HBM4EU science and policy for a healthy future

HORIZON2020 Programme
Contract No. 733032 HBM4EU

REPORT OF THE WP9 interlaboratory comparison

Round 01/2020

UV filters in urine

Version / date of issue	1 /27.02.2020
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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).

The study was performed from January to February 2020.

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

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

Two different test samples consisting of 5 mL urine mixed from burdened native material to obtain two different concentrations (**BP_{R1A}**, **BP_{R1B}**) were prepared and sent to the participating expert laboratories for single analysis.

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

Due to technical problems, stability of the control material could not be assessed in time. As it is essential that stability testing be conducted in the same laboratory as homogeneity testing, for this 1st round, no stability could be evaluated. For the next rounds, however, stability will be tested and provided in the respective reports.

Consensus values were calculated as the arithmetic mean of the values obtained by the expert labs, provided that the relative uncertainty of the mean was within 17.5%.
In order to express the proficiency of the laboratories in a numerical way, Z-scores were calculated using the consensus value and a fixed fit-for-purpose relative target standard deviation (FFP-RSD_R) of 25%.

For **$BP7_{R1A}$ and $BP7_{R1B}$**, consensus values could be calculated from the results of all three experts, the obtained Z-scores were all satisfactory and the relative standard deviation (RSD) was in a range from 7.5% (**$BP7_{R1A}$**) to 25.1% (**$BP7_{R1B}$**).

In case there were only two results for a parameter, the two results were considered comparable if the difference to the mean was ≤35%. Then no Z-scores were calculated.

For **$BP1_{R1B}$**, **$BP2_{R1A}$** and **$BP2_{R1B}$**, no Z-scores could be provided, because the relative uncertainty (u) of the mean concentration from three expert labs was too high and no statistical outlier could be detected. However, the results of two expert laboratories (UEL1 and UEL5) showed a difference from the mean of 1% (**$BP1_{R1B}$**), 34% (**$BP2_{R1A}$**) and 40% (**$BP2_{R1B}$**) and were thus in a good or medium comparable range.

For **$BP1_{R1A}$, $BP3_{R1A}$** and **$BP3_{R1B}$**, only two quantitative results were reported (UEL1 and UEL5). These results showed a difference from the mean of 10% (**$BP1_{R1A}$**), 11% (**$BP3_{R1A}$**) and 2% (**$BP3_{R1B}$**) and were thus in a good comparable range.

Table 1 below gives an overview of the respective number of quantitative results and the consensus/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 1

biomarker	participants	quantitative results	Consensus/*mean* value	satisfactory	questionable	unsatisfactory
BP1 R1A	**3**	**2**	***0.910 ng/mL***	**no**	**no**	**no**
BP1 R1B	**3**	**3**	***2.056 ng/mL***	**no**	**no**	**no**
BP2 R1A	**3**	**3**	***1.247 ng/mL***	**no**	**no**	**no**
BP2 R1B	**3**	**3**	***3.562 ng/mL***	**no**	**no**	**no**
BP3 R1A	**3**	**2**	***1.510 ng/mL***	**no**	**no**	**no**
BP3 R1B	**3**	**2**	***4.470 ng/mL***	**no**	**no**	**no**
BP7 R1A	**3**	**3**	**1.807 ng/mL**	**3 (100%)**	**0**	**0**
BP7 R1B	**3**	**3**	**5.209 ng/mL**	**3 (100%)**	**0**	**0**

no = no Z-score available

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 1st 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 included in this 1st interlaboratory comparison (see **Table 2**).

