	Nc	nc	81	nc	12 (9)	nc	nc	nc	
		H	Nc	nc	62	nc	17 (4)	nc	nc	nc	
	2	L	0.134	16	53	nc	17 (10)	63	7	30	
		H	3.40	4	34	8	28	79	11	10	
	3	L	0.182	8	43	15	21 (5)	73	8	19	
		H	3.35	6	24	12	25 (1)	88	4	8	
	4	L	0.100	4	20	8	14 (8)	50	9	41	
		H	3.42	8	23	15	22	91	9	0	

(C) value: consensus value; (A) value; expert assigned value, nc: not calculated; L: Low; H: High; CM: Control Material, RSD: Relative Standard Deviation; LOQ: Limit of Quantification.

Except for BPF in the 1st round, Z-scores could be calculated for all biomarkers and CMs. In some particular cases, due to non-satisfying stability results (L-CMs for BPA, BPS, BPF in the 1st round and for BPS in the 4th round), Z_i -scores were calculated. In the same way, due to significant uncertainty of the (A), Z' -scores were determined for BPF in L-CM for the 2nd round.

The percentages of satisfactory results was 50% or above for BPA, BPF, and BPS in each of the four rounds. The proportion of satisfactory results ranged from 76% to 97% for BPA, from 61% to 100% for BPS and from 50% to 91% for BPF. As expected, the percentage of satisfactory results was always higher for the H-CM than for L-CM. The best performance of all rounds was achieved for BPS in the H-CM for the 3rd and 4th round where all participants obtained a satisfactory assessment. In contrast, the least successful one was achieved for BPF in the L-CM of the 4th round where only half of the participants obtained a satisfactory evaluation.

As expected, the percentage of satisfactory results was always higher for the high concentration CM than for the corresponding low concentration CM. This difference was however smaller for BPA (83% and 93%) than for BPS (71% and 89%) and BPF (62% and 86%). Moreover, for BPA, the percentage of laboratories with satisfactory results increased between the 1st ICI and the 2nd round (Table 7). The same development could be observed for BPS and for BPF between the 2nd round and the 3rd round. This increase could be explained by the increasing number of participants, but also by the improvement of the analysis of these biomarkers during the ICI/EQUAS scheme.

As shown in Fig A1 B), C), and D) (SM), several labs presented a positive bias (Z -score > 3) compared to the expert assigned values, especially with regard to the analysis of the L-CMs. As this observation is less pronounced for the H-CMs, it might be related to a background contamination or selectivity issue that is still insufficiently addressed in the participating laboratories, which may impair the reliability of results at lowest concentration levels near the LOQ.

Quantification of the three bisphenols at the level of the general population requires a high analytical sensitivity. Several values $< LOQ$ were reported for BPA, BPS and BPF. As shown in Table A7 (SM), laboratories reported more values $< LOQ$ for BPF than for BPS and for BPA (36, 17 and 7 values, respectively) and as expected, more values $< LOQ$ s for low concentrations of the CM than for high concentrations (54 and 6 values, respectively). This observation should be put into perspective considering that the BPF and BPS concentration levels in L-CMs were lower than for BPA. Considering that concentrations of the CMs were based on natural contamination and environmental exposure data, this highlighted the fact that the participants' analytical methods were sensitive enough to determine the levels of exposure to BPA of the general population but still needed higher sensitivity for BPS and especially for BPF.

The uncertainties of consensus values and expert-assigned values were in the same order of magnitude for all bisphenols and all rounds, i. e. between 4% and 13%. As expected, the determined study relative standards deviations (study RSD_R) were higher for L-CMs than for H-CMs (Fig. 1). However, this difference was less pronounced for BPF than

