Prioritised substance group: Bisphenols

Main author	Robert Barouki (INSERM - FR)
Contributors	Charles Persoz, Cathrine Thomsen, Els Heyvaert, Hans Reyders, Karen Van Campenhout, Greet Schoeters, Catherine Ganzleben, Christina Hartmann, Maria Uhl, Jean-Philippe Antignac, Laurent Debrauwer, Nataša Janey Holcer, Coen Graven, Shalenie den Braver-Sewradj, Matthieu Meslin, Christophe Rousselle

Short overview of results of the activities carried out within HBM4EU in 2020 to answer the policy questions with reference to corresponding deliverables.

Policy Question	Short Summary of Results
1. What is the current exposure of the EU population to BPA, BPS and BPF?	Analytical aspects: As a preliminary step to be able to address this policy question, in WP9 a prioritised list with most suitable biomarkers, matrices and analytical methods has been elaborated (see D9.1). The biomarkers of exposure, matrices, analytical methods and method detection limit (MDL) selected for bisphenols are listed below: - Bisphenol A: BPA, Urine (0.5 mL), LC-MS-MS, MDL: 0.02 ng/mL - Bisphenol S: BPS, Urine (NA), LC-MS-MS, MDL: 0.03 ng/mL - Bisphenol F: BPF, Urine (NA), LC-MS-MS, MDL: 0.06 ng/mL For BPA, BPS and BPF urine should be preferred as the matrix of choice for exposure assessment in the general population. The risk of contamination of the samples during the sampling/preparation procedure should be monitored by field and laboratory blank controls. Now the procedure for evaluating and managing this possible external contamination level remains to be harmonised between laboratories as a prerequisite for reliable inter-country data comparison and analysis. Commercially available internal standards and biomarkers for BPS, BPF and other BP's are not yet well implemented in all laboratories proposing bisphenol analyses. More broadly, for BPS, BPF and other BP's there is a general lack of peer-reviewed, published methods, especially in urine, and experience in HBM studies is less well established as compared to BPA. Up to now quantifiable measures in urine with quality assured (labelled) internal standards for BPS and BPF are not available in Europe. The interlaboratory assays (ICI/EQUAS) organised within WP9 in 2018/19 permitted to have a better picture of the current situation on these