Table 2 Benzophenone biomarkers in urine included in this 1st interlaboratory comparison

Abbreviation	Target biomarker
BP1	**2,4-Dihydroxybenzophenone**
BP2	**2,2',4,4'-Tetrahydroxybenzophenone**
BP3	**2-Hydroxy-4-methoxybenzophenone**
BP7	**5-Chloro-2-hydroxybenzophenone**

For this 1st interlaboratory comparison, 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 and tested at IPA. For that purpose, burdened urine samples with different native concentrations of the analytes were mixed to obtain two different control materials (**BP_{R1A}, BP_{R1B}**) with intended concentrations. The two control materials were aliquoted (5 mL each for the participants and 1.5 mL each for homogeneity and stability testing) 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**). In brief, after thawing and homogenisation, an aliquot of urine was mixed with the internal standard and subjected to enzymatic deconjugation of phase II metabolites using β-glucuronidase/aryl sulfatase. The prepared samples were analysed by HPLC-MS/MS coupled to online-SPE for analyte enrichment and matrix depletion. BP2 was only analysed semi-quantitatively, due to the lack of an appropriate (authentic) labelled internal standard. This did not affect homogeneity testing. The measured concentrations are shown in **Appendix 1** of this report.

3.2 Homogeneity of control material

Out of the 16 tubes per concentration for homogeneity and stability testing, ten tubes of each concentration of the control material (BP_{R1A}, BP_{R1B}) were randomly selected from the freezer (≤ -18 °C). The thawed samples were 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**. The conclusion is that no outliers are detected, the homogeneity is adequate and the method is suitable. Homogeneity was tested in 1.5 mL aliquots. According to HBM4EU-SOP-QA-002, homogeneity can be expected for this and higher volumes and can thus be expected also for the 5 mL aliquots.

3.3 Stability of control material

Due to technical problems, stability of the control material could not be assessed in time. As it is essential that stability testing be conducted in the same laboratory as homogeneity testing, for this 1st round, no stability could be evaluated. For the next rounds, however, stability will be tested and provided in the respective reports.

4 Organisational details

4.1 Participants

For the organisation of the 1st interlaboratory comparison, IPASUM contacted the three selected expert laboratories (all from Europe) and sent them announcement letters by e-mail on January 20, 2020 (see **Appendix 3**). 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 to be submitted within the stipulated deadline (February 18, 2020).

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

All laboratories performed the assays and submitted their results. One participant reported their results within the stipulated deadline (February 18, 2020), while two participants reported with a delay (UEL5 on February 19, 2020 and UEL2 on February 20, 2020; see **Appendix 8**).

4.2 Dispatch and instructions

Test materials were dispatched to the participants in frozen state on February 3, 2020. Each participant received two test samples with different native concentrations of the biomarkers, one of each concentration (**BP_{R1A}, BP_{R1B}**). 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 1st interlaboratory comparison, the HBM4EU-QA-SOPs were followed. 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% ($= 0.7 * \sigma_T$).

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 objective 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 ($= 2.8* \sigma_T = 70\%$). 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, three laboratories from three European countries participated as experts in this study. All experts submitted their results, but one laboratory (UEL2) could not detect BP-3 for technical reasons.

Appendix 8 gives an overview of results and LOQs submitted by the participants as well as reasons for delayed submission.

Results indicated as `not detected´ (ND, see **Appendix 8)**:

For **BP1$_{R1A}$**, one participant (UEL2) indicated ND. The number of reported results for this biomarker was < 3 and no consensus value and no Z-scores could be calculated.

False positive results: No participant detected a false positive result.

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 2.000 mL. For deconjugation, one laboratory used ß-glucuronidase and two 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. Two laboratories used online SPE, while one laboratory used LLE. 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 0.05 and 0.3 min.

6.2 Consensus values and (target) standard deviations

The consensus or mean value and its uncertainty, the relative standard deviation as derived from the participant's data, and the fit-for-purpose (FFP) target standard deviation (25%) 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 consensus/mean values is given in **Table 1**.

For BP7, consensus values could be derived for **BP7$_{R1A}$** and **BP1$_{R1B}$** and all three experts obtained satisfactory Z-scores.

For **BP1, BP2 and BP3** no Z-scores could be provided, because the number of quantitative results was too low (**BP1$_{R1A}$**, **BP3**) or the relative uncertainty (u) of the mean concentration from three expert labs was too high (**BP1$_{R1B}$, BP2)**. Thus, no objective and reliable quantitative comparability assessment could be made for these parameters.

