Supplemental Material

Comparison of Serum Bisphenol A Concentrations in Mice Exposed to Bisphenol A through the Diet versus Oral Bolus Exposure

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1.0 BPA-$d_{6}$ Treatments

Body weight was measured and food consumption from the beginning of the dosing period was calculated at each timepoints. Blood samples were collected by cardiac puncture in polypropylene (PP) syringes, placed in PP microcentrifuge tubes, and centrifuged twice (15 min at 13,800 g) (Mikro 22R Microcentrifuge, Hettich Zentrifugen, Germany) for serum collection. The serum samples were stored at -20°C. Individual samples within the same timepoint and treatment group that provided less than the minimum 400 μL needed for the BPA-$d_{6}$ analysis were pooled to yield 9 negative control samples. The number of pooled samples for mice given the oral bolus of BPA-$d_{6}$ sacrificed at each time was: 1 h, n= 3; 4 h, n= 4; 6 h, n= 4; 11 h, n=4; 24 h, n=6; 7 d, n=4. For the BPA-$d_{6}$ diet-exposed group, the number of pooled samples for each timepoint was: 1 h, n= 6; 4 h, n= 4; 6 h, n= 4; 11 h, n=4; 24 h, n=6; 7 d, n=5. The samples were shipped on dry ice to the Wadsworth Center, Albany, NY for analysis.

2.0 Analysis of Unconjugated and Conjugated BPA-$d_{6}$ in Mouse Serum Samples

To assess “freely available” BPA, i.e. that fraction that had not been metabolized to its water-soluble, glucuronyl derivative, each sample was allowed to thaw at room temperature for 30 min and transferred into a 15 mL PP tube. An internal standard (5 ng of deuterated 16-bisphenol A (BPA-$d_{16}$) was added. Five mL of ethyl acetate was then added, and the mixture shaken in an orbital shaker for 30 min. After centrifugation at 4500 g for 3 min (Eppendorf Centrifuge 5804, Hamburg, Germany), the ethyl acetate layer was transferred to a clean PP tube, the residue extracted two more times with 3.5 mL of ethyl acetate by shaking, and the extracts were combined. The extracts were mixed with 3.5 mL of milli-Q water and centrifuged at 5000 rpm for 3 min (Eppendorf Centrifuge 5804). The ethyl acetate layer was collected, dried under a
gentle stream of nitrogen and reconstituted in 0.5 mL of methanol. This fraction that was soluble in ethyl acetate was considered freely available BPA-$d_6$.

After addition of the internal control, the second aliquot of thawed serum was digested with 1 mL of 2 μL/mL β-glucuronidase that also has aryl-sulfatase activity (from Helix pomatia, 145700 unit/mL, Sigma, St Louis, MO) at 37°C for 12 h. The digested sample was processed as described above and referred to as total BPA-$d_6$, which includes the free, i.e. ethyl acetate soluble, plus the conjugated BPA-$d_6$.

The BPA-$d_6$ serum concentrations in samples were measured by a procedure similar to that described earlier, but with some modifications (Padmanabhan et al. 2008). Analyte separation and detection were carried out by using an Agilent 1100 series HPLC interfaced with an Applied Biosystems API 2000 electrospray MS/MS mass spectrometer (Applied Biosystems, Foster City, CA). Samples (10 μL) of the extract were injected onto an analytical column (Betasil® C18, 100 x 2.1 mm column; Thermo Electron Corporation, Waltham, MA), connected in series to a Javelin® guard column (Betasil® C18, 20 x 2.1 mm). The mobile phase was comprised of a gradient of methanol/water (initial 25% methanol; final 99% methanol; 4 min). Flow was maintained under the latter conditions for 10 min before reversing to the initial starting condition. The flow rate and the column temperature were 300 μL/min and 25°C respectively. The MS/MS was operated in the electrospray negative ion mode. Instrument parameters were optimized to transmit the [M-H] ion before fragmentation to one or more product ions. Cone voltage and collision energies were 30 V and 25 V, respectively. Capillary voltage was 4.5 KV,
and desolvation temperature 400°C. Data were acquired by using multiple reaction monitoring (MRM) for the transitions of 233>215 for BPA-\textit{d}_6 and 241>223 for BPA-\textit{d}_{16}.

Quality assurance and control parameters included validation of the method by spiking BPA-\textit{d}_{16} into the sample matrices and passing through the entire analytical procedure to calculate recoveries of BPA-\textit{d}_{16} through the analytical method. The matrix spike recovery was 109% (106-111%) with a standard deviation of 3.5%. A procedural blank, containing milli-Q water in place of serum was analyzed in parallel with the samples to check for interferences that would correlate with the target compound or laboratory contamination. Trace concentrations of BPA present in such blanks (<0.01 ng) were subtracted from sample values for determining the concentrations in samples. The limit of detection, i.e. sensitivity of the assay, was 0.1 ng/mL, which was calculated as twice that of the valid "lowest acceptable calibration standard". The reported concentrations of BPA-\textit{d}_6 for each sample were corrected, based on the recovery value of the surrogate standard, BPA-\textit{d}_{16} (i.e., isotope dilution). The BPA-\textit{d}_6 standard spiked into sample matrices and passed through the entire analytical procedure yielded a mean recovery of 117%. An external calibration curve prepared by injecting 10 μL of 0.05, 0.1, 0.2, 0.5, 1, 2, 5, 10, 20, and 50 ng/mL standards of BPA-\textit{d}_6 and BPA-\textit{d}_{16} yielded a calibration coefficient of 0.99.

