HBM4EU science and policy for a healthy future

HORIZON2020 Programme
Contract No. 733032 HBM4EU

REPORT OF THE WP9 interlaboratory comparison

Round 02/2020

UV filters in urine

Version / date of issue	1 /04.06.2020
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Appendices

1 Summary

Within the framework of the HBM4EU project, an interlaboratory comparison was organized and conducted for the analysis of benzophenones (BP) in urine. Benzophenones correspond to 4 biomarkers: 2,4-Dihydroxybenzophenone (BP1), 2,2',4,4'-Tetrahydroxybenzophenone (BP2), 2-Hydroxy-4-methoxybenzophenone (BP3), 5-Chloro-2-hydroxybenzophenone (BP7). However, the problems caused by COVID-19 and technical problems in one of the participating expert laboratories (UEL1) resulted in the reduction of the tested parameters to BP1 and BP3 from this 2nd round on.

The study was performed from February to June 2020 due to the impact of COVID-19.

The HBM4EU QAU had selected three expert laboratories for benzophenones in urine. All expert laboratories were from different countries in Europe.

The participation in this interlaboratory comparison for benzophenones in urine was mandatory for these laboratories.

During this 2nd round, one expert laboratory (UEL2) withdrew its participation in the interlaboratory comparisons for BP in urine due to the impact of COVID-19.

Two different test samples consisting of 5 mL urine mixed from burdened native material to obtain two different concentrations (**BP_{R2A}**, **BP_{R2B}**) were prepared and sent to the participating expert laboratories for single analysis.

Homogeneity and stability assessment of the control materials confirmed that the materials were adequately homogeneous.

Due to the reduced number of participating expert laboratories, only two results were submitted for each level of each parameter. These two results were considered comparable if the difference to the mean was ≤35% and no Z-scores were calculated.

The results of the two participating expert laboratories (UEL1 and UEL5) showed a difference from the mean of 4.6% **($BP1_{R2A}$)**, 3.8% **($BP1_{R2B}$)**, 8.4% **($BP3_{R2A}$)** and 4.0% **($BP3_{R2B}$)** and were thus in a good comparable range.

Table 1 below gives an overview of the respective number of quantitative results and the mean values for the two different levels of all UV filter biomarkers.

The final evaluation of the comparability of the respective expert laboratories can, however, only take place upon completion of all interlaboratory comparison rounds.

Table 1 Overview of results for benzophenones in urine in interlaboratory comparison/round 2

biomarker	participants	quantitative results	*mean* value	difference from the mean
BP1 R2A	**2**	**2**	***4.234 ng/mL***	**4.6%**
BP1 R2B	**2**	**2**	***1.414 ng/mL***	**3.8%**
BP3 R2A	**2**	**2**	***6.894 ng/mL***	**8.4%**
BP3 R2B	**2**	**2**	***2.424 ng/mL***	**4.0%**

2 Introduction

This interlaboratory comparison is intended to assess the comparability and reliability of analytical methods across the participating expert laboratories. Participation in this exercise forms an integral part of quality control, in addition to initial and ongoing in-house method validation.

This study has been organised within the frame of HBM4EU as part of the Quality Assurance program for biomonitoring analyses. Within HBM4EU, participation in these exercises is mandatory for laboratories that will analyse HBM4EU samples.

This report describes the 2nd round of interlaboratory comparison for benzophenones in urine and was organised by the Institute and Outpatient Clinic of Occupational, Social and Environmental Medicine (IPASUM) at Friedrich-Alexander University of Erlangen-Nuremberg.

The selection of the most relevant benzophenone biomarkers was previously made in WP9, and has been described in Deliverable report 9.5 v2.0. Based on this and upon discussion within the QAU and with proven experts in the field, a set of 4 target biomarkers for benzophenones was selected for the interlaboratory comparisons.

Due to COVID-19 and technical problems in one of the participating expert laboratories (UEL1), only BP1 and BP3 could be included in this 2nd interlaboratory comparison (see **Table 2**).