Policy Question	Short Summary of Results
	analytical aspects and of the real existing capabilities for bisphenol analyses in the different laboratories proposing these analyses. After 3 rounds of ICI/EQUAS, 24 laboratories have been qualified to analyse bisphenol A in HBM4EU (AD9.3). Chemical analyses of BPS and BPF have been improved and a sufficient number of laboratories was achieved after the 3rd ICI/EQUAS round (18 labs BPS and 13 labs BPF). Additional questions in regard to natural occurrences of BPF in mustard (Zoller et al., 2016)1 need to be answered in order to ensure the specificity of this biomarker to represent non-natural exposures to BPF. A more detailed investigation of the isoforms of BPF (4,4'-BPF, 2,2'-BPF and 2,4'-BPF) in terms of exposure biomarker validity might be warranted (D9.7). WP16 is also contributing to refine and/or increase the knowledge regarding the relative proportions of free versus conjugated forms of these contaminants. This aspect is of major importance in consolidated exposure assessment, characterised toxicological impact, and contribution to PBPK modelling for exposure-health studies. It is also important to address the issue of contamination during sample preparation mentioned above. What do we learn from previous surveys? WP5 has prepared a scoping paper on the development of Human Biomonitoring (HBM) indicators on chemical exposure in the European population. A case study on HBM indicators from DEMOCOPHES data has been reported for BPA (see D5.3). This led to the following results (exposure levels in the population): in children, the weighted geometric mean (95% CI) for urinary BPA equaled 1.97 µg/L (1.81-2.15) in the total European study group. In mothers, it equaled 1.78 µg/L (1.62-1.94), suggesting a tendency for higher levels in children compared to their mothers. AD 5.3 reports that HBM based result indicators can provide information relevant to address current exposure of EU population. However, in IPCHEM/repository, there is not enough data available to answer this now (only 4 aggregated data collections on BPA of 3 different countries). Survey planning and harmonisation. In 2018 WP8 has establish a sampling frame for Europe to align ongoing/planned studies to collect HBM data of the prioritised chemicals with EU wide coverage. Due to the scarce availability of recent exposure data on the first set of priority chemicals and due to financial limitations, it was decided to focus on specific chemicals per age group to achieve EU wide coverage of these chemicals within the selected age groups. Therefore, bisphenols (A,S, F) will be analysed in adults (20-39 years) (D8.4). Samples are collected in 11 different European countries. In 2019 specific questionnaires on bisphenols for children and for adolescents where developed under T7.3, available for download at the online library. Statistical plans. WP10 has elaborated, among others, a specific statistic data analysis plan for bisphenols. The plan defines all the necessary variables for the statistical analysis to address bisphenol specific research questions on general exposure levels, time trends, geographic comparisons and exposure determinants (see D10.2).
2. Do different regulatory controls across the EU concerning in particular BPA lead to different exposures?	As a very preliminary step to be able to address this policy question, WP7 has elaborated a study protocol for harmonised recruitment and sampling (see D7.3). This procedure provides the main essential points for the planning of new studies or if an existing study is to be aligned or biobanked samples are to be used in the frame of HBM4EU. A specific questionnaire for bisphenols has been designed to collect all the necessary information concerning individual characteristics of the participants (sociodemographic, dietary, occupational, lifestyle, environmental and health factors). The outcome of these questionnaires might contribute to find out whether there are HBM data or suitable samples available before and after the ban in France, Sweden and Denmark. As an action to guarantee the comparability and the quality of the analytical results within HBM4EU, WP9 is implementing a complete tailor-made ICI/EQUAS program covering until now 74 parameters, including the analysis of BPA, BPF and BPS in urine. The 33 candidate laboratories identified in task 9.2 were invited to join the 1st ICI round and about the 72% of them have participated (different number of

[1] Zoller O, Brüschweiler BJ, Magnin R et al. 2016. Natural occurrence of bisphenol F in mustard. Food Addit. Contam. Part A, Chem. Anal. Control. Expo. Risk Assess. 33:137-146. doi: 10.1080/19440049.2015.1110623.

