HBM4EU science and policy for a healthy future

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ICI/EQUAS REPORT Bisphenols/round 4

Bisphenols in urine

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1 Summary

Within the framework of the HBM4EU project, an Inter-Laboratory Comparison Investigation (ICI) and External Quality Assurance Schemes (EQUAS) was organized and conducted for the analysis of Bisphenol A (BPA), Bisphenol S (BPS) and Bisphenol F (BPF) in human urine. The study was conducted from November 2019 to February 2020.

In total, 27 laboratories from 18 countries participated in this ICI/EQUAS. Among participants, four expert laboratories were included, two from Europe (HBM4EU consortium) and two from outside Europe (USA, Canada). The participation in the ICI/EQUAS was satisfactory, as 27 out of 27 laboratories submitted their results.

Two different test samples consisting of 10 mL urine each were prepared, corresponding to different concentration levels of the targeted biomarkers (naturally contaminated and/or fortified), and sent to the participating laboratories for analysis. These samples were defined as follows:

- 1 sample at low level (L), corresponding to a pool of naturally contaminated individual human urine samples for which the concentrations of the targeted markers were expected to be near the p25 of the concentration distribution of a European general population,

- 1 samples at high level (H), corresponding to a fortified pool of individual human urine samples for which the concentrations of the targeted markers were expected to be near the p95 value of a European general population.

Homogeneity and stability assessment of the control materials confirmed that both low and high concentration levels samples were adequately homogeneous and stable for BPA and BPF. The stability related statistical criteria appeared conversely not met for both samples for BPS.

The HBM4EU QAU selected four expert laboratories for the Bisphenols/urine combination.

Selection criteria included:

- Number of years of experience with the biomarker/matrix combination of interest.
- Application of highly sensitive and selective analytical techniques for the analysis.
- Application of isotopically labelled standards for quantification.
- Availability of in-house validation reports, data on on-going intra-laboratory performance, ISO17025 accreditation for the biomarker of interest.
- Success rate in inter-laboratory comparisons, external quality assessment schemes or at least comparative results in application studies.

The expert-assigned value is the target value based on analysis results obtained from analysis of the control material by at least 3 expert laboratories (see HBM4EU-SOP-QA-001). The expert assigned values were calculated by averaging the values obtained by the expert labs. When the expert-assigned value couldn't be used as the assigned value (as described in 5.2), the consensus value calculated from the results submitted by the participants was used to perform the assessment. Laboratory results were rated using z-scores in accordance with ISO 13528 and ISO 17043. The default standard deviation applied for proficiency assessment (i.e. target standard deviation) was set to FFP = 25 %, as described in 5.3. The table 1 presents a global overview of the proportion of satisfying results (-2 ≤ z-sores ≤ 2).

Table 1: Percentages of satisfying results and number of successful labs for the 4rd round of EQUAS/ICI evaluation for bisphenols

	BPA		BPS		BPF	
	Satisfying Z-scores %	**Nb of successful labs**	**Satisfying Z-scores %**	**Nb of successful labs**	**Satisfying Z-scores %**	**Nb of successful labs**
L sample	85	22	57	12	50	11
H sample	92	24	100	21	91	20

Assessments' scores were calculated for BPA, BPS and BPF for low and high level samples. As a global overview, the proportion of satisfying results (-2 ≤ z-sores ≤ 2) ranged from 57% to 100% for the 3 compounds.

2 Introduction

Interlaboratory Comparison Investigations (ICI) and External Quality Assurance Schemes (EQUAS) are tools to access the proficiency of laboratories, and the comparability and reliability of analytical methods. Participation in ICI / EQUAS forms is full part of Laboratory Quality Assurance system together with initial and on-going in-house method validation.

This ICI/EQUAS study was organised within the frame of HBM4EU as part of the Quality Assurance program for biomonitoring analyses. Within HBM4EU, participation in ICI/EQUAS exercises is mandatory for laboratories that will be further involved in the analytical characterization of the HBM4EU samples.

This report describes the 4th round for Bisphenols in urine and was organised by the LABoratoire d'Etude des Résidus et Contaminants dans les Aliments (LABERCA), a Research Laboratory located in Nantes (France) and affiliated both to Oniris (Nantes-Atlantic National College of Veterinary Medecine, Food Science and Engineering) and to INRA (French National Institute for Agricultural Research).

Bisphenols A, S and F were included in the scope of this ICI/EQUAS. Concentration levels were decided so that to assessing the laboratory performances at two different concentration levels, considering the needed reinforced attention for this particular group of substances especially with regard to the possible external contamination issue.

2.1 Confidentiality

In this report the identity of the participants and the information provided by them is treated as confidential. However, lab codes of the participants will be disclosed to the HBM-QAU for performance assessments.

3 Control material

3.1 Preparation of control material

First, several litres of human urine were collected from several volunteer's individuals at different times of the day (morning, afternoon, evening). These samples were split into two different pools that were then separately filtered in a 2 L Erlenmeyer flask using a vacuum pump and pre-washed pleated filters. The filtered urine pools were transferred to a 1 L glass bottle, placed under magnetic stirring for at least 30 min and then analysed to determine the concentration levels of each target biomarker in this primary naturally contaminated supporting material.

One pool was then directly used to prepare low level (L) samples. Ten millilitres aliquots were distributed into 15 mL tubes immediately closed with a suitable cap (polypropylene, Falcon). The concentrations of the targeted markers were expected to be near the p25 of the concentration distribution of a European general population.

The other pool was fortified with glucuronide-BPA, glucuronide-BPS and glucuronide-BPF at expected concentration levels for coming close to the p95 values typically expected for European general population. It was identified as high (H) concentration level. After being agitated 30 min on a magnetic stir, 10 mL of the spiked materials were introduced in 15 mL tubes equipped with suitable caps (polypropylene, Falcon).

