Supplementary material

Table A1. List of participating laboratories

COUNTRY	LABORATORY GROUP	INSTITUTION
Austria	Global Exposomics & Biomonitoring Group, Dep. of Food Chemistry	University of Vienna
Belgium	Department of Pharmaceutical Sciences	University of Antwerpen
Belgium	Toxicology Lab	CHU Liège
Belgium	Laboratory for Occupational and Environmental Hygiene	KU Leuven
Canada*	Institut National de Santé Publique du Québec	Centre de Toxicologie du Québec
Cyprus	Water and Health Laboratory	Cyprus International Institute for Environmental and Public Health, Cyprus University of Technology
Czech Republic	Unit for Chemical Safety of Products	National Institute of Public Health
Czech Republic	Trace Analytical Laboratory	Research Centre for Toxic Compounds in the Environment (RECETOX)
Denmark*	Chemical Laboratory at Dep. of Growth and Reproduction	Rigshospitalet, Region Hovedstaden (RegionH)
Finland	Environmental health / Chemical risk team	National institute for health and welfare (THL) / Department of Health Security
France*	LABERCA	INRAE, Oniris
France	INRAE Toxalim	INRAE
France	Department Toxicology and Biomonitoring	INRS
France	LABOCEA	LABOCEA
Germany	Institute for Prevention and Occupational Medicine of the German Social Accident Insurance (IPA)	Ruhr-Universität Bochum
Germany	HBM Laboratory	BASF SE - Corporate Health Management
Germany	Institute of Biomonitoring	Currenta GmbH&Co.OHG, SEL-SER-GS
Germany	MVZ Medizinisches Labor Bremen GmbH	Medizinisches Labor Bremen

COUNTRY	LABORATORY GROUP	INSTITUTION
Greece	Health and Exposome Research Centre Center for Transdisciplinary Research and Innovation (KEDEK)	Aristotle University of Thesaloniki
Hungary	Central Laboratory	National Public Health Institute
Italy	Cardiometabolic Risk Unit	Institute of Clinical Physiology CNR
Italy	Laboratory of Environmental and Industrial Toxicology	University of Milan
Norway	Environmental Exposure and Epidemiology	Norwegian Institute of Public Health
Poland	Laboratory of the Department of Toxicology	Medical University of Gdańsk, Faculty of Pharmacy
Slovenia	Department of Environmental Sciences	Jozef Stefan Institute
Slovenia	Center for Chemical Analysis of Food, Water and other Environmental Samples	National Laboratory of Health, Environment and Food
Spain	Public Health Laboratory of Valencia	Public Health Department
Spain	Chromatography laboratory - Biomarkers laboratory	National Center for Environmental Health, Instituto de Salud Carlos III
Sweden	Occupational and enviromental medicine	Laboratory medicine
Switzerland	IST Laboratory	Work and health Insitute
The Netherlands	Vrije Universiteit Amsterdam	Department Environment & Health
UK	Biological Monitoring team	Health & Safety Laboratory
United States of America*	Organic Analytical Toxicology Branch, Division of Laboratory Sciences	Centers for Disease Control and Prevention (CDC)

* Expert laboratories

Table A2. Target concentrations for the three bisphenol biomarkers in the four different ICI/EQUAS rounds.

Target concentration (µg/L)	* BPA_{low}	BPA_{high}	* BPS_{low}	BPS_{high}	* BPF_{low}	BPF_{high}
Round 1	0.600	7.00	0.150	6.00	0.060	1.50
Round 2	0.600	7.00	0.130	6.00	0.040	5.00
Round 3	0.945	7.00	2.45	7.50	0.160	3.15
Round 4	0.600	7.00	0.100	6.00	0.100	3.50

** for the low CMs, no spiking was performed. Concentration corresponds to native content*

Sample preparation and supplementation protocol

For each ICI/EQUAS round, two new different control materials were systematically prepared. The CMs were different for each ICI/EQUAS round.

Preparation of “low control materials”

A sufficient quantity of human urine was collected from volunteers in our laboratory and filtered in an Erlenmeyer flask using a vacuum pump and previously washed pleated filters. The filtered urine was then transferred to a 4 L glass bottle and placed under magnetic stirring for at least 30 min. Using a suitable automatic pipette, 70 aliquots of 10 mL were prepared in 15 mL falcon tubes closed with a suitable stopper.