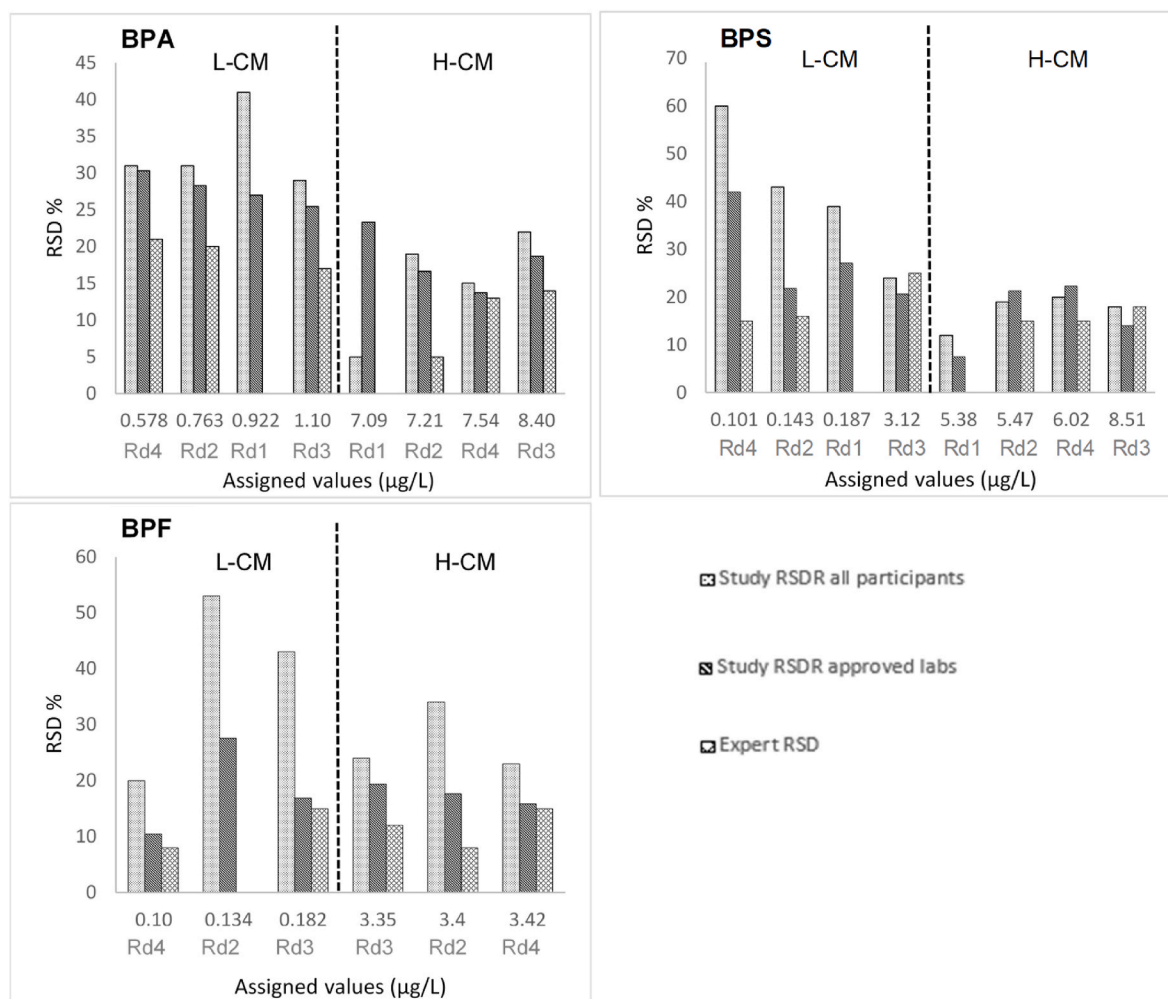


Fig. 1. Graphical representation of the study RSD_R from all participants/the study RSD_R from HBM4EU approved laboratories (i.e. satisfactory rounds in both CMs in at least two rounds)/the RSD from expert laboratories vs assigned values.

for BPA and BPS. Average study RSD_R for BPF were 49% for L-CM and 36% for H-CM, whereas they were 33%/15% and 42%/17% for BPA and BPS, respectively. Overall, the interlaboratory variability of the participants' results increased in the order BPA < BPS < BPF.

Table A8 in SM presents the study RSD_R calculated from all HBM4EU approved laboratories (with satisfactory results in both CMs from at least two rounds) for BPA (between 14% and 30%), BPS (between 8% and 42%), and BPF (between 10% and 28%). The highest RSD_R (42%) was obtained for the analysis of BPS in L-CM (round 4) and the lowest value (8%) for the analysis of BPS in H-CM (round 1). Overall, the differences in variability were higher between concentration levels than between the three bisphenols.

All Z-scores were compared with regard to the different analytical methods used by the participants. As shown in Table 5, LC-MS/MS, GC-MS/MS and GC-MS were applied by 75%, 22% and 6% of the participants, respectively. Fig. 2 presents the distribution of Z-scores associated with the three instrumental techniques. No statistically significant differences were observed in terms of results generated with these different analytical techniques.