The tubes were stored in the freezer (≤ -18 °C) until transportation. The two different concentrations (L and H) were measured by the ICI/EQUAS organiser using GC-MS/MS (applied analytical method described in Appendix 5).

3.2 Homogeneity of control material

Ten tubes of each concentration of the control material (L and H) were randomly selected from the freezer (≤ -18 °C). The thawed samples were re-homogenised by vortex shaking and analysed in duplicate using GC-MS/MS (analysis method see Appendix 5). The homogeneity was evaluated according to HBM4EU-SOP-QA-002 "Preparation of control materials for Interlaboratory Comparison Investigations (ICI) and External Quality Assurance Schemes (EQUAS)", ISO 13528:2015, Fearn et al [2001] and Thompson [2000]. The full data are presented in Appendix 1.

The results are summarized in the table 2 below.

Table 2: conclusions associated to the homogeneity test

		HOMOGENEITY CRITERIA		
	Concentration Level	$s_s < 0.3*\sigma H$	$s_w < 0.5*\sigma_H$	***Outliers ?***
BPA	Low (L)	ACCEPT	ACCEPT	NO
	High (H)	ACCEPT	ACCEPT	NO
BPS	Low (L)	ACCEPT	ACCEPT	YES
	High (H)	ACCEPT	ACCEPT	NO
BPF	Low (L)	ACCEPT	ACCEPT	NO
	High (H)	ACCEPT	ACCEPT	NO

Homogeneity assessment of the control materials confirmed that the low and high concentration levels samples were adequately homogeneous for BPA, BPS and BPF.

3.3 Stability of control material

In accordance with HBM4EU-SOP-QA-002 “Preparation of control materials for Interlaboratory Comparison Investigations (ICI) and External Quality Assurance Schemes (EQUAS)” and with ISO 13528:2015, three randomly selected test samples from each concentration (L and H) were analysed in duplicate by GC-MS/MS (see analysis method in Appendix 5). The randomly selected control materials were stored at ≤ -18 °C.

The stability was evaluated using the Excel-sheet “HBM4EU ICI-EQUAS stability test CM v1”. The results are presented in Appendix 2. The conclusions are summarized in table 3.

Table 3: conclusions associated to the homogeneity test

		STABILITY CRITERIA	
	Concentration Level	**X-Y < 0.3*σH**	**X-Y < 0.3*σFFP Fit-for-purpose (25%)**
BPA	Low (L)	ACCEPT	ACCEPT
	High (H)	ACCEPT	ACCEPT
BPS	Low (L)	NOT ACCEPT	NOT ACCEPT
	High (H)	ACCEPT	ACCEPT
BPF	Low (L)	ACCEPT	ACCEPT
	High (H)	ACCEPT	ACCEPT

Stability assessment of the control materials confirmed that the low and high concentration levels samples were adequately stable for BPA and BPF. The stability related statistical criteria appeared conversely not met for both samples for BPS. This result was taken into account to calculate Z-score (described in 5.4).

4 Organisational details

4.1 Participants

A list of 46 candidate laboratories from different countries previously identified as potential candidates for the analysis of Bisphenols had been compiled by the Work Package (WP) Task 9.2 leaders and made available to the institution organizing the respective ICI.

Invitation letters were sent by e-mail to all those 46 candidate laboratories on 21st October 2019 (see Appendix 3), indicating that participation would be free of charge.

Twenty-seven laboratories from 16 countries indicated their interest in participating in this ICI/EQUAS and sent their registration form to LABERCA, with their agreement to abide by the conditions for participation. Among participants, four expert laboratories were included, two from Europe (HBM4EU consortium) and two from outside Europe (USA, Canada). All these laboratories received an individual laboratory code to report their measurement results.

The deadline to submit the test results was fixed to 24th January 2020. Of the 27 participants, 27 performed the assays and submitted results.

4.2 Dispatch and instructions

Two test materials consisting of 10 mL urine tube each were shipped to participants under frozen conditions (package shipping from LABERCA, 26th November 2019). The characteristics of the two test samples are described in the section 3.1.

Moreover, a letter with instructions related to sample handling (instruction letter, see Appendix 4), an acknowledgment of receipt, as well as a result submission/method information form were sent to the participants by e-mail the 22nd November 2019. Information related to the analytical method used for quantification was compiled in Appendix 5. Participants were asked to perform for each sample a single analysis using the same procedure they will routinely use in HMB4EU. Participants were asked to report results according to the instructions given.

5 Data evaluation

5.1 False negatives and false positives

Classification of results as false negatives or false positives was done as described in HBM4EU-SOP-QA-003.

A result was assigned as false positive when the following conditions all applied:

1) the biomarker was below the LOQ value as applied by the organiser, the expert laboratories, and the majority of the participants.
2) the biomarker was reported by the participant at a level clearly exceeding the LOQs mentioned under 1).

When a biomarker is reported as "<LOQ-value", AND an assigned value could be established for the biomarker in the control material, a further assessment was done to verify whether this result might be a false negative and to judge whether the LOQ is considered adequate (low enough) for analysis in the frame of HBM4EU. A result is a false negative when the LOQ of a biomarker is well below the assigned value, but the laboratory did not report a quantitative value. The LOQ is considered not adequate (too high) when:

1) the LOQ is substantially above the assigned value
2) the assigned value represents a realistic concentration of real samples in the frame of HBM4EU
3) quantitatively determination is feasible by the majority of the laboratories

In order to judge "<LOQ" results in a quantitative way, 'proxy-Z-scores' were calculated as described in 5.5

5.2 Assigned value

The expert-assigned value is the target value based on analysis results obtained from analysis of the control material by at least 3 expert laboratories (see HBM4EU-SOP-QA-001). The results obtained by the expert laboratories will be submitted to the EQUAS organiser who will calculate the means and (relative) standard deviation for each expert laboratory. Using the individual means of the expert laboratories, the mean of the means will be calculated, its relative standard deviation, and the relative uncertainty of the mean of the means which is given by:

u = RSD / sqrt(N)

with u = relative uncertainty of the mean of the mean concentrations from the expert labs

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

N = the number of expert labs (after exclusion of outliers if applicable)

The mean concentration derived from the expert laboratories is considered suitable for use as assigned value in EQUAS studies when $u \leq 0.7 * \sigma_T$ (σ_T = 25% as described in 5.3).