Table 2 Benzophenone biomarkers in urine included in this 2nd interlaboratory comparison

Abbreviation	Target biomarker
BP1	**2,4-Dihydroxybenzophenone**
BP3	**2-Hydroxy-4-methoxybenzophenone**

For the interlaboratory comparisons, expert laboratories were selected according to the following selection criteria described in HBM4EU-SOP-QA-005 and in agreement with the QAU.

The selection criteria included:

1. Experience in analysis of all selected parameters in (the selected) human matrices at levels expected in the general population (proven experience, papers, reports, etc.)
2. Capacity for analysis (number of samples/time for analysis)
3. Limit of quantification of the method sufficiently low for HBM4EU samples (indicate how the LOQ was determined)
4. Historical data of the successful participation in interlaboratory comparison exercises for the target substance (selected parameters)

The interlaboratory comparison assesses the comparability of analysis results for the same sample analysed by multiple expert laboratories in the same time frame. As measure of proficiency, Z-scores are calculated using the mean value derived from the experts' results as consensus value, and a pre-set target standard deviation (e.g. fit-for-purpose standard deviation). Expert laboratories are requested to apply the same procedure as they will use for analysis of samples in the frame of HBM4EU.

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 assessment.

3 Control material

3.1 Preparation of control material

Control material was prepared at IPA. For that purpose, burdened urine samples with different native concentrations of the analytes were mixed to obtain two different control materials (**BP_{R2A}, BP_{R2B}**) with intended concentrations. The two control materials were aliquoted (5 mL each for the participants into tubes with caps (120x17 mm, polypropylene, Sarstedt). The tubes were stored in a freezer (≤ -18 °C) until transportation. According to HBM4EU-SOP-QA-002, the samples were tested for homogeneity (see **Section 3.2**) and stability (see **Section 3.3**). These tests were conducted by the Chemical Laboratory at the Department of Growth and Reproduction of Rigshospitalet, Region Hovedstaden. The two different concentrations (**BP_{R2A}, BP_{R2B}**) were measured using ICP-MS (see analysis method in **Appendix 5**).

3.2 Homogeneity of control material

Ten randomly selected tubes of each concentration of the control material (BP_{R2A}, BP_{R2B}) were thawed from the freezer (≤ -18 °C), re-homogenised by vortex shaking and analysed in duplicate. The homogeneity was evaluated according to the procedure described in HBM4EU-SOP-QA-002, based on ISO 13528:2015, Fearn et al [2001] and Thompson [2000]. The results are presented in **Appendix 1** of this report. The conclusion is that no outliers are detected, the homogeneity is adequate and the method is suitable.

3.3 Stability of control material

Six randomly selected tubes of each concentration of the control material (BP_{R2A}, BP_{R2B}) were stored after preparation under conditions representative for transport and storage at the participant's laboratory (frozen, <-18°C). These samples were then thawed, re-homogenised by vortex shaking and analysed using the method shown in **Appendix 5**. Assessment of the stability was done by comparing the mean of the stored samples and the mean of the homogeneity testing. The stability was evaluated according to HBM4EU-SOP-QA-002 and using the Excel-sheet "HBM4EU ICI-EQUAS stability test CM v1". The results are presented in **Appendix 2**. No consequential instabilities and no statistical differences were detected.

4 Organisational details

4.1 Participants

For the organisation of the 2nd interlaboratory comparison, IPASUM contacted the three selected expert laboratories (all from Europe) and sent them instruction letters by e-mail on February 27, 2020 (see **Appendix 4**). It was indicated that participation would be free of charge and that participants would receive a kit containing the test materials needed for analysis. Test results had originally to be submitted within the stipulated deadline (March 30, 2020). Due to COVID-19, this deadline could not be met and was extended to the 3rd of June.

The laboratories received an individual laboratory code to report their measurement results (see **Appendix 8**).

Two of three laboratories performed the assays and submitted their results. UEL5 withdrew its participation for the 2nd and the following round.

4.2 Dispatch and instructions

Test materials were dispatched to the participants in frozen state on February 26, 2020. Each participant received two test samples with different native concentrations of the biomarkers, one of each concentration (**BP_{R2A}, BP_{R2B}**). Each sample consisted of approximately 5 mL urine.