For **BP1$_{R1A}$** and **BP3**, only two quantitative results (UEL1 and UEL5) were reported. These results showed a difference from the mean of 10% **(BP1$_{R1A}$)**, 11% **(BP3$_{R1A}$)** and 2% **(BP3$_{R1B}$)** and were thus in a good comparable range.

For **BP1$_{R1B}$** and **BP2**, the results of two expert laboratories (UEL1 and UEL5) were in a comparable range with a difference from the mean of 1% (**BP1$_{R1B}$**), of 34% (**BP2$_{R1A}$)**. The comparability of UEL1

and UEL2 as well as of UEL2 and UEL5 for **$BP1_{R1B}$** and for **$BP2_{R1A}$** was also calculated, but was above the reproducibility limit of ±35% (see 5.2).

$BP2_{R1B}$, the difference from the mean was above the reproducibility limit of ±35% for all different combinations of two expert laboratories. The lowest value was obtained by the comparison of UEL1 and UEL5 (difference form the mean of 40%).

6.4 Conclusions and recommendations

The participation in the 1st HBM4EU interlaboratory comparison for benzophenones was successful. All three expert laboratories reported results, representing a participation rate of 100%.

However, the LOQ requirements were not fully met. UEL2 should try to lower their LOQ for **BP1** and **BP7**.

The evaluation of laboratory performance and comparability using derived consensus values and calculated Z-scores was only possible for **BP7**, where all three experts obtained satisfactory Z-scores.

For **BP1, BP2 and BP3,** no Z-scores could be calculated**,** because there were only two quantitative expert results or the relative uncertainty (u) of the mean concentration from three expert labs was too high.

All in all, the three participating expert laboratories were in a comparable range for **BP7. For BP1, $BP2_{R1A}$** and **BP3**, the results of two expert laboratories (UEL1 and UEL5) showed a good comparability.

For **$BP2_{R1B}$**, the comparability was best between UEL1 and UEL5, although it was above a reproducibility limit of 35%.

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				BP2			
	R1A [ng/mL]		R1B [ng/mL]		R1A [ng/mL]		R1B [ng/mL]	
	replicate 1	replicate 2	replicate 1	replicate 2	replicate 1	replicate 2	replicate 1	replicate 2
1	1.050	1.060	2.980	2.850	1.090	1.110	2.790	2.980
2	0.984	1.020	2.960	2.940	1.110	1.130	2.860	2.900
3	1.020	0.978	2.900	2.810	1.110	1.190	2.780	2.760
4	0.953	1.040	2.840	2.840	1.100	1.080	2.840	2.920
5	0.992	0.952	2.810	2.870	1.150	1.120	2.800	2.850
6	0.928	1.000	2.690	2.810	1.100	1.140	2.860	2.900
7	1.010	0.957	2.860	2.830	1.130	1.090	2.850	2.830
8	0.977	0.990	2.870	2.890	1.120	1.110	2.940	2.850
9	1.000	1.000	2.750	2.810	1.190	1.130	2.890	3.170
10	0.971	1.040	2.760	2.790	1.280	1.130	2.880	2.870
grand mean	1.000		2.840		1.130		2.880	
Cochran`s test								
C	0.300		0.343		0.594		0.578	
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}:	0.250		0.710		0.280		0.720	
s_x	0.020		0.060		0.030		0.070	
s_w	0.040		0.050		0.040		0.080	
s_s	0.000		0.050		0.010		0.030	
Critical=0.3 σ_{FFP}	0.070		0.210		0.080		0.220	
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 1 Homogeneity data (continued)