When $u > 0.7 * \sigma_T$, the individual means are checked for outliers (Grubbs' outlier test). When an individual expert mean is identified as Grubbs' outlier, it is rejected from the data set and the relatively uncertainty is calculated again. If the condition $u \leq 0.7 * \sigma T$ is still not met, then the uncertainty of the

expert-derived mean is too high to be used as assigned value. In this case, no EQUAS assessment of participants' performance is possible for the applicable biomarker. This is also the case when the number of (remaining) individual expert means is less than three.

Only if the EQUAS assessment of participants' performance is not possible, the consensus value of the ICI is used as assigned value and calculated as described in SOP HBM4EU-SOP-QA-003. In brief, the consensus values and its uncertainty were calculated from the results submitted by the participants using robust statistics to minimize the influence of outliers. Two criteria were evaluated before calculating performance's score:
- if the uncertainty was not significant ($u \leq 0.3\sigma_T$), z-scores were then calculated, otherwise another kind of performance's scores were calculated taking into account the uncertainty.
- if the uncertainty did not meet the following criteria : $u \leq 0.7\sigma_T$, no kind of scores were provided.

5.3 Target standard deviation

A pre-set fit-for-purpose target standard deviation is used rather than the standard deviation obtained from the participants' results. The fit-for-purpose target standard deviation reflects the maximum variability that is considered acceptable for a certain biomarker/ (concentration)/matrix. The value is set based on expert opinion, taking into account what is technically feasible and realistic in current routine practises. By default, the value is set at: $\sigma_T = 0.25C$

5.4 Z-scores

Z-scores were calculated according to SOP HBM4EU-SOP-QA-003.

$$Z = \frac{x - C}{\sigma_T}$$

with: Z = Z-score for the submitted analysis result;
x = result submitted by the laboratory;
C = expert-assigned value;
σ_T = target standard deviation, here 0.25*C

In accordance with ISO 13528 and ISO 17043 and the deliverable D 9.4 "*The Quality Assurance/Quality Control Scheme in the HBM4EU project*, Z-scores are classified as presented in Table 4.

Table 4: Classification of Z-scores

$\lvert Z \rvert \leq 2$	Satisfactory
$2 < \lvert Z \rvert < 3$	Questionable
$\lvert Z \rvert \geq 3$	Unsatisfactory

When the biomarker turned out to be not stable in the test material (for stability testing see HBM4EU-QA-002), it is still possible to calculate a Z-score, but the instability has to be taken into account, and the Z-score (Z_i) is calculated as follows:

$$Z_i = \frac{x - C}{\sqrt{\sigma_T^2 + \Delta^2}}$$

With: Z_i = Z-score for the submitted analysis result;
x = result submitted by the participant;
C = consensus or expert-assigned value, determined according to 5.2;
σ_T = target standard deviation, here 0.25*C;
Δ = difference between mean concentrations of the biomarker at t=0 and t=end.

5.5 Proxy-Z-scores

'Proxy-Z-score' are used here to judge "<LOQ" results in a quantitative way (see 5.1). The proxy-Z-scores' is calculated using the LOQ-value as result and equation (1). When no LOQ was specified, zero was used.

Proxy-Z-scores are classified as follows:

- proxy-Z ≤ -3 false negative. Based on the LOQ provided, the laboratory should have been able to detect and quantify the biomarker. Performance is considered 'unsatisfactory'.
- proxy-Z ≥3 the LOQ is considered too high to be fit-for-purpose in the frame of HBM4EU analysis. It also means that the LOQ is too high in comparison with other laboratories. (Note: proxy-Z can only be calculated when an assigned value could be established. When this is the case, this inherently means that reliable quantitative determination at a certain low level is feasible). Performance is considered 'unsatisfactory'.
- -3 ≤ proxy-Z < -2 possible false negative. Performance is considered 'questionable'.
- 2 < proxy-Z ≤ 3 the LOQ is relatively high in relation to HBM4EU analysis and compared to other laboratories. Performance is considered 'questionable'.
- -2 ≤ proxy-Z ≤ 2 LOQ is within an acceptable range relative to the assigned value, adequate for HBM4EU analysis, and in line with the LOQs of the majority of the participating laboratories. Performance is considered 'satisfactory'.

6 Results and discussion

6.1 Results submitted by participants

In total 27 laboratories from 16 countries agreed to participate in this study; 27 participants submitted results.

Laboratories were also asked to provide LOQs. This information is compiled in appendix 9. An overview of results submitted by the participants is included in appendix 6.

More precisely, and with regard to the total set of two test materials to be analysed, 26 participants reported results for BPA, while 21 reported results for BPS and 22 for BPF.

The LOQs were generally in the range 0.010-1.150 ng/mL, higher for few laboratories (see appendix 9). For the test materials of this ICI/EQUAS these LOQs were found adequate in most cases.

6.2 Assigned values and (target) standard deviations

The expert assigned value and its uncertainty, the standard deviation as derived from the expert laboratory's data, and the fit-for-purpose (FFP) target standard deviation for each of the analytes/control materials are included in appendix 6.

The robust relative standard deviation was calculated as described in HBM4EU-SOP-QA-003 and was compared to the FFP target standard deviation, in order to evaluate whether the FFP fitted well with the variability actually observed. All these observations are presented in the appendix 6.

The used expert-assigned values, their associated uncertainty and standard deviation are presented in the table 5.