Policy Question	Short Summary of Results
	laboratories for the different bisphenols). The 1st round finished in July 2018 and the 2nd ICI round at the end of 2018. A 3rd ICI/EQUAS took place and based on the combined results of the ICI/EQUAS scheme 24 laboratories have been qualified to analyse bisphenols in HBM4EU (AD9.3). WP5 is contributing to guide decision makers for using HBM data in a broad sense (awareness raising, remediating measures, and improved regulation), trajectories towards participatory processes have been initiated. A first case study on policy uptake of HBM results has focused on bisphenols and phthalates (see D 5.4). The bisphenols case (mainly focusing on BPA) is characterised by a persistent controversy, fuelled by the discrepancy between standardised regulatory studies (used for formal risk assessments) that do not report health effects, and an increasing number of academic studies reporting effects at current exposure levels (low doses), but lacking reproducibility and therefore not meeting the quality standards for regulatory risk assessment. A first goal of HBM4EU for the bisphenols is therefore to guarantee quality controlled HBM data measurements via interlaboratory assays (ICI/EQUAS) organised within WP9.
3. Are bisphenols exposure levels of concern for health?	Establishing Health-based Guidance values. For BPA, there are different HBM-HBGVs (Human Biomonitoring derived Health-Based Guidance Values): 1) the German HBM-I-value for children and the German HBM-I-value for adults2 2) the BE value based on the one hand on the pTDI from Health Canada and on the other the BE value based on the US EPA RfD and EFSA TDI3. The German HBM-I value was used here as primary health-based guidance value in the absence of an HBM HBGV derived in HBM4EU. The use of the German HBM-I-value indicated that in <5% of the Danish children and in <5% of the mother-child pairs in Belgium the measured urinary BPA concentrations exceeded the German HBM-I-guidance value. Exceeding the HBM-I-value implies that the occurrence of a certain health risk cannot be excluded with sufficient certainty. As the percentage of the population exceeding the HBM HBGV was <5% in Danish mothers, Belgian children and mothers, the extent of exceedance indicator, based here on the ratio of the 95% percentile over the HBM-I-guidance value, was <1. None of the participants in the six European countries exceeded the BE values for urinary BPA which are older and higher than the HBM-I. The BE value corresponding to the oral provisional tolerable daily intake (pTDI) of 25 μg/kg-d from Health Canada is 1 mg/L (1.3 mg/g creatinine); value corresponding to the US EPA reference dose (RfD) and EFSA tolerable daily intake (TDI) estimates (both of which are equal to 50 μg/kg-d) is 2 mg/L (2.6 mg/g creatinine). Towards the end of 2018, in WP5, ANSES and UBA started working on the establishment of new HBM4EU health-based guidance values for BPA both in the general population and in occupational settings. These values have been reported in D5.14 and are detailed below under policy question 8. Selecting effect markers. A strategy for the selection of effect biomarkers for their potential implementation in HBM4EU aligned studies has been presented and exemplified in three case studies in relation to the bisphenols family of compounds. Because bisphenols have complex mode of action (MoA), implementation of effect biomarkers at different levels of biological organisation (e.g., DNA, RNA, proteins or metabolites) seems necessary. The suitability of using effect biomarkers from the WP14 inventory (D14.3) to represent possible AOPs identified by WP13 has been explored for bisphenol A and female reproductive health, glucose homeostasis and neurological effects. The technical and scientific limitations have also been discussed (AD14.3). A comprehensive review of the literature was performed, creating the first inventory of effect biomarkers for bisphenols. Several epigenetics, gene transcription, oxidative stress, reproductive, glucocorticoid and thyroid hormones, metabolic and allergy/immune biomarkers were first studied, and then, promising effect biomarkers related to altered neurodevelopment and reproductive outcomes including brain-derived neurotrophic factor (BDNF), kisspeptin (KiSS), and gene expression of

[2] German Human Biomonitoring Commission, 2012. Stoffmonographie bisphenol-A (BPA) – Referenz- und Human-Biomonitoring-(HBM)-Werte für BPA im Urin. Bundesgesundheitsblatt, Gesundheitsforschung, Gesundheitsschutz 55 (9), 1215-1231.
[3] Krishnan, K., Gagne, M., Nong, A., Aylward, L.L., Hays, S.M., 2010. Biomonitoring Equivalents for bisphenol A (BPA). Regulatory toxicology and pharmacology: RTP 58, 18-24.