	BP3				BP7			
	R1A [ng/mL]		R1B [ng/mL]		R1A [ng/mL]		R1B [ng/mL]	
	replicate 1	replicate 2	replicate 1	replicate 2	replicate 1	replicate 2	replicate 1	replicate 2
1	1.760	1.740	4.830	4.650	1.910	2.030	5.560	5.640
2	1.620	1.740	4.610	4.640	1.910	1.970	5.900	5.560
3	1.660	1.710	4.600	4.630	1.910	1.980	5.600	5.490
4	1.680	1.790	4.860	4.620	1.920	2.010	5.670	5.740
5	1.750	1.640	4.360	4.430	2.020	1.960	6.070	5.860
6	1.680	1.630	4.680	4.300	1.910	1.930	6.380	5.760
7	1.580	1.640	4.420	4.550	1.900	1.970	5.830	5.750
8	1.640	1.590	4.700	4.340	1.940	2.050	5.980	6.070
9	1.620	1.530	4.570	4.570	1.880	1.960	5.890	5.990
10	1.650	1.650	4.700	4.480	2.000	2.010	5.950	5.740
grand mean	1.670		4.580		1.960		5.820	
Cochran`s test								
C	0.247		0.331		0.246		0.604	
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}:	0.420		1.140		0.490		1.460	
s_x	0.060		0.110		0.030		0.180	
s_w	0.050		0.150		0.050		0.180	
s_s	0.040		0.040		0.000		0.130	
Critical=0.3 σ_{FFP}	0.120		0.340		0.150		0.440	
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 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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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 1

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 03, 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 February 18, 2019

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

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Prof. Dr. Thomas Göen (for the organisers)

Appendix 5 HBM4EU: Method information form for participation in interlaboratory comparison UV-filters in urine/Round 1-3

Laboratory code		
ISO17025 accredited	yes/no	
SAMPLE PREPARATION		
amount sample extracted		g or ml
Deconjugation	No / yes	
- chemical	Reagent / pH/ temp / time	
- enzymatic	Enzyme / pH / temp / time	
Extraction		
- pH adjustment		
- LLE;	solvent(s) / time / shaking	
- SPE; material	Material	
Cleanup		
- LLE; solvent(s)		
- SPE; material		
Derivatisation		
- reagent		
INSTRUMENTAL ANALYSIS		
HPLC		
- injection volume		µl
- column stationary phase		
- column L (mm) x ID (mm); dp (µm)		
- temperature		
- mobile phase A		
- mobile phase B		
- flow rate		ml/min
GC		
- injector	splitless/PTV/....	
- injection volume		
- column stationary phase		
- column L (m) x ID (mm) df (µm)		
- carrier		
- flow rate / inlet pressure		
Detection		
MS	single quad/triple quad/Q-Orbitrap/Q-TOF	
other		
Quantification		
Use of internal standard (IS)	yes/no	

- isotopic label	yes/no	
- other	specify	
- moment of addition	e.g. before deconjugation, to final extract..	
- response normalised to IS	yes/no	
Calibration	isotope dilution (addition to sample before extraction) isotope dilution (addition to final extract) standard addition (addition to sample before extraction) standard addition (addition to final extract) matrix-matched (addition to blank matrix before extraction) matrix-matched (addition to blank extract) solvent standards	
	single level / multi level	
Correction for recovery	Yes / no	
Identification criteria used		
- retention time tolerance	min or % deviation from reference standard	
- number of ions/transitions		
- ion ratio tolerance	% relative/absolute deviation from reference standard	

Further remarks/observations:

Date:

Signature:

Appendix 6 Consensus values and participant's performance

HBM4EU 01/2020	BP1 (urine)			
control material	$BP1_{R1A}$		$BP1_{R1B}$	
mean value from two experts (R1A), from three experts (R1B)	*0.910 ng/mL*		*2.056 ng/mL*	
expert standard deviation	*0.127 ng/mL*		*1.289 ng/mL*	
uncertainty of assigned value (u)	na		36.2%	
study RSD	*14.0%*		*62.7%*	
difference of UEL1 and UEL5 from the mean value (2.800 ng/mL for R1B)	*10%*		*1%*	
laboratory code	value	Z-score	value	Z-score
UEL1	1.000	no	2.840	no
UEL2	ND	no	0.568	no
UEL5	0.820	no	2.760	no