Table 5: Expert assigned values, associated uncertainty and standard deviations

	EXPERT ASSIGNED VALUES					
	L SAMPLE			H SAMPLE		
	BPA	BPS	BPF	BPA	BPS	BPF
Mean Expert Lab 1	0.393	0.087	0.096	6.14	5.10	2.64
Mean Expert Lab 2	0.604	0.100	0.108	7.65	5.58	3.71
Mean Expert Lab 3	0.650	0.117	< 0.200	7.93	6.13	3.58
Mean Expert Lab 4	0.667	0.237*	0.096	8.44	7.25	3.73
Mean of the means without outliers (ng/mL)	**0.578**	**0.101**	**0.100**	**7.54**	**6.02**	**3.42**
Uncertainty of expert assigned value (ng/mL)	0.063	0.009	0.004	0.494	0.463	0.262
Expert Standard deviation (ng/mL)	0.127	0.069	0.007	0.99	0.93	0.523
Target standard deviation 25% (ng/mL)	0.145	0.025	0.025	1.89	1.50	0.854
0.7 * Target standard deviation (ng/mL)	0.101	0.018	0.017	1.26	0.957	0.595

** declared outlier*

6.3 Assessment of laboratory performance

Z-scores calculated for all target biomarkers and analysed samples are reported in appendix 6. Graphical representations of the z-scores are provided in appendix 8. A summary of number of laboratories that reported results and the number of acceptable/questionable/unacceptable scores are presented in tables 6, 7 and 8.

Table 6: Summary of BPA results assessment

	BPA	
	Low Level	**High Level**
Nb of reported quantitative results	26	26
Nb of reported <LOQ	2	0
Nb of acceptable score	22	24
Nb of questionnable score	3	2
Nb of unacceptable score	1	0

Table 7: Summary of BPS results assessment

	BPS	
	Low Level	**High Level**
Nb of reported quantitative results	21	21
Nb of reported <LOQ	5	0
Nb of acceptable score	12	21
Nb of questionnable score	3	0
Nb of unacceptable score	6	0

Table 8: Summary of BPF results assessment

	BPF	
	Low Level	**High Level**
Nb of reported quantitative results	22	22
Nb of reported <LOQ	8	0
Nb of acceptable score	11	20
Nb of questionnable score	2	2
Nb of unacceptable score	9	0

As a global overview, the proportion of satisfying results ($-2 \leq$ Z-score ≤ 2) was from 57 % to 100 %. The summary of participant's scores are included in the Appendix 7.

6.4 Conclusions and recommendations

The participation was 27 out of 46 potential candidate labs. All the registered candidate laboratories reported results.

For BPA, BPS and BPF, satisfactory results associated to the low / high sample were 85% / 92%, 57% / 100% and 50% / 91% respectively. Unsurprisingly the percentage of satisfactory results increases with the concentration level, mainly for BPF and BPS. At low level, the quantification of these 2 biomarkers require a very good analytical sensitivity and thus very low detection/quantification limits. Several LOQs were associated to BPF and BPS by the participants.

Globally, these results indicate the reality of a quite significant core network of competent laboratories for BPA, BPS and BPF. However, compared to BPA, there is a lower number of competent labs for BPF and BPS.

The percentage of satisfactory laboratory was found to be similar the between this 4th round and the 3rd ICI/EQUAS. The organizer thanks all the laboratories for their participation in the different ICI/EQUAS campaign.

7 References

HBM4EU-SOP-QA-001 "Organisation of Interlaboratory Comparison Investigations (ICI) and External Quality Assurance Schemes (EQUAS) of interlaboratory studies”

HBM4EU-SOP-QA-002 “Preparation of control materials for Interlaboratory Comparison Investigations (ICI) and External Quality Assurance Schemes (EQUAS)”

HBM4EU-SOP-QA-003 “Evaluation of results from Interlaboratory Comparison Investigations (ICI) and External Quality Assurance Schemes (EQUAS)”

HBM4EU-SOP-QA-004 “Reporting of results of Interlaboratory Comparison Investigations (ICI) and External Quality Assurance Schemes (EQUAS)”

ISO/IEC 17043:2010, Conformity assessment – General requirements for proficiency testing

ISO 13528, 2015, Statistical methods for use in proficiency testing by interlaboratory comparison.

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Analytical Methods Committee, 1989a, Robust statistics - How not to reject outliers Part 1. Basic concepts, Analyst, 114, 1693-1697.

Analytical Methods Committee, 1989b, Robust statistics - How not to reject outliers Part 2. Interlaboratory trials, Analyst, 114, 1699-1702.

Official Methods of Analysis Program Manual, 2002, Appendix D: Guidelines for Collaborative Study Procedures To Validate Characteristics of a Method of Analysis. Association Of Analytical Communities International. http://www.aoac.org/vmeth/Manual_Part_6.pdf.

