Supplementary Information

Urinary concentrations of BPA and analogous bisphenols (BPF and BPS) among school children from Poland: Exposure and Risk Assessment in the REPRO_PL cohort.

Mercè Garí, Daniel Bury, Rebecca K. Moos, Monika Weteska, Agnieszka Jankowska, Agnieszka Brzozowska, Joanna Jerzynska, Stephan Bose-O'Reilly, Holger M. Koch, Kinga Polanska

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Section S-1 Supplementary information regarding the analysis of bisphenols A, F, and S

Chemicals and reagents

Acetonitrile and methanol CHROMASOLV™ LC-MS grade and ammonium fluoride for LC-MS were purchased from Honeywell (Seelze, Germany). Water LC-MS grade was obtained from Carl Roth (Karlsruhe, Germany) and acetic acid (LiChropur, 100%, for LC-MS) was obtained from Merck (Darmstadt, Germany). Bisphenol A ('BPA'; ≥99%), ammonium acetate BioXtra ≥98%, and β-Glucuronidase from *H. pomatia* (with aryl sulfatase activity) were obtained from Sigma-Aldrich (Steinheim, Germany). Bisphenol A-d_{16} (BPA-d_{16}; chemical purity 99%, isotopic purity 99.1%) was purchased from Dr. Ehrenstorfer (Augsburg, Germany). Isomerically pure 4,4'-Bisphenol F ('BPF'; >98%) was purchased from TCI Deutschland (Eschborn, Germany) and isomerically pure 4,4'-

bisphenol F-d_{10} (1,1-Bis(4-hydroxyphenyl-2,3,5,6-d_4)methane-d_2; 'BPF-d_{10}'; chemical purity: 95%, isotopic purity: 97.0%) was obtained from Toronto Research Chemicals (North York, Ontario, Canada). Bisphenol S-$^{13}C_{12}$ ('BPS-$^{13}C_{12}$'; isotopic purity: 98%) and bisphenol S ('BPS') (both: chemical purity: 98%; 100 µg/mL in methanol) were obtained from Cambridge Isotope Laboratories (Tewksbury, MA, USA). Standard HPLC vials (Macherey-Nagel, Düren, Germany) with silicone/PTFE screw caps (VWR, Leuven, Belgium) were used for LC-MS analysis.

Method details

For calibration, eight different aqueous solutions containing each of the three bisphenols at 0, 0.05, 0.1, 0.25, 2.5, 10, 25, and 50 µg/L, respectively, were used.

For online-SPE-LC-MS/MS an Agilent 1260 Infinity II HPLC system (Agilent Technologies, Waldbronn, Germany) coupled to a 5500 triple quadrupole mass spectrometer (Sciex, Darmstadt, Germany) was used. The HPLC consisted of a G7167A thermostatted autosampler, two G7112B binary high-pressure gradient pumps (used as loading pump and LC pump), a G7111B quaternary low-pressure gradient pump ('diverter pump'; used to keep flow into the MS source during diversion of the LC flow into waste), and a G7116A thermostatted column compartment with a ten-port switching valve. The instrument setup was according to Ye *et al*. (2008), except that the loading pump was used to elute the analytes from the enrichment column and the LC pump to provide weak eluent for peak focusing (instead of vice versa) and additionally the integrated 6-port valve of the MS was used to divert the LC flow into waste outside the retention time windows of the analytes (see figure S-1).

For online sample clean-up and analyte enrichment a 20 x 2.1 mm column custom packed with AFFINIMIP® Bisphenols material (Affinisep, Petit-Couronne, France) was used and for HPLC separation a Luna Omega PS-C18 mixed mode phase (50x3 mm; 5µm; Phenomenex, Aschaffenburg, Germany). An inline filter (porosity 0.5 µm; 3.0mm diameter; Phenomenex, Aschaffenburg, Germany) was placed between the autosampler and the column compartment to capture particles from the injected samples and two further inline filters were placed at the inlet and outlet of the enrichment column to avoid issues caused by potential sorbent particle bleeding. The injection volume was 50 µL. As part of the injection procedure, the needle was rinsed with methanol/water 8:2 (v/v) for 10 s

after sample aspiration. For the solvent gradients and valve switching times, see Tables S-1 through S-4. The column compartment and autosampler were kept at 25 and 6 °C, respectively.