The distribution of Z-scores for each bisphenol biomarker by all participants in round 1 to round 4 regarding the use of derivatisation is presented in Fig A2 (SM). No statistically significant difference was observed for any of the analysed biomarkers in terms of results generated with or without derivatisation (relevant for both GC and LC methodology, see Table 5).

ns: non-significant.

Finally, laboratories from 17 European countries were approved for the analysis of bisphenols in the HBM4EU project according to the criterion of satisfactory Z-scores in both concentration levels in at least two rounds. In total, 24 of the 32 participants (75%) for BPA, 18 of the 27 participants (67%) for BPS and 13 of the 28 participants (46%) met this criterion in the ICI/EQUAS programme of the HBM4EU project. Globally, these results indicate that a significant core network of competent laboratories could be established through HBM4EU for the human biomonitoring of BPA and BPS in Europe, while the number of competent laboratories for human biomonitoring of BPF is smaller. This also demonstrated a high analytical comparability and accuracy of the data generated under HBM4EU by the successful participants of the ICI/EQUAS programme.

4. Conclusions

One of the objectives of the HBM4EU project was to build a network of laboratories able to answer to the requirements of European human biomonitoring studies. From this perspective, a QA/QC programme was

designed in HBM4EU for a range of exposure markers, including bisphenols. International proficiency tests have been proposed for bisphenols in food, spirit drinks or waste water (organized by FAPAS and BIPEA for example). To our knowledge, only one program, called OSE-QAS (Organic Substances in urine Quality Assessment Scheme) initiated by the Centre de Toxicologie du Quebec, offers the possibility to participate in a proficiency test dedicated to BPA, BPS, BPF and bisphenol Z (BPZ) in human urine. However, the number of participants in this program is typically low (below eight laboratories). G-EQUAS (<https://app.g-equas.de/web/>) is also offering inter-laboratory comparison but only for BPA. In the present work, the targeted compounds included BPA, BPF, and BPS, which were monitored in human urine at high and low concentration levels reflecting P95 and P25 values of the real exposure in the EU general population.

The results of the ICI/EQUAS programme for bisphenols led to a total of 24, 18 and 13 European laboratories being approved for the determination of BPA, BPS and BPF, respectively, in HBM4EU, confirming a significant core network of laboratories for the analysis of BPA, and to a lesser extent for BPS and BPF. The study has indicated challenges with the analyses of the low concentration samples. A positive bias at the low level suggests issues with background contamination or selectivity, which need further attention to ensure accurate measurements of low-level samples. LOQs were not always sufficiently low, in particular for BPF, and showed a high interlaboratory variability.

Importantly, the diversity of analytical approaches used by the participating laboratories, including the analysis of the total deconjugated forms *versus* the direct measurement of the glucuronide form, as well as the use of naturally contaminated and/or fortified samples with conjugated forms, permitted to confirm a good consistency of the produced results with regard to the efficiency of the employed deconjugation methods. Bisphenols present one of few compound groups where both LC- and GC-based methods are employed in human biomonitoring. LOQs tended to be lower for LC-MS/MS compared to GC-MS/MS, but no significant difference was observed in laboratory performance.

These proficiency tests developed and conducted in HBM4EU permitted to assess the performances of the existing analytical capacity in the European Union, to assess the comparability of the generated data, and then to use the knowledge gained to improve the capacity building of the HBM4EU laboratory network for the future biomonitoring studies.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence

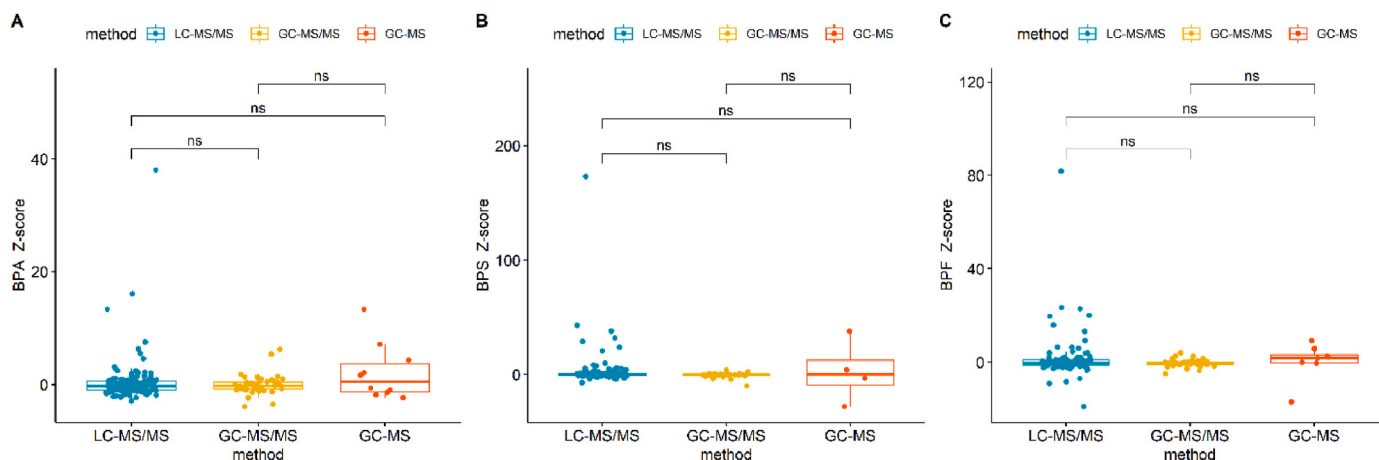


Fig. 2. Boxplots of the Z-scores obtained with different analysis methods for A) BPA, B) BPS and C) BPF by all participants. The box of the boxplots ranged from the P25 to the P75 percentiles with the horizontal line showing the mean, the whiskers showing the P5 to the P95 percentiles. Groups of Z-scores for all CMs over all rounds were compared using Kruskal-Wallis test.

the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envres.2022.112933>.