Preparation of “high control materials”

A sufficient quantity of human urine was filtered in an Erlenmeyer flask using a vacuum pump and previously washed pleated filters. The filtered urine was then transferred to a 4 L glass bottle, fortified with individual solutions of glucuronide-BPA, glucuronide-BPS and glucuronide-BPF at 100 µg/L purchased by Toronto Research Chemicals (Toronto, Canada), and placed under magnetic stirring for at least 30 min. Using a suitable automatic pipette, 70 aliquots of 10 mL were prepared in 15 mL falcon tubes closed with a suitable stopper.

Table A3. Conclusions associated with the homogeneity testing of the two control materials for the four ICI/EQUAS rounds.

		Round 1			Round 2			Round 3			Round 4		
Control material	Biomarker	$s_s < 0.3*\sigma H$	$s_w < 0.5*\sigma_H$	Outliers	$s_s < 0.3*\sigma H$	$s_w < 0.5*\sigma_H$	Outliers	$s_s < 0.3*\sigma H$	$s_w < 0.5*\sigma_H$	Outliers	$s_s < 0.3*\sigma H$	$s_w < 0.5*\sigma_H$	Outliers
L	BPA	accept	accept	no	accept	accept	no	accept	accept	no	accept	accept	no
	BPS	accept	**not acceptable***	**yes**	accept	accept	no	accept	accept	no	accept	accept	**yes**
	BPF	accept	accept	no	accept	accept	no	accept	accept	no	accept	accept	no
H	BPA	accept	accept	no	accept	accept	no	accept	accept	no	accept	accept	no
	BPS	accept	accept	no	accept	accept	no	accept	accept	no	accept	accept	no
	BPF	accept	accept	no	accept	accept	no	accept	accept	**yes**	accept	accept	no

s_s = *between-sample standard deviation*
sw = within-sample standard deviation
σH (Horwitz standard deviation) = 22%
**Note: For technical reasons, a batch of analyses did not meet the required quality control criteria for being included in the calculation of homogeneity results for BPS at L level. Nevertheless, based on the satisfactory results obtained for BPS in the H sample and for BPA, BPF in the L sample, by extrapolation the L material was considered homogeneous for BPS.*

Table A4. Conclusions associated with the stability testing on the two control materials for the four ICI/EQUAS rounds.

		Round 1		Round 2		Round 3		Round 4	
Control Material	Biomarker	X-Y < 0.3*σH	X-Y < 0.3*σFFP	X-Y < 0.3*σH	X-Y < 0.3*σFFP	X-Y < 0.3*σH	X-Y < 0.3*σFFP	X-Y < 0.3*σH	X-Y < 0.3*σFFP
L	BPA	**not acceptable**	**not acceptable**	accept	accept	accept	accept	accept	accept
	BPS	**not acceptable**	accept	accept	accept	accept	accept	**not acceptable**	**not acceptable**
	BPF	**not acceptable**	**not acceptable**	accept	accept	accept	accept	accept	accept
H	BPA	accept	accept	accept	accept	accept	accept	accept	accept
	BPS	accept	accept	accept	accept	accept	accept	accept	accept
	BPF	accept	accept	accept	accept	accept	accept	accept	accept

X = concentration average value of homogeneity analysis (t=0)
Y = concentration average value of stability analysis (t= 65 days, 68 days, 59 days, 77 days for rounds 1 to 4, respectively)
σH (Horwitz standard deviation) = 22%
σFFP (fit-for-purpose standard deviation) = 25%

Table A5. Analytical method used to test homogeneity and stability of the two control materials (L and H) sent to the participants.

Laboratory	LABERCA - France
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)
Extraction method	SPE offline
Type of column used for SPE offline	Chromabond HR-X / Affinimip
Derivatisation agent	N-methyl-N-(trimethylsilyl)-trifluoroacetamide (MSTFA)
Instrumental technique	GC-MS/MS
Name and version of mass spectrometer	Agilent 7010
Type of GC column	Optima 17 MS
Response normalised to IS (yes/no)	Yes
Internal standards	BPA ^{13}C; BPS ^{13}C; BPF ^{13}C purchased by Toronto Research Chemicals (Toronto, Canada)
Calibration type	Isotopic dilution before extraction

Z'-score, Z_i-score and Z'_i-score calculations procedures

Uncertainty of consensus value NOT negligible / biomarker in control material stable

When the uncertainty of the consensus value cannot be considered negligible, the uncertainty of the consensus value is taken into account and the Z-score (Z') is calculated as follows:

$$Z'_a = \frac{x - C}{\sqrt{\sigma_T^2 + u^2}} \quad (1)$$

With: Z' = Z-score for the submitted analysis result;
x = result submitted by the participant;
C = consensus value;
σ_T = target standard deviation;
u = uncertainty of the consensus value.