Moreover, a letter with instructions on sample handling (instruction letter, see **Appendix 4**), a sample receipt form to be sent back to IPASUM upon receipt of the test material as well as a result submission form and a method information form were sent to the participants by e-mail. The latter form was used to extract relevant information related to the analytical method used for quantification.

Participants were asked to perform a single analysis of each sample using the same procedure as will be used for analysis of samples in the frame of HMB4EU and to report results following the instructions given.

4.3 Deviations from SOPs

For this 2nd interlaboratory comparison, the HBM4EU-QA-SOPs were followed, but the suggested timelines could not be met. Besides this, there were no deviations from the relevant SOPs.

5 Data evaluation

5.1 False positives and <LOQ

Classification of false positives and biomarkers reported as "<LOQ-value" or "not detected" (ND) was done as described in HBM4EU-SOP-QA-003.

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

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

If a biomarker is reported as "<LOQ-value", AND a consensus 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 within the frame of HBM4EU. A result is a false negative if the LOQ of a biomarker is well below the assigned value, but the laboratory did not report a quantitative value.

5.2 Consensus value (A)

The minimum number of expert laboratories required for establishment of a consensus value (A) in these interlaboratory comparisons is three.

The results obtained by the expert laboratories will be used to calculate the mean of all expert values, the respective relative standard deviation, and the relative uncertainty of the mean, which is given by:

$u = RSD / \sqrt{N}$

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

RSD = relative standard deviation of the mean concentration

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

The mean concentration derived from the expert laboratories is considered an acceptable consensus value in interlaboratory comparison studies if the relative uncertainty of the mean is ≤17.5% (= 0.7 * σ_T).

Only if u>17.5%, are the results of the expert laboratories checked for outliers. If an individual expert value is identified as an outlier, it is rejected from the data set and the relative uncertainty is calculated again when N is still ≥3. If u is still >17.5%, then no meaningful consensus expert value can be derived, and no objectively reliable quantitative comparability assessment can be done.

It is recognised that with the small number of participants it is unlikely that outliers can be identified through statistical tests.

In case there are only results from two expert labs, a mean value can be calculated using the results of these two experts.

Then the comparability of the results of the two expert laboratories is evaluated using the reproducibility limit (= 2.8* σ_T = 70%). Thus, the results are considered comparable when the difference to the mean is ≤35%. In that case, the calculation of Z-scores cannot be applied.

5.3 Target standard deviation (σ_T)

For calculation of the Z-scores, a fit-for-purpose relative target standard deviation (FFP-RSD_R) of 25% of the consensus value was used as target standard deviation.

5.4 Relative standard deviation

To gain insight into the actual inter-laboratory variability of the biomarker analysis in this study, the relative standard deviation (RSD) was calculated based on the participants' results.

5.5 Z-scores

The quantitative results from all participating expert laboratories are used to calculate a consensus value based on the participants' results (see 5.2).

This consensus value (A) is then used to calculate the Z-scores of the participants' mean results (x) using a target standard deviation (σ_T) of 25%.

The Z-score (Z) is calculated as follows:

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

Z-scores are classified as presented in **Table 3**.

Table 3 Classification of Z-scores

$\lvert Z \rvert \leq 2$	satisfactory
$2 < \lvert Z \rvert < 3$	questionable
$\lvert Z \rvert \geq 3$	unsatisfactory

When the Z-score is within -2 and +2 (-2 ≤ Z≤ 2), the results are considered sufficiently comparable.

6 Results and discussion

6.1 Results submitted by participants

In total, two laboratories from three European countries participated as experts in this study. Both experts submitted their results and UEL 1 declared that it will no longer be able to submit results for BP2 and BP7. Consequently, only the results for BP1 and BP 3 were included in this report.

Appendix 8 gives an overview of results and LOQs submitted by the participants.

Methods: The method details provided by the laboratories are included in **Appendix 9**.