References

- Bernier, M.R., Vandenberg, L.N., 2017. Handling of thermal paper: implications for dermal exposure to bisphenol A and its alternatives. *PLoS One* 12. <https://doi.org/10.1371/JOURNAL.PONE.0178449>.
- Buscher, B., van de Lagemaat, D., Gries, W., Beyer, D., Markham, D.A., Budinsky, R.A., Dimond, S.S., Nath, R.V., Snyder, S.A., Hentges, S.G., 2015. Quantitative analysis of unconjugated and total bisphenol A in human urine using solid-phase extraction and UPLC-MS/MS: method implementation, method qualification and troubleshooting. *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.* 1005, 30–38. <https://doi.org/10.1016/j.jchromb.2015.09.020>.
- Calafat, A.M., Ye, X., Wong, L.-Y., Reidy, J.A., Needham, L.L., 2007. Exposure of the U.S. Population to bisphenol A and 4-tertiary-Octylphenol: 2003–2004. *Environ. Health Perspect.* 116, 39–44.
- Christensen, K.L., Lorber, M., Koslitz, S., Brüning, T., Koch, H.M., 2012. The contribution of diet to total bisphenol A body burden in humans: results of a 48 hour fasting study. *Environ. Int.* 50, 7–14. <https://doi.org/10.1016/j.envint.2012.09.002>.
- Covaci, A., Hond, E., Den, Geens, T., Govarts, E., Koppen, G., Frederiksen, H., Knudsen, L. E., Morck, T.A., Gutleb, A.C., Guignard, C., Cocco, E., Horvat, M., Heath, E., Kosjek, T., Majej, D., Tratnik, J.S., Castaño, A., Esteban, M., Cutanda, F., Ramos, J.J., Berglund, M., Larsson, K., Jönsson, B.A.G., Biot, P., Castejón, L., Joas, R., Joas, A., Bloemen, L., Sepai, O., Exley, K., Schoeters, G., Angerer, J., Kolossa-Gehring, M., Fiddicke, U., Aerts, D., Koch, H.M., 2015. Urinary BPA measurements in children and mothers from six European member states: overall results and determinants of exposure. *Environ. Res.* 141, 77–85. <https://doi.org/10.1016/j.envres.2014.08.008>.
- Deceuninck, Y., Bichon, E., Marchand, P., Boquien, C.Y., Legrand, A., Boscher, C., Antignac, J.P., Le Bizec, B., 2015. Determination of bisphenol A and related substitutes/analogues in human breast milk using gas chromatography-tandem mass spectrometry. *Anal. Bioanal. Chem.* 407, 2485–2497. <https://doi.org/10.1007/s00216-015-8469-9>.
- Deceuninck, Y., Bichon, E., Gény, T., Veyrand, B., Grandin, F., Viguié, C., Marchand, P., Le Bizec, B., 2019. Quantitative method for conjugated metabolites of bisphenol A and bisphenol S determination in food of animal origin by ultra high performance liquid chromatography–tandem mass spectrometry. *J. Chromatogr. A* 1601, 232–242. <https://doi.org/10.1016/j.chroma.2019.05.001>.
- Dekant, W., Völkel, W., 2008. Human exposure to bisphenol A by biomonitoring: methods, results and assessment of environmental exposures. *Toxicol. Appl. Pharmacol.* 228, 114–134. <https://doi.org/10.1016/j.taap.2007.12.008>.
- Dvorakova, D., Pulkrabova, J., Gramblícka, T., Polachova, A., Buresova, M., López, M.E., Castaño, A., Nübler, S., Haji-Abbas-Zarrabi, K., Klausner, N., Göen, T., Mol, H., Koch, H.M., Vaccher, V., Antignac, J.P., Haug, L.S., Vorkamp, K., Hajslova, J., 2021. Interlaboratory comparison investigations (ICIs) and external quality assurance schemes (EQUASs) for flame retardant analysis in biological matrices: results from the HBM4EU project. *Environ. Res.* 202, 111705. <https://doi.org/10.1016/j.envres.2021.111705>.
- EC, 2016. Commission Regulation (EU) No 2016/2235 of 12 December 2016 Amending Annex XVII to Regulation (EC) No 1907/2006 of the European Parliament and of the Council Concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) as Regards Bisphenol A.
- ECHA (European Chemicals Agency), 2015. Committee for Risk Assessment (RAC) and Committee for Socio-Economic Analysis (SEAC) Background Document to the Opinion on the Annex XV Dossier Proposing Restrictions on 4,4'-Isopropylidenediphenol (Bisphenol A; BPA). Available at: <https://echa.europa.eu/documents/10162/d52d2c6b-2f1c-4ddf-bb44-4e3e42ea1820>.
- EFSA (European Food Safety Authority), 2008. Scientific opinion of the Panel on food additives, flavourings, processing aids and materials in contact with food (AFC) on a request from the commission on the toxicokinetics of bisphenol A. *EFSA J.* 759, 1–10.
- EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2015. Scientific Opinion on the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs: executive summary. *EFSA J.* 13 (1), 3978.
- Erler, C., Novak, J., 2010. Bisphenol A exposure: human risk and health policy. *J. Pediatr. Nurs.* 25, 400–407. <https://doi.org/10.1016/j.pedn.2009.05.006>.
- Esteban López, M., Göen, T., Mol, H., Nübler, S., Haji-Abbas-Zarrabi, K., Koch, H.M., Kasper-Sonnenberg, M., Dvorakova, D., Hajslova, J., Antignac, J.-P., Vaccher, V., Elbers, I., Thomsen, C., Vorkamp, K., Pedraza – Díaz, S., Kolossa-Gehring, M., Castaño, A., 2021. 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. <https://doi.org/10.1016/j.ijheh.2021.113740>.