Uncertainty of consensus value negligible / biomarker in control material NOT stable

When the biomarker turned out to be not stable in the test material, 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:

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

With: Z_i = Z-score for the submitted analysis result;
x = result submitted by the participant;

C = consensus value;

σ_T = target standard deviation;

Δ = difference between mean concentrations of the biomarker at t=0 and t=end.

Uncertainty of consensus value NOT negligible / biomarker in control material NOT stable

In case the biomarker turned out to be not stable in the test material and the uncertainty of the consensus value cannot be neglected, then the Z-score (Z'_i) is calculated as follows:

$$Z'_i = \frac{x - C}{\sqrt{\sigma_T^2 + u^2 + \Delta^2}} \quad (3)$$

With: Z'_i = Z-score for the submitted analysis result;

x = result submitted by the participant;

C = consensus value;

σ_T = target standard deviation;

u = uncertainty of the consensus value;

Δ = difference between mean concentrations of the biomarker at t=0 and t=end.

Z'-score, Z_i-score and Z'_i-score are classified as Z-scores

Table A6. Mean LOQ values obtained by all the participants (excluding experts) over all rounds according their chromatographic method and comparison with previous biomonitoring studies focused on bisphenols.

	LOQ mean value (µg/L)											
	BPA				**BPS**				**BPF**			
	Rd 1	**Rd 2**	**Rd 3**	**Rd 4**	**Rd 1**	**Rd 2**	**Rd 3**	**Rd 4**	**Rd 1**	**Rd 2**	**Rd 3**	**Rd 4**
LC participant's method	0.544	0.210	0.202	0.229	0.973	0.186	0.166	0.143	0.808	0.287	0.205	0.215
GC participant's method	0.888	0.274	0.255	0.278	0.097	0.423	0.346	0.316	0.128	0.306	0.195	0.104
LC-MS/MS (Karrer et al., 2020)	0.100				0.400				0.200			
LC-MS/MS (Sanchis et al., 2020)	0.200				0.200				0.200			
GC-MS/MS (Gys et al., 2020)	0.300				0.040				0.020			

Rd: Round; nc: not calculated

Table A7. Overview of the <LOQ results for BPA, BPS and BPF for the 2nd, 3rd and 4th rounds.

				LOQ << assigned value	LOQ < assigned value	LOQ within acceptable range to the assigned value	LOQ > assigned value	LOQ >> consensus/assigned value
	Round	**CM**	**No of -LOQ results**	**LOQ-Z ≤ -3**	**-3 < LOQ-Z < -2**	**-2 ≤ LOQ-Z ≤ 2**	**2 < LOQ-Z < 3**	**LOQ-Z ≥ 3**
BPA	**1 - ICI***	low	3					
		high						
	2 - EQUAS	low	1		1			
		high						
	3 - EQUAS	low	1		1			
		high						
	4 - EQUAS	low	2			2		
		high						
	Total number		**7**		**2**	**2**		
BPS	**1 - ICI***	low	3					
		high						
	2 - EQUAS	low	8	1		3		4
		high	1					1
	3 - EQUAS	low						
		high						
	4 - EQUAS	low	5			2	2	1
		high						
	Total number		**17**	**1**		**5**	**2**	**6**
BPF	**1 - ICI***	low	9					
		high	4					
	2 - EQUAS	low	9			4		4
		high						
	3 - EQUAS	low	5			4		1
		high	1			1		1
	4 - EQUAS	low	8			1		7
		high						
	Total number		**36**			**10**		**13**

**LOQ-Z-SCORE were not calculated for the 1st round*

Table A8. Study RSD_R (%) of all HBM4EU approved laboratories (satisfactory Z-scores for both low and high concentration levels in at least two ICI/EQUAS rounds) for BPA, BPS and BPF.