Policy Question	Short Summary of Results
	nuclear receptors were prioritised, providing mechanistic insights based on in vitro, animal studies and AOP information. Finally, the potential of omics technologies for biomarker discovery and its implications for risk assessment were also discussed (Mustieles et al., 2020)4. AOPs and BPA. WP13 has produced a report on adverse outcome pathways (AOPs) for the first set of prioritised substances within HBM4EU. However, most of the effort was devoted to BPA substituents (see below). PBTK. Within task 12.3, biological half-lives (t1/2) in human have been compiled that contribute to the refinements of the PBTK models and estimation of internal doses for 1st set of priority compounds. The t1/2 values (hours) compiled for bisphenols [BPA and its substitutes (BPS, BPF)] in blood/serum or urine were generally low, with median values lower than 7 h (D12.3). WP12 has also assessed exposure pathways leading to internal dose using a combination of external exposure determinants/modifiers and, in the case of bisphenol A, a dedicated model was developed by WP12 partners. Furthermore, tissue levels were determined based on blood levels and blood/tissue partition coefficient. For bisphenols, the BPA exposure of the Elfe study pregnant women was estimated individually from the HBM data (here, the urinary concentration of total BPA) using a reverse dosimetry approach based on PBPK modelling. The aim was to estimate the internal exposure in the mothers and their foetuses. Two scenarios of exposure corresponding to the same amount of administered BPA over the pregnancy were tested. The scenario I assumes a constant and continuous exposure to BPA via ingestion and dermal contact over the whole pregnancy. The scenario II models three diet intakes and 2 dermal intakes by PCPs each day of the pregnancy. Our modelling results showed that the urinary and plasma concentrations in mothers evolves over the day according to the BPA intakes (3 meals and 2 dermal contacts). On the contrary, the foetal plasma concentration is quite stable due to transfer rates that are quite low within a day. The maternal and foetal plasma concentrations were of the same order. Available HBM data for Europe on bisphenols have been reviewed, as well as the regulatory thresholds available for bisphenols. Using toxicokinetic modelling, environmental (i.e., non-occupational) exposure to bisphenols has been estimated for the reviewed HBM data. The uncertainty interval of the external exposure ranged from 0.01 to <1 µg/kg bw/day for the different European cohorts. All reconstructed exposures were below the regulatory threshold selected, i.e. the temporary tolerable daily intake set by EFSA in 2015 (4 µg/kg/day). Thus, the obtained risk characterisation ratios from the 95th percentile of the population exposure were below 1, indicating that no risks were associated with BPA exposure in the studied European cohorts.
4. Is occupational exposure of cashiers a health concern?	WP5 has elaborated a concept document on the strategy for the derivation of health-based guidance values for the general population and for occupationally exposed adults (see D5.1). In the case of occupational BPA exposure of cashiers, HBM data can be used to support modelling data, giving a stronger basis for the assessment. However, BPA HBM based risk assessment included some uncertainties related for example on the fraction of free BPA available for systemic distribution after dermal exposure. As of January 2020, BPA in thermal paper is restricted because of health risks for pregnant workers and consumers exposed to it in thermal paper (France, 2014). The analyses of biomonitoring studies performed by ANSES and by EFSA (EFSA, 2015) were included into an updated version of the restriction dossier. A draft on the derivation of Human Biomonitoring Guidance Values (HBM-GV) for BPA is currently under development. HBM-GV will help interpretation of the potential health impact of internal chemical exposures measured in workers and in the general population through HBM. At this stage, urinary BPA concentration distributions have been reconstructed based on published EU HBM studies for further comparison with the derived HBM-GV on BPA. Information on BPA HBM data in occupational settings has already been collected.

[4] Mustieles V, D'Cruz SC, Couderq S, Rodríguez-Carrillo A, Fini J-B, Hofer T, Steffensen IL, Dirven H, Barouki R, Olea N, Fernández MF, David A. Bisphenol A and its analogues: a comprehensive review to identify and prioritize effect biomarkers for human biomonitoring. Environment International. 2020. In Press.