Detection of the bisphenols was performed with electrospray-tandem mass spectrometry with time-programmed multiple reaction monitoring (MRM) in negative ion mode. The instrument gases (nitrogen in all cases) were set to 40 psi nebulizer and curtain gas, 50 psi heater gas, and 8 arbitrary units collision gas. The source heater temperature was 650 °C and the ion spray voltage was 4.5 kV. Entrance potential and collision cell exit potential were both set to -10 V. The target scan time was 0.3 s and the MRM detection window was set to 60 s. For further MS conditions, see Table S-5. Analyst 1.6 (Sciex, Darmstadt, Germany) was used for instrument control and quantitative data analysis.

To prevent loss in separation power for BPF and an interfering compound eluting shortly after BPF, the HPLC column had to be re-equilibrated after each analytical batch by flushing it with 10 column volumes of water/acetonitrile 50:50 (v:v) containing 0.05% acetic acid and storing it in this solvent overnight. To enable an undisturbed analysis, two different HPLC columns were used in alternation.

Method validation:

The accuracy of the method was investigated by analyzing 20 different urine samples (with creatinine levels ranging from 0.13 to 2.31 g/L) spiked with BPA, BPF, and BPS at three different spiking levels (0.5, 2, and 10 µg/L), as well as without spiking. The relative recoveries were calculated after subtraction of the native bisphenol concentrations. The average relative recoveries for the three spiking levels were in the ranges of 101-104% (BPA), 93-97% (BPF), and 99-103% (BPS).

For the determination of the method's precision, two different pooled urine samples were prepared and analyzed six times in one analytical batch (intraday precision), as well as on 13 different days (interday precision) (see Table S-6). For all analytes and concentration levels, the method's imprecision was ≤15%. This material was used as quality control material and analyzed in each analytical batch.

Figure S-1. Instrument setup for the analysis of bisphenols A, F, and S.