- EU-RAR, 2003. (European Union Risk Assessment Report) for 4,4'-isopropylidenediphenol (Bisphenol A). Available at: <https://oehha.ca.gov/media/downloads/cmr/eubisphenolareport325.pdf>.
- Fearn, T., Thompson, M., 2001. A new test for "sufficient homogeneity. *Analyst* 126, 1414–1417. <https://doi.org/10.1039/b103812p>.
- Fillol, C., Oleko, A., Saoudi, A., Zeghnoun, A., Balicco, A., Gane, J., Rambaud, L., Leblanc, A., Gaudreau, É., Marchand, P., Le Bizec, B., Bouchart, V., Le Gléau, F., Durand, G., Denys, S., 2021. Exposure of the French population to bisphenols, phthalates, parabens, glycol ethers, brominated flame retardants, and perfluorinated compounds in 2014–2016: results from the Esteban study. *Environ. Int.* 147, 106340. <https://doi.org/10.1016/j.envint.2020.106340>.
- Ganzleben, C., Antignac, J.P., Barouki, R., Castaño, A., Fiddicke, U., Klánová, J., Lebre, E., Olea, N., Sarigiannis, D., Schoeters, G.R., Sepai, O., Tolonen, H., Kolossa-Gehring, M., 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>.
- Geens, T., Goeyens, L., Covaci, A., 2011. Are potential sources for human exposure to bisphenol-A overlooked? *Int. J. Hyg Environ. Health* 214, 339–347.
- Gilles, L., Govarts, E., Rambaud, L., Vogel, N., Castaño, A., Esteban López, M., Rodríguez Martín, L., Koppen, G., Remy, S., Vrijheid, M., Montazeri, P., Birks, L., Sepai, O., Stewart, L., Fiddicke, U., Loots, I., Knudsen, L.E., Kolossa-Gehring, M., Schoeters, G., 2021. HBM4EU combines and harmonises human biomonitoring data across the EU, building on existing capacity – the HBM4EU survey. *Int. J. Hyg Environ. Health* 237, 113809. <https://doi.org/10.1016/J.IJHEH.2021.113809>.
- Ginsberg, G., Rice, D.C., 2009. Does rapid metabolism ensure negligible risk from bisphenol A? *Environ. Health Perspect.* 117, 1639–1643. <https://doi.org/10.1289/EHP.0901010>.
- Gys, C., Ait Bamai, Y., Araki, A., Bastiaensen, M., Caballero-Casero, N., Kishi, R., Covaci, A., 2020. Biomonitoring and temporal trends of bisphenols exposure in Japanese school children. *Environ. Res.* 191, 110172. <https://doi.org/10.1016/j.envres.2020.110172>.
- Haines, D.A., Murray, J., 2012. Human biomonitoring of environmental chemicals—early results of the 2007–2009 Canadian Health Measures Survey for males and females. *Int. J. Hyg Environ. Health* 215, 133–137.
- Ivry Del Moral, L., Le Corre, L., Hélène, P., Isabelle, N., Caroline, T., Jean-François, M., Patrick, R., Philippe, B., Roger, R., Marie-Christine, C., 2016. Obesogen effects after perinatal exposure of 4,4'-sulfonyldiphenol (Bisphenol S) in C57BL/6 mice. *Toxicology* 357–358, 11–20. <https://doi.org/10.1016/j.tox.2016.05.023>.
- Karrer, C., Andreassen, M., von Goetz, N., Sonnet, F., Sakhi, A.K., Hungerbühler, K., Dirven, H., Husøy, T., 2020. The EuroMix human biomonitoring study: source-to-dose modeling of cumulative and aggregate exposure for the bisphenols BPA, BPS, and BPF and comparison with measured urinary levels. *Environ. Int.* 136, 105397. <https://doi.org/10.1016/J.ENVINT.2019.105397>.
- Kolla, S.D.D., Morcos, M., Martin, B., Vandenberg, L.N., 2018. Low dose bisphenol S or ethinyl estradiol exposures during the perinatal period alter female mouse mammary gland development. *Reprod. Toxicol.* 78, 50–59. <https://doi.org/10.1016/j.reprotox.2018.03.003>.
- Kommission Human-Biomonitoring des Umweltbundesamtes, 2012. Stoffmonographie Bisphenol A (BPA) - referenz- und Human-Biomonitoring-(HBM)-Werte für BPA im Urin. *Bundesgesundheitsblatt* 55, 1215–1231. <https://doi.org/10.1007/s00103-012-1525-0>.
- Lehmler, H.J., Liu, B., Gadogbe, M., Bao, W., 2018. Exposure to bisphenol A, bisphenol F, and bisphenol S in U.S. Adults and children: the national health and nutrition examination survey 2013–2014. *ACS Omega* 3, 6523–6532. <https://doi.org/10.1021/acsomega.8b00824>.
- Liu, J., Wattar, N., Field, C.J., Dinu, I., Dewey, D., Martin, J.W., 2018. Exposure and dietary sources of bisphenol A (BPA) and BPA-alternatives among mothers in the APrON cohort study. *Environ. Int.* 119, 319–326. <https://doi.org/10.1016/j.envint.2018.07.001>.
- Longnecker, M.P., Harbak, K., Kissling, G.E., Hoppin, J.A., Eggesbo, M., Jusko, T.A., Eide, J., Koch, H.M., 2013. The concentration of bisphenol A in urine is affected by specimen collection, a preservative, and handling. *Environ. Res.* 126, 211–214. <https://doi.org/10.1016/j.envres.2013.07.002>.
- Nübler, S., López, M.E., Castaño, A., Mol, H., Schäfer, M., Haji-Abbas-Zarrabi, K., Bury, D., Koch, H.M., Vaccher, V., Antignac, J.-P., Dvorakova, D., Hajslova, J., Thomsen, C., Vorkamp, K., Göen, T., 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>.
- Park, J.C., Lee, M.C., Yoon, D.S., Han, J., Kim, M., Hwang, U.K., Jung, J.H., Lee, J.S., 2018. Effects of bisphenol A and its analogs bisphenol F and S on life parameters, antioxidant system, and response of defenseome in the marine rotifer *Brachionus koreanus*. *Aquat. Toxicol.* 199, 21–29. <https://doi.org/10.1016/j.aquatox.2018.03.024>.