	BPA_{low}	**BPA_{high}**	**BPS_{low}**	**BPS_{high}**	**BPF_{low}**	**BPF_{high}**
Round 1	27%	23%	27%	8%	nc	nc
Round 2	28%	17%	22%	21%	28%	18%
Round 3	25%	19%	21%	14%	17%	19%
Round 4	30%	14%	42%	22%	10%	16%
Mean all rounds	**28%**	**18%**	**28%**	**16%**	**18%**	**18%**

nc : not calculated

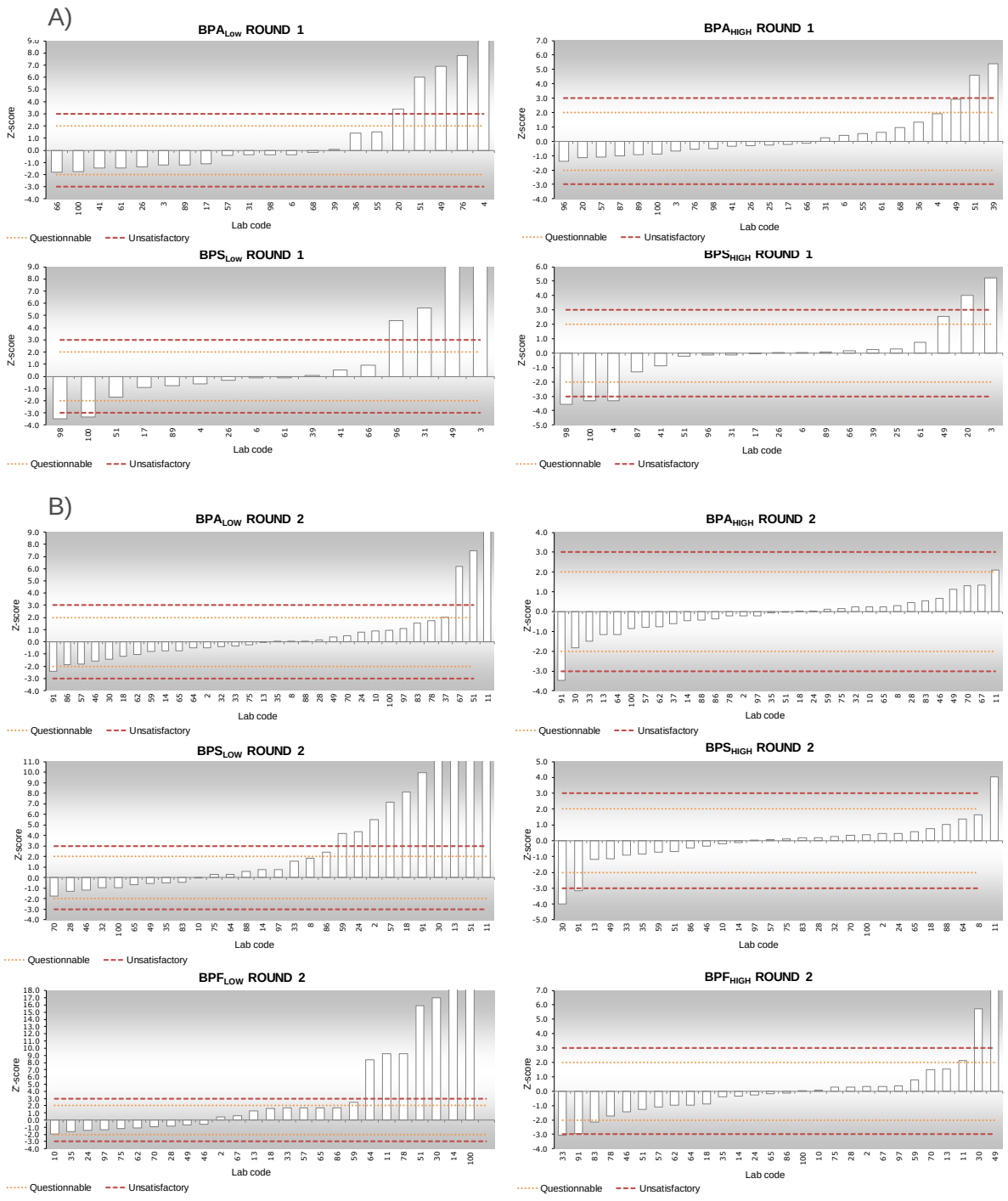

Fig. A1 Z-scores of the participants` results in the ICI/EQUAS rounds 1-4 for BPA, BPS and BPF