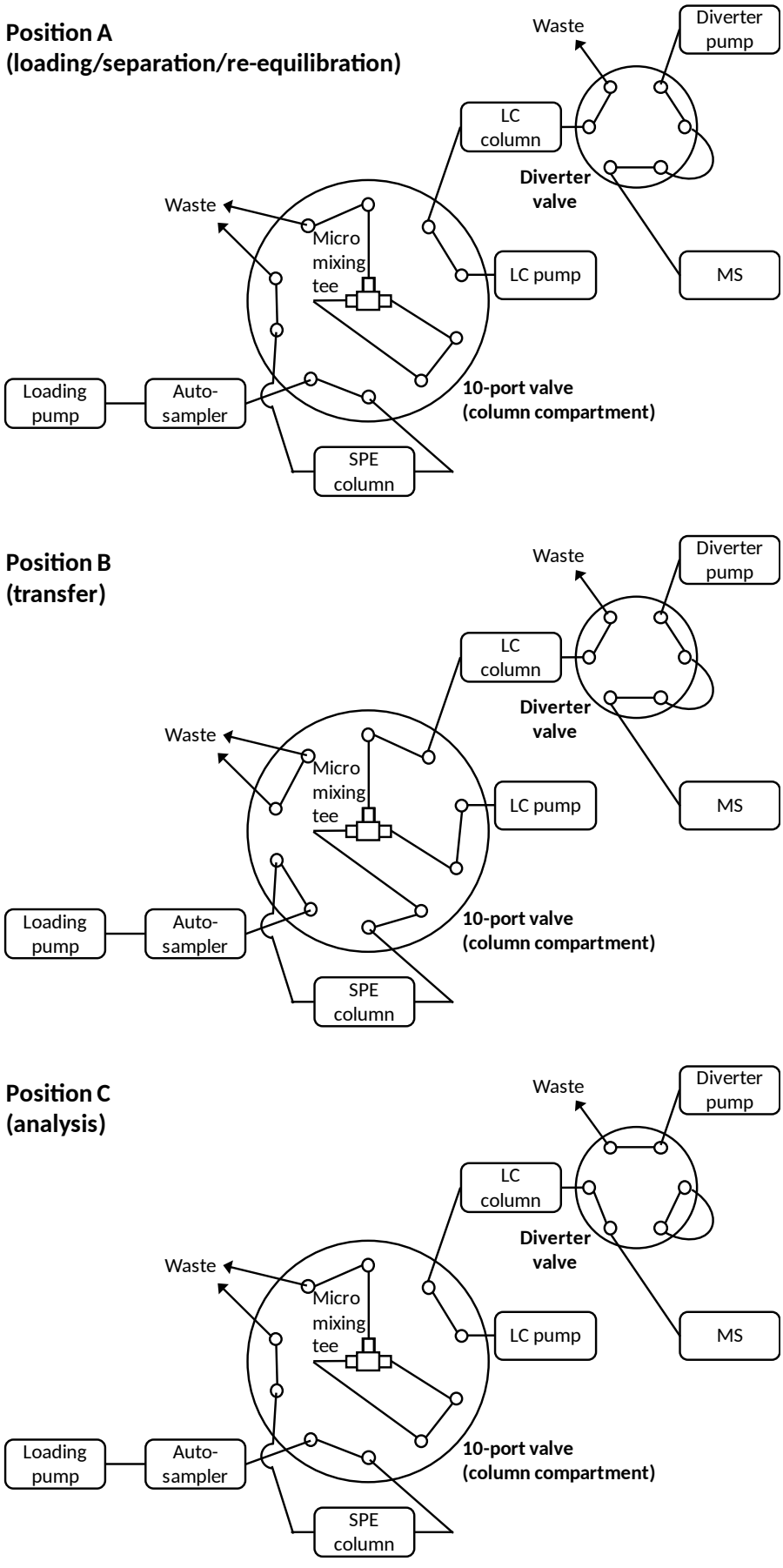

Table S-1. Time table for valve switching events of the 10-port switching valve and the diverter valve.

Time [min]	Switch to valve position
0	A
2.5	B
7.5	A
10	C
12.5	A

Table S-2. Solvent gradient for the loading pump. Eluent A and B were water and acetonitrile, respectively, both containing 0.05 % acetic acid

Time [min]	Flow rate [µL/min]	%A	%B
0	300	60	40
2	300	60	40
2.5	200	35	65
7.4	200	35	65
7.5	50	35	65
8	300	25	75
11.5	300	25	75
12	300	5	95
14	300	5	95
14.5	300	60	40
17.5	300	60	40

Table S-3. Solvent gradient for the LC pump. Eluent A was 0.2 mM ammonium fluoride in water and B was acetonitrile.

Time [min]	Flow rate [µL/min]	%A	%B
0	300	100	0
2	300	100	0
2.1	1500	100	0
7.4	1500	100	0
7.5	300	100	0
8.5	300	70	30
13	300	5	95
15	300	5	95
15.3	300	100	0
17.5	300	100	0

Table S-4. Solvent gradient for the diverter pump. Eluent A and B were water and acetonitrile, respectively, both containing 0.05 % acetic acid

Time [min]	Flow rate [µL/min]	%A	%B
0	300	50	50
10	300	50	50
10.5	10	50	50
12	10	50	50
12.5	300	50	50
17.5	300	50	50

Table S-5. MRM conditions. For each analyte and internal standard, the retention time (t_R), precursor and product ions, declustering potential (DP) and collision energies (CE) are given (values for the qualifier ion are in parentheses).

Analyte	t_R [min]	Precursor ion	Product ion	DP [V]	CE [eV]
BPA	11.65	227	133 (93)	-130	-36 (-57)
BPA-d_{16}	11.60	241	98 (222)	-130	-60 (-35)
BPF	11.20	199	105 (77)	-130	-29 (-34)
BPF-d_{10}	11.15	209	82 (128)	-130	-36 (-36)
BPS	10.60	249	156 (108)	-130	-38 (-50)
BPS-$^{13}C_{12}$	10.60	261	114 (98)	-130	-50 (-63)

Table S-6. Method precision. The coefficients of variation of the intra- and interday precision are shown with measured bisphenol concentrations (arithmetic means) shown in parentheses.

	BPA	BPF	BPS
Intraday precision (n=6)	2.8% (0.78 µg/L) 2.0% (5.40 µg/L)	7.0% (0.19 µg/L) 1.6% (4.69 µg/L)	1.3% (0.453 µg/L) 1.0% (2.06 µg/L)
Interday precision (n=13)	5.4% (0.81 µg/L) 5.6% (5.05 µg/L)	15% (0.18 µg/L) 5.8% (4.54 µg/L)	5.7% (0.495 µg/L) 3.9% (2.05 µg/L)

References

Ye X, Tao LJ, Needham LL, Calafat AM (2008) Automated on-line column- switching HPLC-MS/MS method for measuring environmenta l phenols and parabens in serum. Talanta 76:865-71. https://doi.org/10.1016/j.talanta.2008.04.034