Policy Question	Short Summary of Results
5. What is the toxicity of BPA substitutes and are current exposure level of concern?	Computational Tool development. In order to assess the putative toxicological impact of BPS and BPF, new tools were developed. These tools are based on text mining using artificial intelligence as well as on systems biology tools (Carvalho et al, EHP, 2019)5. Using different combinations of these tools it was possible to identify the most likely toxic outcomes of exposure to these substituents. Putative toxic effects of BPA substituents. Using the tools mentioned above it was possible to highlight obesity as one of the major potential health endpoint of BPS which was related to the biological activity of adipogenesis. The characteristic key events were decreased lipolysis, increased adipocyte formation, fatty acid uptake and lipogenesis. These are initiated by the disruption of the activity of 1) several transcription factors including estrogen receptors or ERR gamma, 2) enzymes such as hormone-sensitive lipase, or 3) expression of adipogenic biomarkers including PGC-1 alpha & perilipin 4 (D13.4). Using refined computational tools applied to bisphenol F allowed us to link BPF stressor to an AOP network for thyroid cancer (Rugard et al. 2020)6 A research study on (Bishenols A, S, and F) male fertility endpoints and endocrine disruptive effects in cohort studies is currently ongoing (AD13.3). To further investigate the potential of seminal plasma as a key resource for study of male exposure to environmental pollutants and effect on general health, bisphenols will be measured in the seminal plasma of sub-fertile (case) and fertile (control) males. More than 150 samples (75 pairs) have been collected, characterised and stored. Quantification of bisphenols in all samples is expected to be completed by end 2020 and comparative analysis between fertile and sub-fertile males undertaken early 2021.
6. Are health risks age and gender dependent?	WP7 has run a NHCPs online consultation on existing HBM surveys. The outcome from 124 questionnaires has been analysed in D7.1. For bisphenols, an analysis by European-defined region showed that the identified studies included predominantly the North (Denmark, Estonia. Finland, Iceland, Ireland, Latvia, Lithuania, Norway, Sweden, UK) and the West (Austria, Belgium, France, Germany, Luxembourg, The Netherlands, Switzerland). For the studies reported as having a national representativeness level, the majority involved children and newborns. In studies with children, bisphenols were among one of the most analysed substances. As previously described above, in WP12, the BPA exposure of the Elfe pregnant women was estimated individually from the HBM data (here, the urinary concentration of total BPA) using a reverse dosimetry approach based on PBPK modelling. Two scenarios of exposure corresponding to the same amount of administered BPA over the pregnancy were tested. (Detailed results in D12.4)
7. Can we find evidence for low-dose effects within mixtures?	During 2018 WP13 addressed exploration of available cohort data for bisphenols and (neuro) developmental and reproductive outcomes (D13.3). Further studies were outlined addressing mixture effects of PFAS, bisphenols and/or phthalates in children.

[5] Carvaillo, J-C., Barouki, R., Coumoul, X., Audouze, K., 2019. Linking bisphenol S as an environmental chemical stressor to key events and adverse outcomes using a text mining-based computational approach. EHP- Environmental Health Perspectives. https://doi.org/10.1289/EHP4200

[6] Rugard M., Coumoul X., Carvaillo J-C., Barouki R., Audouze K. (2020) Deciphering Adverse Outcome Pathway Network Linked to Bisphenol F Using Text Mining and Systems Toxicology Approaches, Toxicological Sciences, 173 (1), 32–40, https://doi.org/10.1093/toxsci/kfz214