Appendix 1. Homogeneity data

	LOW LEVEL SAMPLE (L)						HIGH LEVEL SAMPLE (H)					
	BPA		BPS		BPF		BPA		BPS		BPF	
	Replicate 1	Replicate 2	Replicate 1	Replicate 2	Replicate 1	Replicate 2	Replicate 1	Replicate 2	Replicate 1	Replicate 2	Replicate 1	Replicate 2
1	0.410	0.400	0.105	0.097	0.094	0.075	5.24	5.87	6.19	6.50	2.23	2.32
2	0.393	0.397	0.114	0.107	0.071	0.068	5.23	7.45	6.22	7.05	2.25	2.79
3	0.410	0.384	0.105	0.103	0.081	0.075	5.35		6.16	7.54	2.28	2.50
4	0.455	0.389	0.111	0.104	0.087	0.072	6.04	6.31	6.59	6.78	2.51	2.73
5	0.410	0.362	0.138	0.089		0.076	5.76	5.63	6.47	6.44	2.36	2.42
6	0.353	0.455	0.093	0.112	0.064	0.087	5.18	6.23	5.79	6.64	2.05	2.56
7	0.377	0.334	0.095	0.096	0.076	0.073	4.98	4.52	5.62	5.43	2.01	2.00
8	0.429	0.323	0.108	0.091	0.081	0.061	5.55	6.42	6.31	6.96	2.40	2.75
9	0.476	0.425	0.117	0.110	0.087	0.087	6.71	6.21	6.98	6.87	2.81	2.64
10	0.399	0.368	0.098	0.102	0.077	0.079	5.36	6.25	5.91	6.85	2.14	2.67
Grand mean	0.397		0.105		0.077		5.80		6.46		2.42	
Cochran's test												
C	0.322		0.730		0.347		0.577		0.395		0.266	
Ccrit	0.602		0.602		0.638		0.602		0.602		0.602	
C < Ccrit ?	no outliers		outliers		no outliers		no outliers		no outliers		no outliers	
σT	0.099		0.026		0.077		1.45		1.62		0.605	
s_x	0.026		0.006		0.006		0.482		0.400		0.205	
s_w	0.041		0.013		0.009		0.652		0.491		0.234	
s_s	0		0		0		0.139		0.197		0.121	
critical	0.030		0.008		0.006		0.435		0.485		0.181	
Ss < critical ?	**Homogeneity adequate**		**Homogeneity adequate**		**Homogeneity adequate**		**Homogeneity adequate**		**Homogeneity adequate**		**Homogeneity adequate**	
Sw < 0.5*σT ?	**Method suited**		**Method suited**		**Method suited**		**Method suited**		**Method suited**		**Method suited**	

Appendix 2. Stability data

	LOW LEVEL SAMPLE (L)						HIGH LEVEL SAMPLE (H)					
	BPA		BPS		BPF		BPA		BPS		BPF	
	Replicate 1	Replicate 2	Replicate 1	Replicate 2	Replicate 1	Replicate 2	Replicate 1	Replicate 2	Replicate 1	Replicate 2	Replicate 1	Replicate 2
1	0.405	0.446	0.101	0.084	0.084	0.088	5.556	5.803	6.345	5.047	2.275	2.390
2	0.395	0.371	0.111	0.069	0.069	0.073	6.343	6.383	6.636	5.254	2.521	2.650
3	0.397	0.374	0.104	0.070	0.078	0.078	6.174	5.852	6.852	5.328	2.390	2.378
4	0.422	0.393	0.108	0.064	0.080	0.070	5.694	5.346	6.689	5.336	2.619	2.229
5	0.386	0.399	0.114	0.075	0.075	0.082	5.702	5.277	6.451	4.653	2.387	2.068
6	0.404	0.471	0.103	0.092	0.074	0.096	4.749	5.298	6.214	5.167	2.306	2.085
Average	0.402	0.409	0.107	0.076	0.077	0.081	5.703	5.660	6.531	5.131	2.416	2.300
Std deviation	0.012	0.041	0.005	0.010	0.005	0.010	0.559	0.438	0.237	0.258	0.131	0.220
Difference	-0.007		0.031		-0.004		0.043		1.400		0.116	
0.3 sigma h	0.027		0.007		0.005		0.376		0.431		0.159	
instability ?	NO		YES		NO		NO		YES		NO	
0.3 sigma FFP	0.030		0.008		0.006		0.428		0.490		0.181	
instability ?	NO		YES		NO		NO		YES		NO	

Appendix 3. Copy of letter of invitation

HBM4EU science and policy for a healthy future

HBM4EU: Announcement / invitation to participate in ICI / EQUAS study

Bisphenols/Round 4.

Title of ICI/EQUAS: Bisphenols in urine

Dear Colleague,

within the frame of HBM4EU, the

LABERCA (LABoratoire d'Etude des Résidus et des Contaminants dans les Aliments), UMR 1329 Oniris-INRA,

Route de Gachet – CS50707

44307 NANTES Cedex

France

announces the 4th round of ICI/EQUAS for the determination of Bisphenols in urine.
The aim of ICI/EQUAS exercises is to provide laboratories with an assessment of their analytical performance and reliability of their data in comparison with other laboratories and/or expert laboratories. This will aid in the quality improvement of analysis in human biomonitoring at each of the laboratories.

Participation is mandatory for laboratories analysing samples in the frame of HBM4EU.

Test samples
The matrix will be human urine. The participants will receive:

- 2 materials of urine (2 samples of 10 mL each) for determination of Bisphenols in urine

Target biomarkers
Please see registration form for Bisphenols/Round 4 for the biomarkers potentially present in the test samples. We would be pleased if your laboratory could participate with the analysis of as most as possible Bisphenols.
LOQs should allow the analysis of Bisphenols in samples of the general population

HBM4EU science and policy for a healthy future

Calendar:

Deadline registration	04-11-2019
Distribution of test samples (projected)	25-11-2019
Deadline for reporting the results (projected)	24-01-2020

Registration
For registration, please find attached a registration form for parameter Bisphenols in urine. Please send it back to us by mail in case you want to register.
Upon registration, the participant will receive a lab-code to be used for submission of results.

Fee
For partners and linked-third parties of HBM4EU, participation is free of charge. Please note that the participant is responsible for custom clearance and associated costs if applicable.

Confidentiality:
All laboratory specific information will be treated confidentially, and will never be disclosed to third parties (government, accreditation bodies) except the HBM4EU QAU, without permission of the laboratory

Contact information organiser:

Coordinator:
Vincent Vaccher

LABERCA (LABoratoire d'Etude des Résidus et des Contaminants dans les Aliments), UMR 1329 Oniris-INRA,
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Appendix 4. Copy of letter/instructions sent together with test samples

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HBM4EU: Instruction letter ICI / EQUAS study Bisphenols/Round 4

Title of ICI/EQUAS: Bisphenols in urine

Dear participant,

Thank you for participation in HBM4EU ICI/EQUAS study Bisphenols in urine/Round 4 for the determination of Bisphenols in urine.