A) ROUND 1 ; B) ROUND 2

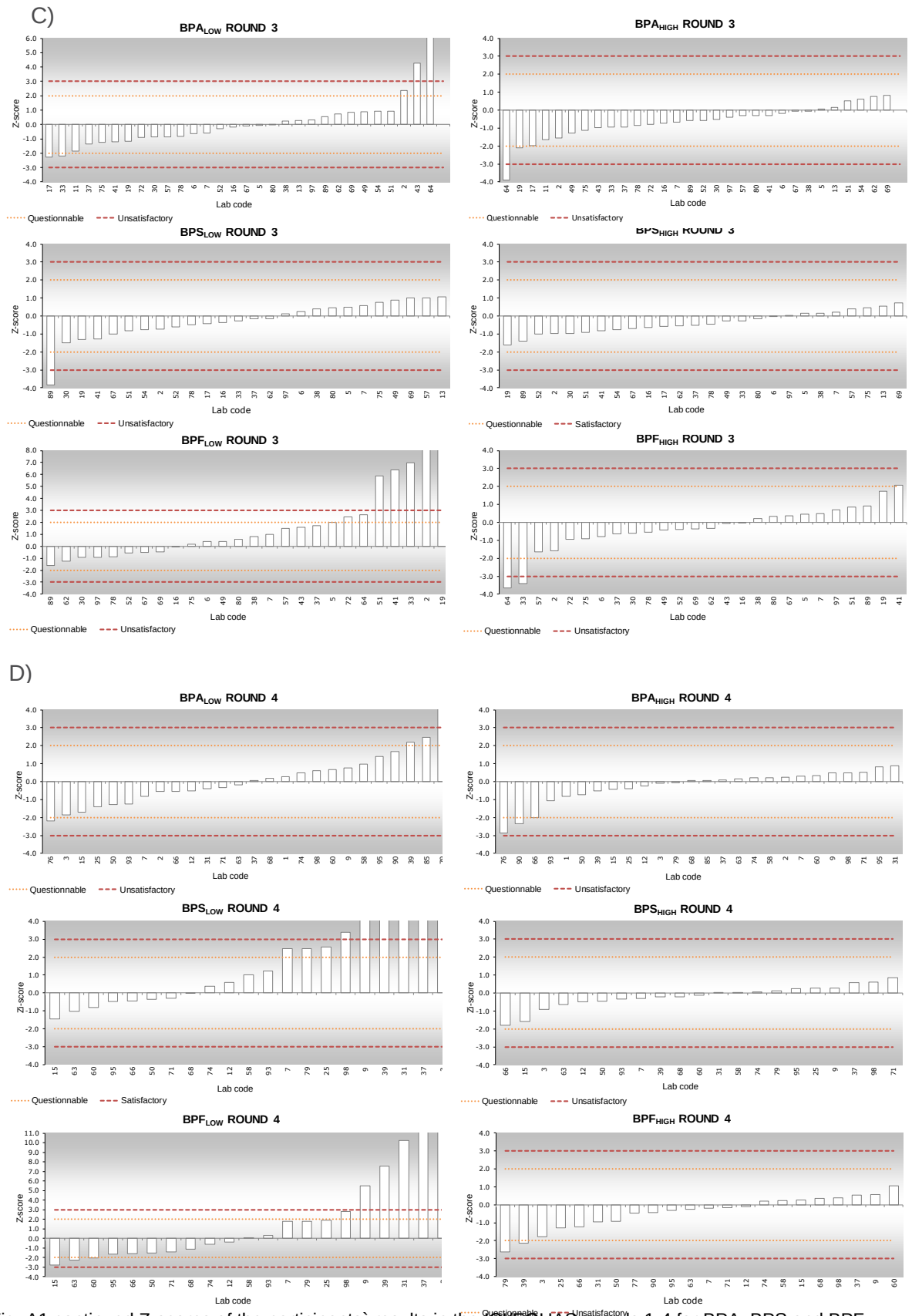

Fig. A1 continued Z-scores of the participants` results in the ICI/EQUAS rounds 1-4 for BPA, BPS and BPF