na: not applicable; ND= not detected; no: no Z-score available

HBM4EU 01/2020	BP2 (urine)			
control material	$BP2_{R1A}$		$BP2_{R1B}$	
mean value from three experts	1.247 ng/mL		3.562 ng/mL	
expert standard deviation	0.989 ng/mL		2.888 ng/mL	
uncertainty of assigned value (u)	45.8%		46.8%	
study RSD	79.3%		81.1%	
difference of UEL1 and UEL5 from the mean value (1.710 ng/mL)	*34%*		*40%*	
laboratory code	value	Z-score	value	Z-score
UEL1	1.130	no	2.880	no
UEL2	0.322	no	1.075	no
UEL5	2.290	no	6.730	no

no: no Z-score available

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

HBM4EU 01/2020	BP3 (urine)			
control material	$BP3_{R1A}$		$BP3_{R1B}$	
mean value from two experts	*1.510 ng/mL*		*4.470 ng/mL*	
expert standard deviation	*0.226 ng/mL*		*0.156 ng/mL*	
uncertainty of assigned value (u)	na		na	
study RSD	*15%*		*3.5%*	
difference of UEL1 and UEL5 from the mean value	*11%*		*2%*	
laboratory code	value	Z-score	value	Z-score
UEL1	1.670	no	4.580	no
UEL2	NA*	no	NA*	no
UEL5	1.350	no	4.360	no

NA* = not analysed due to methodical problems; na: not applicable; no: no Z-score available

HBM4EU 01/2020	BP7 (urine)			
control material	$BP7_{R1A}$		$BP7_{R1B}$	
consensus value from three experts	1.807 ng/mL		5.209 ng/mL	
expert standard deviation	0.136 ng/mL		1.308 ng/mL	
uncertainty of assigned value (u)	4.3%		14.5%	
study RSD	7.5%		25.1%	
laboratory code	value	Z-score	value	Z-score
UEL1	1.960	0.338	5.820	0.469
UEL2	1.762	-0.100	3.708	-1.153
UEL5	1.700	-0.238	6.100	0.684

Appendix 8 Results and LOQs and reasons for delayed submission

HBM4EU 1/2020 BP1 in urine [ng/mL]				
Lab.code	**R1A**	**R1B**	**LOQ**	**delayed reporting**
UEL1	1.000	2.840	0.200	
UEL2	ND	0.568	0.500	shipment problems
UEL5	0.820	2.760	0.010	no reason

HBM4EU 1/2020 BP2 in urine [ng/mL]				
Lab.code	**R1A**	**R1B**	**LOQ**	**delayed reporting**
UEL1	1.130	2.880	0.200	
UEL2	0.322	1.075	0.200	shipment problems
UEL5	2.290	6.730	0.080	no reason

HBM4EU 1/2020 BP3 in urine [ng/mL]				
Lab.code	**R1A**	**R1B**	**LOQ**	**delayed reporting**
UEL1	1.670	4.580	0.200	
UEL2	NA*	NA*	-	shipment problems
UEL5	1.350	4.360	0.030	no reason

HBM4EU 1/2020 BP7 in urine [ng/mL]				
Lab.code	**R1A**	**R1B**	**LOQ**	**delayed reporting**
UEL1	1.960	5.820	0.200	
UEL2	1.762	3.708	1.200	shipment problems
UEL5	1.700	6.100	0.050	no reason

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
UEL2	2.000 mL	beta-glucuronidase	5.0	3.0 h / 38°C	LLE	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	50	3.0 mm x 150 mm; 2.6 µm	triple quad
UEL2	HPLC / GC	10	2.1 mm x 100 mm; 2.7 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
UEL2	yes	before deconjugation	isotope dilution (addition to sample before extraction)	0.05 min	2 per compound	+/-30%
UEL5	yes	before deconjugation	isotope dilution (addition to sample before extraction)	0.2 min	1-2	/