You will receive a parcel containing 2 test samples. Each sample consists of 10 mL urine.

The parcel will be shipped 26/11/2019 under frozen conditions.

Instructions:

- Upon receipt, please check the content for any damage/leaking of the containers, complete the sample receipt form and return it to the organizer.
- Store the test samples under frozen (-18°C) conditions until analysis.
- Analyze the samples for the biomarkers indicated in the invitation letter.
- Thaw the samples and re-homogenize them according to your own procedure.
- Analyze the samples using the same procedure as will be used for analysis of samples in the frame of HBM4EU.
- Carry out a single analysis for each sample.
- For submission of results and method information, use the forms provided by email.
- The deadline for submission of analysis results and method details is **24.01.2020**

If you have any questions or need any assistance, please contact:

Vincent Vaccher

Email: vincent.vaccher@oniris-nantes.fr

Tel: +33 2 40 68 78 80

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Appendix 5. GC-MS/MS method LABERCA

Laboratory	LABERCA - France
ISO17025 accredited	yes
Volume of urine used to perform the analysis	2 mL
Type of deconjugation	enzymatic deconjugation
Enzyme used (ref number)	Abalonase purified enzymatic formula Bglucu (beta gluc 10)
SPE offline (yes/no)	Yes
Type of column used for SPE offline	Chromabond HR-X / Affinimip
Derivatisation agent	MSTFA
Name and version of mass spectrometer	Agilent 7010
GC-MS/MS (yes/no)	Yes
Type of column (GC or LC)	GC (Optima 17 MS)
Response normalised to IS (yes/no)	Yes
Internal standard	BPA 13C; BPS 13C; BPF 13C
Calibration type	Isotopic dilution before extraction

Appendix 6. Expert assigned value and participant's performance

Control material		LOW LEVEL SAMPLE (L)					
Parameter		BPA		BPS		BPF	
Number of participants		26		21		22	
Number of quantitative results		23		10		14	
Expert assigned value ng/mL		0.578		0.101		0.100	
Uncertainty of expert assigned value ng/mL		0.063		0.009		0.004	
Δ Instability		-		0.031		-	
Expert RSD (%)		21		15		8	
Study RSD (%)		31		60		20	
Relative target standard deviation (%)		25		25		25	
Laboratory code	ID sample	Value	Z-score	Value	Z-score	Value	Z-score
1	19-BP4-222	0.620	0.3	NA	NA	NA	NA
2	19-BP4-671	0.500	-0.5	NA	NA	NA	NA
3	19-BP4-649	0.311	-1.8	1.80	43	0.030	-2.8
7	19-BP4-585	0.460	-0.8	< 0.200	(2.5)	< 0.200	(4.0)
9	19-BP4-134	0.690	0.8	0.330	5.7	< 0.200	(4.0)
12	19-BP4-664	0.502	-0.5	0.125	0.6	0.112	0.5
15	19-BP4-13	0.330	-1.7	0.043	-1.5	0.104	0.2
25	19-BP4-617	0.376	-1.4	0.204	2.6	< 0.196	(3.8)
31	19-BP4-233	0.519	-0.4	0.494	10	0.212	4
37	19-BP4-838	0.585	0.0	1.05	24	0.428	13
39	19-BP4-643	0.895	2.2	< 0.400	(7.5)	< 0.600	(20)
50	19-BP4-511	0.393	-1.3	0.087	-0.4	0.096	-0.2
58	19-BP4-318	0.720	1.0	0.142	1.0	0.139	1.6
60	19-BP4-45	0.677	0.7	0.069	-0.8	< 0.150	(2.0)
63	19-BP4-974	0.550	-0.2	0.060	-1.0	0.090	-0.4
66	19-BP4-316	< 0.500	(-0.5)	< 0.083	(-0.5)	< 0.260	(6.4)
68	19-BP4-198	0.604	0.2	0.100	0.0	0.108	0.3
71	19-BP4-188	0.531	-0.3	0.090	-0.3	0.098	-0.1
74	19-BP4-970	0.650	0.5	0.117	0.4	< 0.200	(4.0)
76	19-BP4-778	0.260	-2.2	NA	NA	NA	NA
77	19-BP4-436	NA	NA	NA	NA	0.088	-0.5
79	19-BP4-536	2.90	16	< 0.200	(2.5)	< 0.200	(4.0)
85	19-BP4-920	0.933	2.5	NA	NA	NA	NA
90	19-BP4-179	0.820	1.7	NA	NA	0.160	2.4
93	19-BP4-23	< 0.400	(-1.2)	< 0.150	(1.2)	NA	NA
95	19-BP4-859	0.779	1.4	0.082	-0.5	0.053	-1.9
98	19-BP4-252	0.667	0.6	0.237	3.4	0.096	-0.2