- Rubin, B.S., 2011. Bisphenol A: an endocrine disruptor with widespread exposure and multiple effects. *J. Steroid Biochem. Mol. Biol.* 127, 27–34. <https://doi.org/10.1016/J.JSBMB.2011.05.002>.
- Rudel, R.A., Gray, J.M., Engel, C.L., Rawsthorne, T.W., Dodson, R.E., Ackerman, J.M., Rizzo, J., Nudelman, J.L., Brody, J.G., 2011. Food packaging and bisphenol A and bis (2-ethylhexyl) phthalate exposure: findings from a dietary intervention. *Environ. Health Perspect.* 119, 914–920.
- Sanchis, Y., Coscollà, C., Corpas-Burgos, F., Vento, M., Gormaz, M., Yusà, V., 2020. Biomonitoring of bisphenols A, F, S and parabens in urine of breastfeeding mothers: exposure and risk assessment. *Environ. Res.* 185, 109481. <https://doi.org/10.1016/j.envres.2020.109481>.
- Santé Publique France, 2019. Impregnation of the French Population by Bisphenols A, S and F. National Biomonitoring Program, p. 57. Esteban 2014-2016. www.sante-publiquefrance.fr.
- Sasso, A.F., Pirow, R., Andra, S.S., Church, R., Nachman, R.M., Linke, S., Kapraun, D.F., Schurman, S.H., Arora, M., Thayer, K.A., Bucher, J.R., Birnbaum, L.S., 2020. Pharmacokinetics of bisphenol A in humans following dermal administration. *Environ. Int.* 144, 106031. <https://doi.org/10.1016/j.envint.2020.106031>.
- Schöringhumer, K., Cichna-Markl, M., 2007. Sample clean-up with sol-gel enzyme and immunoaffinity columns for the determination of bisphenol A in human urine. *J. Chromatogr. B. Analyt. Technol. Biomed. Life Sci.* 850, 361–369. <https://doi.org/10.1016/J.JCHROMB.2006.12.002>.
- Skledar, D.G., Schmidt, J., Fic, A., Klopčič, I., Trontelj, J., Dolenc, M.S., Finel, M., Masič, L.P., 2016. Influence of metabolism on endocrine activities of bisphenol S. *Chemosphere* 157, 152–159. <https://doi.org/10.1016/j.chemosphere.2016.05.027>.
- Thayer, K.A., Doerge, D.R., Hunt, D., Schurman, S.H., Twaddle, N.C., Churchwell, M.I., Garantzotis, S., Kissling, G.E., Easterling, M.R., Bucher, J.R., Birnbaum, L.S., 2015. Pharmacokinetics of bisphenol A in humans following a single oral administration. *Environ. Int.* 83, 107–115. <https://doi.org/10.1016/j.envint.2015.06.008>.
- Thompson, M., Ellison, S.L.R., Wood, R., 2006. THE international harmonized protocol for the proficiency testing OF analytical chemistry laboratories (IUPAC technical report). *Pure Appl. Chem.* 78, 145–196. <https://doi.org/10.1351/pac200678010145>.
- Völkel, W., Colnot, T., Csanady, G., Filser, J., Dekant, W., 2002. Metabolism and kinetics of bisphenol A in humans at low doses following oral administration. *Chem. Res. Toxicol.* 15, 1281–1287.
- 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., Pérez Luzardo, O., 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>.
- Ye, X., Bishop, A.M., Reidy, J.A., Needham, L.L., Calafat, A.M., 2007. Temporal stability of the conjugated species of bisphenol A, parabens, and other environmental phenols in human urine. *J. Expo. Sci. Environ. Epidemiol.* 17, 567–572. <https://doi.org/10.1038/SJ.JES.7500566>.
- Ye, X., Zhou, X., Hennings, R., Kramer, J., Calafat, A.M., 2013. Potential external contamination with bisphenol A and other ubiquitous organic environmental chemicals during biomonitoring analysis: an elusive laboratory challenge. *Environ. Health Perspect.* 121 (3), 283–286.