For the determination of BP, all laboratories used methods involving enzymatic deconjugation. The volume of urine used for the analysis varied from 0.100 to 0.300 mL. For deconjugation, both laboratories used ß-glucuronidase/arylsulfatase, after adjustment of the pH to a certain value (5.0 to 5.5). Deconjugation was performed at 37 °C or 38 °C for 1.5 to 4.0 h. In all cases, a clean-up step was performed. Both laboratories used online SPE. All extracts were analysed by triple quadrupole mass spectrometry and by using internal standards added before deconjugation. All laboratories used an isotope dilution (addition to sample before extraction) for calibration. The retention time tolerance varies between 0.2 and 0.3 min.

6.2 Consensus values and (target) standard deviations

The mean value and the difference from the mean for each of the control materials are included in **Appendix 6**.

6.3 Assessment of laboratory performance

A summary of the number of quantitative results and the respective mean values is given in **Table 1**.

For **BP1 and BP3**, no Z-scores could be provided, because the number of quantitative results was too low. Thus, no objective and reliable quantitative comparability assessment could be made for these parameters.

For **BP1** and **BP3**, two quantitative results (UEL1 and UEL5) were reported. These results showed a difference from the mean of 4.6% **($BP1_{R2A}$)**, 3.8% **($BP1_{R2B}$)**, 8.4% **($BP3_{R2A}$)** and 4.0% **($BP3_{R2B}$)** and were thus in a good comparable range.

6.4 Conclusions and recommendations

The participation in the 2nd HBM4EU interlaboratory comparison for benzophenones was successful. Both expert laboratories reported results.

The evaluation of laboratory performance and comparability using derived consensus values and calculated Z-scores was not possible, because there were only two quantitative expert results.

All in all, both participating expert laboratories were in a good comparable range for **BP1** and **BP3**.

The final evaluation of the comparability of the respective expert laboratories can, however, only take place upon completion of all interlaboratory comparison rounds.

7 References

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

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

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

[4] HBM4EU-SOP-QA-002 “Preparation of test materials for ICI / EQUAS”

[5] HBM4EU-SOP-QA-003 “Evaluation of ICI / EQUAS results”

[6] HBM4EU-SOP-QA-004 “Reporting of ICI / EQUAS studies”

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

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

[9] 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.

[10] Thompson, M., 2000, Recent trends in inter-laboratory precision at ppb and sub-ppb concentrations in relation to fitness for purpose criteria in proficiency testing, Analyst, 125, 385-386.

[11] Thompson M., Ellison R. and Wood, R., 2006, The International Harmonized Protocol for the Proficiency Testing of Analytical Chemistry Laboratories, Pure Appl. Chem, 78(1), 145-196.

Appendix 1 Homogeneity data

	BP1				BP3			
	R2A [ng/mL]		R2B [ng/mL]		R2A [ng/mL]		R2B [ng/mL]	
	replicate 1	replicate 2	replicate 1	replicate 2	replicate 1	replicate 2	replicate 1	replicate 2
1	1,536	1,436	4,518	4,663	2.339	2.368	6.612	6.200
2	1,591	1,548	4,936	5,161	2.303	2.231	6.685	6.577
3	1,548	1,474	4,773	4,811	2.215	2.235	6.362	6.362
4	1,277	1,388	4,542	4,596	2.430	2.235	6.375	6.146
5	1,520	1,483	4,625	4,335	2.297	2.433	6.422	6.436
6	1,575	1,483	4,254	4,763	2.328	2.269	6.767	6.895
7	1,343	1,356	4,654	4,386	2.191	2.300	6.649	6.191
8	1,386	1,340	4,673	4,522	2.176	2.352	6.530	6.561
9	1,384	1,466	4,621	4,477	2.070	2.270	6.935	6.619
10	1,541	1,363	4,545	4,502	2.276	2.079	6.508	6.567
grand mean	1,452		4,618		2,270		6,520	
Cochran`s test								
C	0,396		0,483		0,213		0,372	
Ccrit	0,602		0,602		0,602		0,602	
C < Ccrit?	no outliers detected		no outliers detected		no outliers detected		no outliers detected	
target σ_{FFP}:	0,621		1,660		0,908		2,225	
s_x	0,082		0,174		0,068		0,182	
s_w	0,063		0,164		0,097		0,168	
s_s	0,069		0,130		0,000		0,139	
Critical=0.3 σ_{FFP}	0,109		0,346		0,170		0,489	
s_s < critical?	Homogeneity adequate		Homogeneity adequate		Homogeneity adequate		Homogeneity adequate	
s_w< 0.5*σ_{FFP}?	Method suited		Method suited		Method suited		Method suited	