Control material		HIGH LEVEL SAMPLE (H)					
Parameter		BPA		BPS		BPF	
Number of participants		26		21		22	
Number of quantitative results		26		21		22	
Expert assigned value ng/mL		7.54		6.02		3.42	
Uncertainty of expert assigned value ng/mL		0.49		0.46		0.26	
Δ Instability		-		1.40		-	
Expert RSD (%)		13		15		15	
Study RSD (%)		15		20		23	
Relative target standard deviation (%)		25		25		25	
Laboratory code	ID sample	Value	Z-score	Value	Z-score	Value	Z-score
1	19-BP4-735	5.97	-0.8	NA	NA	NA	NA
2	19-BP4-329	8.00	0.2	NA	NA	NA	NA
3	19-BP4-237	7.37	-0.1	4.15	-0.9	1.89	-1.8
7	19-BP4-457	8.14	0.3	5.38	-0.3	3.26	-0.2
9	19-BP4-361	8.44	0.5	6.61	0.3	3.90	0.6
12	19-BP4-966	7.07	-0.3	4.99	-0.5	3.33	0
15	19-BP4-34	6.72	-0.4	2.76	-1.6	3.65	0
25	19-BP4-750	6.77	-0.4	6.57	0.3	2.31	-1.3
31	19-BP4-987	9.21	0.9	6.02	0.0	2.60	-1.0
37	19-BP4-241	7.74	0.1	7.21	0.6	3.88	0.5
39	19-BP4-515	6.56	-0.5	5.55	-0.2	1.58	-2.2
50	19-BP4-852	6.14	-0.7	5.10	-0.4	2.64	-0.9
58	19-BP4-910	7.94	0.2	6.05	0.0	3.61	0.2
60	19-BP4-814	8.19	0.3	5.79	-0.1	4.31	1.0
63	19-BP4-963	7.82	0.1	4.73	-0.6	3.21	-0.2
66	19-BP4-788	3.77	-2.0	2.31	-1.8	2.37	-1.2
68	19-BP4-451	7.65	0.1	5.58	-0.2	3.71	0.3
71	19-BP4-230	8.54	0.5	7.79	0.9	3.27	-0.2
74	19-BP4-290	7.93	0.2	6.13	0.1	3.58	0.2
76	19-BP4-414	2.12	-2.9	NA	NA	NA	NA
77	19-BP4-489	NA	NA	NA	NA	3.02	-0.5
79	19-BP4-575	7.44	-0.1	6.24	0.1	1.16	-2.6
85	19-BP4-6	7.69	0.1	NA	NA	NA	NA
90	19-BP4-931	3.15	-2.3	NA	NA	3.04	-0.4
93	19-BP4-628	5.53	-1.1	5.32	-0.3	NA	NA
95	19-BP4-959	9.08	0.8	6.53	0.2	3.14	-0.3
98	19-BP4-888	8.44	0.5	7.25	0.6	3.73	0.4

Appendix 7. Summary of participant's score

$|Z| \leq 2$ Satisfactory

$2 < |Z| < 3$ Questionable

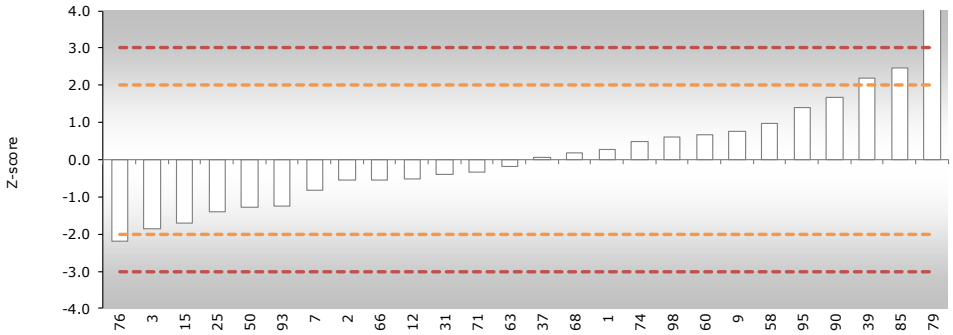
$|Z| \geq 3$ Unsatisfactory

	Z-SCORES					
Laboratory code	BPA		BPS		BPF	
	L sample	H sample	L sample	H sample	L sample	H sample
1	0.3	-0.8	NA	NA	NA	NA
2	-0.5	0.2	NA	NA	NA	NA
3	-1.8	-0.1	43.0	-1	-2.8	-1.8
7	-1	0.3	(2.5)	-0.3	(4.0)	-0.2
9	0.8	0.5	5.7	0.3	(4.0)	0.6
12	-0.5	-0.3	0.6	-0.5	0.5	-0.1
15	-1.7	-0.4	-1.5	-1.6	0.2	0
25	-1.4	-0.4	2.6	0.3	(3.8)	-1.3
31	-0.4	0.9	9.9	0.0	4.5	-1.0
37	0.0	0.1	23.8	0.6	13.1	0.5
39	2.2	-0.5	(7.5)	-0.2	(20)	-2.2
50	-1.3	-0.7	-0.4	-0.4	-0.2	-0.9
58	1.0	0	1.0	0.0	1.6	0.2
60	0.7	0.3	-0.8	-0.1	(2.0)	1.0
63	-0.2	0.1	-1.0	-0.6	-0.4	-0.2
66	(-0.5)	-2.0	(-0.5)	-1.8	(6.4)	-1.2
68	0.2	0.1	0.0	-0.2	0.3	0.3
71	-0.3	0.5	-0.3	0.9	-0.1	-0.2
74	0.5	0.2	0.4	0.1	(4.0)	0.2
76	-2.2	-2.9	NA	NA	NA	NA
77	NA	NA	NA	NA	-0.5	-0.5
79	16	-0.1	(2.5)	0.1	(4.0)	-2.6
85	2.5	0.1	NA	NA	NA	NA
90	1.7	-2.3	NA	NA	2.4	-0.4
93	(-1.2)	-1.1	(1.2)	-0.3	NA	NA
95	1.4	0.8	-0.5	0.2	-1.9	-0.3
98	0.6	0.5	3.4	0.6	-0.2	0.4

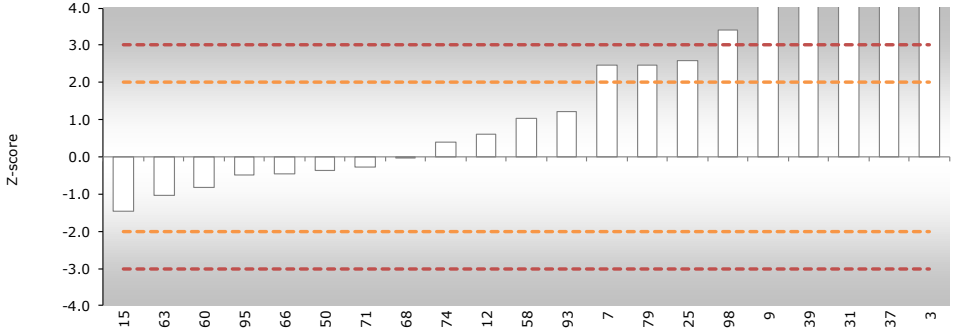
Appendix 8. Graphical representation of the Z-scores