C) ROUND 3 ; D) ROUND 4

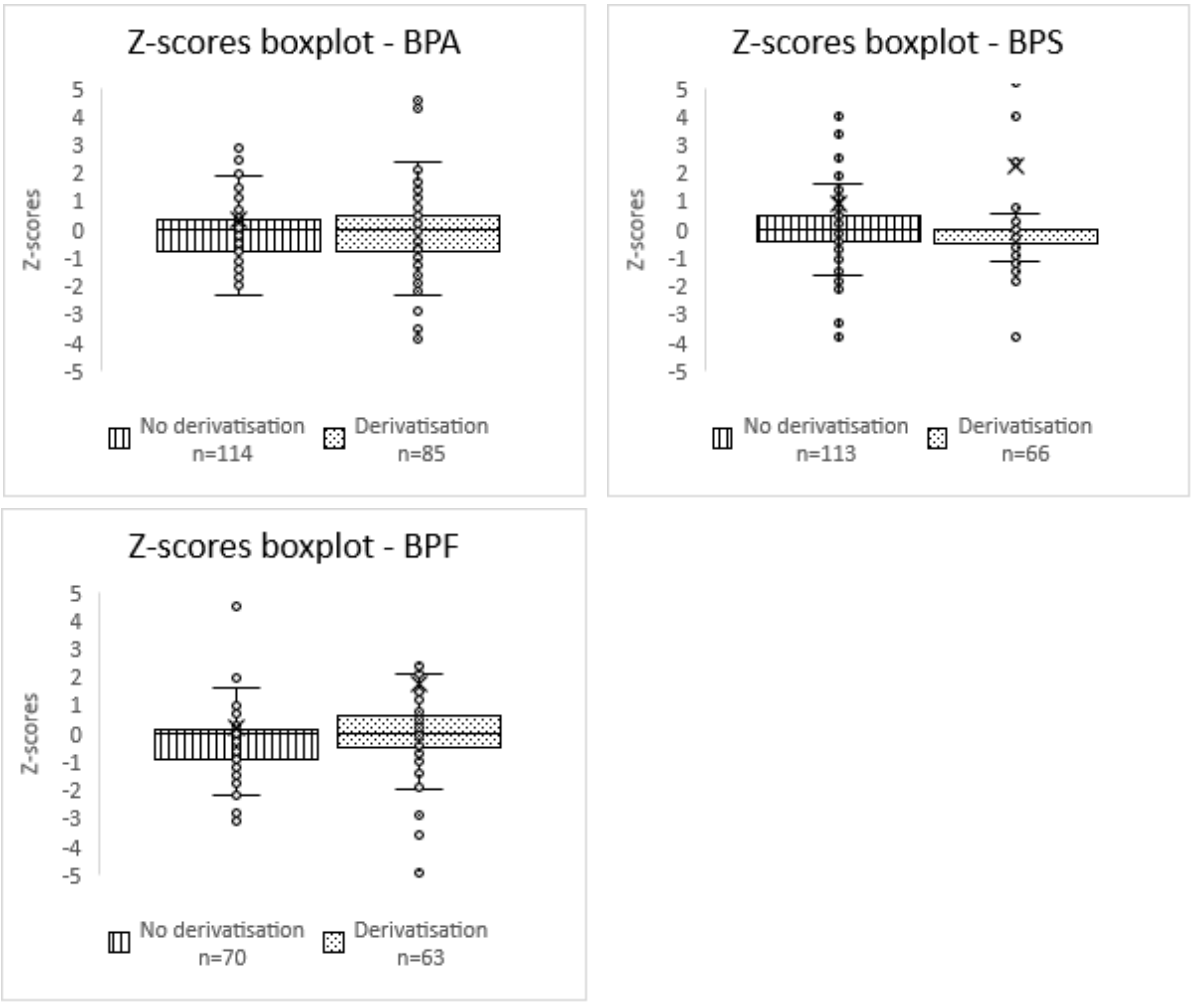

Fig. A2. Boxplots of the Z-scores obtained for BPA, BPS and BPF in round 1–4 by participants using derivatisation or no derivatisation. The box of the boxplots ranged from the 25th to the 75th percentile with the horizontal line showing the mean, the whiskers showing the 5–95 percentiles.