Appendix 2 Stability data

	BP1				BP3			
	R2A [ng/mL]		R2B [ng/mL]		R2A [ng/mL]		R2B [ng/mL]	
	-80°C	-18°C	-80°C	-18°C	-80°C	-18°C	-80°C	-18°C
1	4.522	4.500	1.340	1.401	6.561	5.490	2.352	2.650
2	4.444	4.219	1.328	1.451	6.161	6.377	2.222	2.309
3	4.477	4.472	1.466	1.465	6.619	6.865	2.270	2.365
4	4.780	4.282	1.563	1.479	6.422	6.696	2.180	2.538
5	4.595	4.568	1.400	1.510	6.413	6.594	2.235	2.133
6	4.502	4.521	1.363	1.506	6.567	5.888	2.079	1.973
average	4.553	4.427	1.410	1.468	6.457	6.318	2.223	2.328
stdev	0.122	0.141	0.090	0.040	0.167	0.528	0.091	0.251
difference	0.126		-0.058		0.139		-0.105	
critical=0.3 σ_{FFP}	0.342		0.106		0.484		0.167	
consequential instability	no		no		no		no	
t	1.658		1.453		0.616		0.962	
tcrit	2.228		2.228		2.228		2.228	
Significant difference	no		no		yes		yes	

Appendix 3 Copy of announcement letter

HBM4EU: Announcement to participate in three rounds of interlaboratory comparisons for <u>UV FILTER biomarkers</u> as an expert laboratory

Title: UV filter biomarkers in urine

Dear Colleagues,

within the frame of HBM4EU the

Institute and Outpatient Clinic of Occupational, Social and Environmental Medicine (IPASUM), Friedrich-Alexander University Erlangen-Nuremberg

in collaboration with

Institute for Prevention and Occupational Medicine of the German Social Accident Insurance - Institute of the Ruhr-Universität-Bochum (IPA)

announces 3 rounds of interlaboratory comparisons for the determination of **UV filters in urine**. The aim of these exercises is to provide laboratories with an assessment of their analytical performance and reliability of their data in comparison with other expert laboratories. This will aid in the quality improvement of analysis in human biomonitoring at each of the laboratories.

IPASUM will be the coordinator and organiser of these interlaboratory comparisons, will perform the data evaluation and the reporting.

Urine samples to be analysed for the UV-filter biomarkers will be prepared by IPA and sent directly from IPA to the participates.

Test samples
The matrix will be urine. Accordingly, the participants will receive **in each round**:

- 2 different materials of urine (**2 samples of 5 mL each**) for determination of UV filters in urine

Target biomarkers
Please analyse all of the following target biomarkers in both samples:

- **2,4-Dihydroxybenzophenone (BP1)**
- **2,2',4,4'-Tetrahydroxybenzophenone (BP2)**
- **2-Hydroxy-4-methoxybenzophenone (BP3)**
- **5-Chloro-2-hydroxybenzophenone (BP7)**

LOQs should allow the analysis of benzophenones in samples of the general population.
Please try to reach the LOQ requirements as follows:

BP1, BP2, BP3 and BP7:	0.2 µg/L or lower

Calendar: projected dates

Distribution of test samples for round 1	03-02-2020
Deadline for submission of results for round 1	18-02-2020
Report for round 1	21-02-2020
Distribution of test samples for round 2	26-02-2020
Deadline for submission of results for round 2	16-03-2020
Report for round 2	20-03-2020
Distribution of test samples for round 3	16-03-2020
Deadline for submission of results for round 3	03-04-2020
Report for round 3	09-04-2020
Letters of approval and certificates sent to participants	21-04-2020

Fee
For partners and linked-third parties of HBM4EU, participation is free of charge. Please note that the participants are responsible for custom clearance and associated costs if applicable and that they will not be reimbursed.