LOW LEVEL SAMPLE (L)

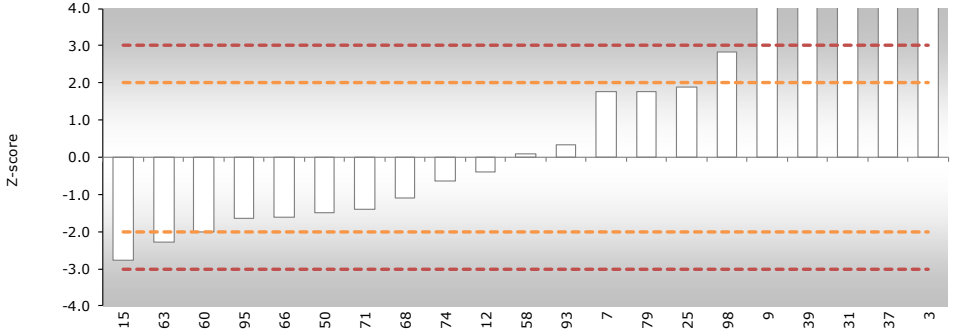
BPA - LOW LEVEL in urine



BPS - LOW LEVEL in urine

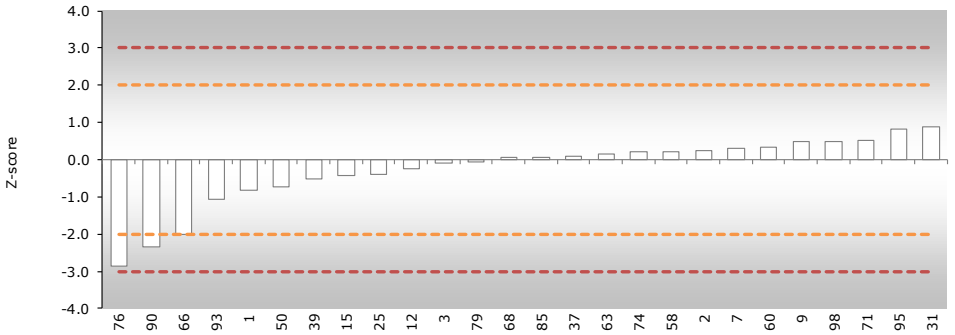


BPF - LOW LEVEL in urine

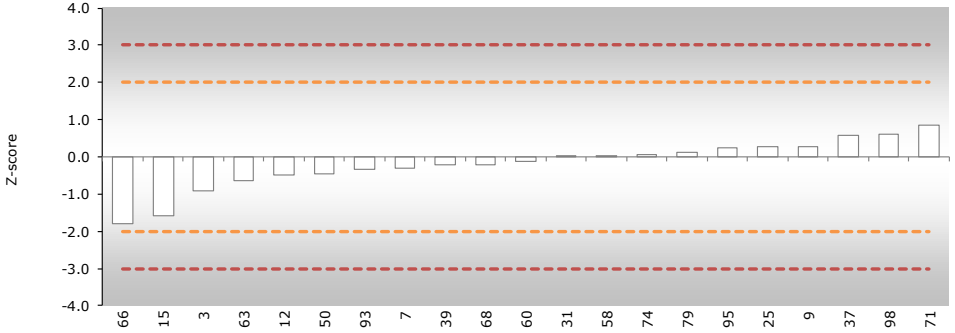


HIGH LEVEL SAMPLE (H)

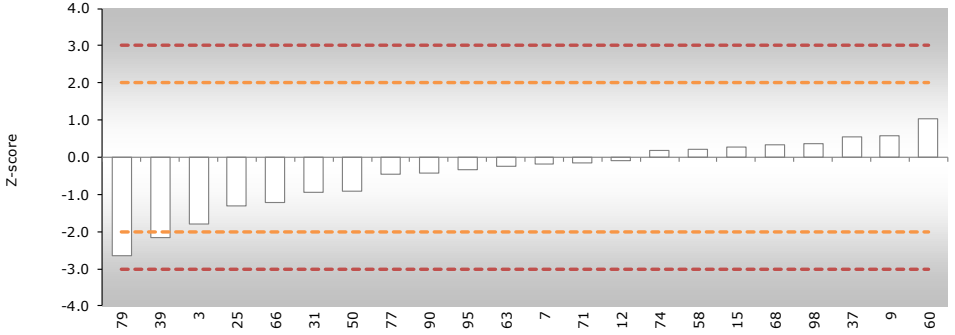
BPA - HIGH LEVEL in urine



BPS - HIGH LEVEL in urine



BPF - HIGH LEVEL in urine



Appendix 9. Summary of participant's LOQ

	LOQ (ng/mL)		
Lab code	**BPA**	**BPS**	**BPF**
1	0.500	-	-
2	0.100	-	-
3	0.500	0.500	0.500
7	0.200	0.200	0.200
9	0.200	0.200	0.200
12	0.200	0.040	0.020
15	0.100	0.030	0.100
25	0.200	0.100	0.250
31	0.100	0.030	0.100
37	0.250	0.050	0.100
39	0.100	0.400	0.600
50	0.090	0.010	0.030
58	0.200	0.100	0.030
60	0.250	0.050	0.150
63	0.030	0.030	0.090
66	0.500	0.083	0.260
68	0.031	0.190	0.085
71	0.290	0.090	0.070
74	0.200	0.100	0.200
76	0.200	-	-
77	-	-	0.039
79	0.200	0.200	0.200
85	0.500	-	-
90	0.280	1.150	0.200
93	0.400	0.150	-
95	0.027	0.031	0.027
98	0.070	0.020	0.050