<table>
<tr><th>Policy Question</th><th>Short Summary of Results</th></tr>
<tr><td>8. How can HBM feed into assessment of the Tolerable Daily Intake (TDI) for BPA, as set by the European Food Safety Authority (EFSA)?</td><td>
WP12 has developed a roadmap for PBTK/TD model refinement and analysis for priority substances including bisphenols (see AD12.2)
This PBPK model can reproduce the BPA chemical-specific pharmacokinetic data for oral exposure though solid form (cookie) and is reliable with regard to its predictions of BPA in serum (Thayer et al 2015, N=3 volunteers), BPAG in serum (Thayer et al. 2015, N=3 volunteers), cumulative excretion of BPAG in urine (Thayer et al 2015, N=3 volunteers and Volkel et al. 2002, 2005).
For oral exposure though liquid form (soup), the PBPK model has been revised (re-calibrated by optimisation of the oral uptake constant) however not evaluated with new data.
The model should be further evaluated, in particular towards the biological relevance of modelling the enterohepatic recirculation.
WP12 has optimised the methodology for exposure reconstruction (AD12.6), which was applied in available HBM data.
For bisphenols, in particular BPA: the EU population was estimated to have an average daily intake of 0.05 µg/kg_bw•d, a value that is much lower than the corresponding temporary tolerable daily intake set by the European Food Safety Authority (EFSA). Even the highest level of HBM measurements (corresponding to Italian adult population) corresponded to a daily intake of 0.77 µg/kg_bw•d, still significantly lower than the EFSA t-TDI. HBM data for different age groups including young children, adults and pregnant mothers were used for the assessment. Both a life course PBTK model and a pregnancy PBTK model were used to allow for more accurate reconstruction of external exposure taking note of the physiological and metabolic differences characteristic of different age windows during the life course and in utero.(AD12.5). In WP5, ANSES and UBA have worked on the derivation of HBM-GVs for BPA both in the general population and in occupational settings. (D5.14).
The table below shows the derived boundary values for total BPA in urine consistent with the concentration of free BPA in plasma after 100% oral exposure to the t-TDI of 4 µg/kg bw/d.
<table>
<tr><th>Population group</th><th>High boundary value for total BPA in urine (100% oral exposure to BPA) → HBM-GVGenPop</th><th>Corresponding concentration of free BPA in plasma</th></tr>
<tr><td>Adult (45 years, 70 kg)</td><td>233 µg/L</td><td>6.9.10-3 µg/L</td></tr>
<tr><td>Child (5 years, 19 kg)</td><td>137 µg/L</td><td>13.7.10-3 µg/L</td></tr>
</table>
Despite the fact that inhalation seems to be the most important route of BPA exposure for workers, data characterising the toxicokinetic of BPA after inhalation in humans is lacking. It is presumed that a fraction of inhaled BPA is actually absorbed by the oral route and is thus subject to first-pass hepatic metabolism. However, due to the paucity of kinetic data regarding the inhalation route and the fact that all existing OELs are based on non-systemic respiratory effects, it is not possible to derive a HBM-GVworker based on atmospheric levels to BPA likely to induce toxic effects at the workplace.
Taking advantage of the modified PBPK model from Karrer et al. (2018)7, which includes the oral and dermal route of exposure, the concentration of total BPA in urine after dermal exposure to BPA was rather estimated based on the plasmatic concentration of free BPA (e.g. the toxicologically-relevant chemical form) generated after 24h average oral exposure to the oral DNEL for workers of 8 µg/kg bw
Regarding BPA exposure at the workplace, the level of urinary total BPA was estimated after the dermal uptake of BPA which would generate the same free BPA concentration in plasma (considered as the bioactive form) as a 24h-averaged intake to the ECHA’s DNEL for oral uptake of 8 µg/kg bw for workers. The estimated concentration of urinary total BPA is equivalent to, or exceeds the 95th percentile of total BPA in urine
</td></tr>
</table>

[7] Karrer C, Roiss T, von Goetz N, Gramec Skledar D, Peterlin Mašič L, Hungerbühler K., 2018. Physiologically Based Pharmacokinetic (PBPK) Modeling of the Bisphenols BPA, BPS, BPF, and BPAF with New Experimental Metabolic Parameters: Comparing the Pharmacokinetic Behavior of BPA with Its Substitutes. Environ Health Perspect.;126(7):077002. doi:10.1289/EHP2739

Policy Question	Short Summary of Results
	measured in different European HBM studies conducted in the general population. Thus, no HBM-GVworker was proposed, as the high background level of BPA coming from environmental exposure - mostly through food intake - is making the discrimination with the occupational exposure to BPA difficult
9. Is it important to eliminate legacy BPA from material cycles (i.e. waste till receipt rolls) when implementing a circular economy in order to protect human health?	Based on the studies carried out in HBM4EU but also in several other projects that were analysed by HBM4EU, it is possible to state that legacy can possibly have health impact. However, this will be better assessed when Guidance values will be obtained for BPs.