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:

Thomas Göen, Stefanie Nübler, Karin H. A. Zarrabi

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Contact information IPA:

Holger M. Koch, Rebecca Moos, Daniel Bury

Institute for Prevention and Occupational Medicine of the German Social Accident Insurance - Institute of the Ruhr-Universität-Bochum (IPA))

Bürkle-de-la-Camp-Platz 1
44789 Bochum

Germany

Email: wp9@ipa-dguv.de

Phone: 0049 234 302 4647

Please complete the following sheet and send it back to ipasum-hbm4eu@fau.de:

Participating laboratory:

name of the institution

address of the laboratory

name of 1st contact person, telephone number and email address

name of 2nd contact person, telephone number and email address

Address for delivery of the test samples:

name of (the contact person and) the institution

address of the laboratory

The above laboratory will participate in the interlaboratory comparisons for benzophenones in urine**.**
I agree with the conditions mentioned in this letter, and that the laboratory will analyse the samples using the same procedure as will be used for analysis of samples in the frame of HBM4EU, and submit results before the indicated deadlines.

Name: Signature:

Date:

Appendix 4 Copy of letter of instructions sent together with test samples

HBM4EU: Instruction letter interlaboratory comparison UV-filters in urine/Round 2

Dear participant,

Thank you for participation in the HBM4EU interlaboratory comparison for the determination of **UV-filters in urine**.

You will receive a parcel containing **2 test samples** spiked with the biomarkers. Each sample consists of approximately **5 mL urine**.
The parcel will be shipped on February 26, 2020 under frozen conditions.

Instructions:

- Upon receipt, please check the content for any damage/leakage and for the frozen condition of the containers, **complete the sample receipt form and return it to the organiser as soon as possible**.

- Store the test samples under frozen (-18°C) conditions until analysis.

- Analyse both samples for the biomarkers:

  - **2,4-Dihydroxybenzophenone (BP1)**
  - **2,2',4,4'-Tetrahydroxybenzophenone (BP2)**
  - **2-Hydroxy-4-methoxybenzophenone (BP3)**
  - **5-Chloro-2-hydroxybenzophenone (BP7)**

- Thaw the samples and re-homogenise them according to your own procedure.

- Analyse 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**.

- The deadline for submission of analysis results and method details is March 30, 2020

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

Stefanie Nübler, Karin Zarrabi

Email: ipasum-hbm4eu@fau.de; Tel.: + 49 (0)9131/8526145, /8526146

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Friedrich-Alexander University Erlangen-Nuremberg
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91054 Erlangen, Germany

Prof. Dr. Thomas Göen (for the organisers)

Appendix 5 HBM4EU: Method information form for participation in interlaboratory comparison UV-filters in urine

Laboratory code		
ISO17025 accredited	no	
SAMPLE PREPARATION		
amount sample extracted	0.100	ml
Deconjugation	yes	
- chemical	NH4Ac/pH 5.5/37°C/90 min	
- enzymatic	ß-glucoronidase/ acrylsulfatase	
Extraction		
- pH adjustment		
- LLE;	solvent(s) / time / shaking	
- SPE; material	Material	
Clean-up		
- LLE; solvent(s)		
- SPE; material	ON-LINE TURBO FLOW purification, Loading column: P-Cyclone column (0.5 x 50mm) with mobile phase A: 10mM NH4Ac (pH 9) and B: MeOH added 0.1%HCOOH	
Derivatisation		
- reagent	No	
INSTRUMENTAL ANALYSIS		
HPLC		
- injection volume	40	µl
- column stationary phase	Hypersil Gold aQ	
- column L (mm) x ID (mm); dp (µm)	4 x 50mm, 3µm particle size	
- temperature	30	°C
- mobile phase A	milli Q H_2O	
- mobile phase B	MeOH	
- flow rate	0.7	ml/min
GC		
- injector		
- injection volume		
- column stationary phase		
- column L (m) x ID (mm) df (µm)		
- carrier		
- flow rate / inlet pressure		
Detection		
MS	triple quad	
other		

Quantification		
Use of internal standard (IS)	yes	
- isotopic label	yes	
- other		
- moment of addition	before deconjugation	
- response normalised to IS	no	
Calibration	isotope dilution (addition to sample before extraction)	
	multi level	
Correction for recovery	no	
Identification criteria used		
- retention time tolerance	0.2 min	
- number of ions/transitions	1-2	
- ion ratio tolerance	% relative/absolute deviation from reference standard	

Further remarks/observations:

Further method specifications are described in

Frederiksen H, Nielsen O, Skakkebaek NE, Juul A, Andersson AM. UV filters analyzed by isotope diluted TurboFlow-LC-MS/MS in urine from Danish children and adolescents. *Int J Hyg Environ Health*. 2017;220(2 Pt A):244-253. doi:10.1016/j.ijheh.2016.08.005, PMID: **27637469**

Date:

Signature:

Appendix 6 Consensus values and participant's performance

HBM4EU 02/2020	BP1 (urine)	
control material	**$BP1_{R2A}$**	**$BP1_{R2B}$**
mean value from two experts	***4.234 ng/mL***	***1.414 ng/mL***
expert standard deviation	***0.274 ng/mL***	***0.076 ng/mL***
study RSD_R	***6.5%***	***5.4%***
difference from the mean value	***4.6%***	***3.8%***
laboratory code	**value**	**value**
UEL1	4.040	1.360
UEL5	4.427	1.468

Appendix 6 Consensus values and participant's performance (continued)

HBM4EU 02/2020	BP3 (urine)	
control material	**$BP3_{R2A}$**	**$BP3_{R2B}$**
mean value from two experts	***6.894 ng/mL***	***2.424 ng/mL***
expert standard deviation	***0.815 ng/mL***	***0.136 ng/mL***
study RSD	***11.8%***	***5.6%***
difference from the mean value	***8.4%***	***4.0%***
laboratory code	**value**	**value**
UEL1	7.470	2.520
UEL5	6.318	2.328

Appendix 8 Results and LOQs and reasons for delayed submission

HBM4EU 2/2020 BP1 in urine [ng/mL]			
Lab.code	**R2A**	**R2B**	**LOQ**
UEL1	4.040	1.360	0.200
UEL5	4.427	1.468	0.010

HBM4EU 2/2020 BP3 in urine [ng/mL]			
Lab.code	**R2A**	**R2B**	**LOQ**
UEL1	7.470	2.520	0.200
UEL5	6.318	2.328	0.030

ND = not detected

NA* = not analysed due to methodical problem

Appendix 9: Method details for determination of benzophenones in urine, provided by the laboratories

Lab.code	Pretreatment					
	amount sample extracted	deconjugation	pH adjustment	time (h) / temp (°C)	extraction / clean-up	derivatisation
UEL1	0.300 mL	beta-glucuronidase / aryl sulfatase	5.0	4.0 h / 37°C	online SPE	no
UEL5	0.100 mL	beta-glucuronidase / aryl sulfatase / NH4AC	5.5	1.5 h / 37°C	online SPE	no

Lab.code	Instrumental analysis			
	separation	injection volume (µL)	column	detection
UEL1	HPLC	10	3.0 mm x 150 mm; 2.6 µm	triple quad
UEL5	HPLC	40	4.0 mm x 50 mm; 3.0 µm	triple quad

Lab.code	Quantification			Criteria used for identification		
	use of internal standard	moment of addition	calibration	retention time tolerance	number of ions/transitions	ion ratio tolerance
UEL1	yes	before enzymatic deconjugation	isotope dilution (addition to sample before extraction)	0.3 min	2 per analyte	30% relative deviation from reference standard
UEL5	yes	before deconjugation	isotope dilution (addition to sample before extraction)	0.2 min	1-2	/