3.0 Pharmacokinetic analysis

3.1 Non-compartmental analysis of total and unconjugated BPA-\textit{d}_6 concentrations after oral and diet exposure

Extrapolation to infinity to obtain \textit{AUC}_{(0-infinity)} was calculated by dividing the last observed quantifiable serum concentrations by the slope of the terminal phase as estimated by linear regression by using the best fit option of WinNonlin.
Mean Residence Time (MRT), which refers to the average total time BPA-\textit{d}_6 molecules of a given BPA-\textit{d}_6 dose spend in the body, was obtained with and without extrapolation to infinity by using statistical moments (Gibaldi and Perrier, 1982). MRT can be viewed as the arithmetic mean of times that each BPA-\textit{d}_6 molecule spends in the body, and it is a metric of persistency of BPA in the body because it is a stochastic view of BPA-\textit{d}_6 pharmacokinetics in the body.

The apparent oral clearance (Cl/F) was obtained by dividing the administered BPA-\textit{d}_6 dose by the corresponding AUC(0-\text{Clast}), \text{C}_{\text{last}} being the 24h serum BPA-\textit{d}_6 concentration. The sparse data option of WinNonlin was used, allowing computation of the different standard errors (SE) associated with estimated parameters. Definitions of the different computed parameters are given in Supplemental Material, Table 3.

Results of the NCA for unconjugated and total after oral bolus and diet exposure are presented in Supplemental Material, Tables 4 and 5.

3.2 Compartmental analysis of unconjugated BPA-\textit{d}_6 concentrations after oral bolus exposure

The terminal half-life (T\textsubscript{1/2}) was defined as \( \frac{\ln(2)}{K_{10}} \) and T\textsubscript{max}, time at the maximal concentration (C\textsubscript{max}) as \( \frac{\ln(K_{01} / K_{10})}{K_{01} - K_{10}} \). The area under the concentration curve \textit{versus} time (AUC) from time zero to the last detectable concentration was calculated as \( AUC = \frac{D}{V \times K_{10}} \) with \( \frac{V}{F} \times K_{10} \) being
the apparent clearance (Clearance/F). The estimated final BPA parameters are presented in Supplemental Material, Table 6.

### 3.3 Deconvolution analysis

The curve representing mean unconjugated BPA-$d_6$ serum concentrations over time (0-11 h) was presented with automatic smoothing procedure in Supplemental Material, Figure 3, and these are the data that were analyzed by deconvolution.

Deconvolution computed for each discretization step (here 0.08h or 5.04 min, Supplemental Material, Table 7) was the amount of BPA-$d_6$ that entered the blood from the feed. For example, at 1.93 h, the BPA-$d_6$ input rate was 1.23 mg/h and at that time the total amount of BPA-$d_6$ that had entered the bloodstream was 0.448 mg *in toto* representing 2.87 % of the dose that was actually bioavailable at the end period of observation. The shape of the input rate during the night is given by Supplemental Material, Figure 4. Visual inspection of the curve indicates that after time 0 (19:00 h in the trial), there was a lag time of about 1 h before BPA-$d_6$ entered the bloodstream. This delay was presumably due to food consumption and gastrointestinal transit times, release of BPA-$d_6$ from food, and BPA-$d_6$ absorption. Thereafter, the BPA-$d_6$ input rate increased rapidly to reach a maximum value at 4.2 h (23:12 h in the trial) and then returned to base line after a delay of 7 to 8 h, after the beginning of the evening meal (i.e at about 02:00 to 03:00 h the next day). The cumulative input rate indicates that 50 % of the bioavailable BPA-$d_6$ dose had entered the bloodstream at 4.11h (23:00 h) and that 90 % of the dose gained access to blood at 5.46 h after the beginning of the meal, i.e. before 1:00 AM the next day. The total
estimated absorbed dose between 0 and 11 h by deconvolution analysis was 15.6 mg/kg over the first 11 h.

**4.0 References**

Gibaldi, M. and Perrier, D. (1982) *Pharmacokinetics*, 2nd edn., Marcel Dekker, New York.

Padmanabhan V, Siefert K, Ransom S, Johnson T, Pinkerton J, Anderson L, et al. 2008.

Maternal bisphenol-A levels at delivery: a looming problem? J Perinatol 28(4): 258-263.
Supplemental Material, Table 1: Components of AIN-93G control diet with 7% corn oil from Harlan-Teklad

| Diet Component                        | g/kg  |
|--------------------------------------|-------|
| Casein                               | 200.0 |
| L-Cystine                            | 3.0   |
| Corn Starch                          | 397.386 |
| Maltodextrin                         | 132.0 |
| Sucrose                              | 100.0 |
| Corn Oil                             | 70.0  |
| Cellulose                            | 35.0  |
| Mineral Mix, AIN-93G-MX (94046)       | 35.0  |
| Vitamin Mix, AIN-93-VX (94047)        | 10.0  |
| Choline Bitartrate                   | 2.5   |
| TBHQ, Antioxidant                    | 0.014 |

Selected Nutrient Information

|                        | % by Weight | % kcal |
|------------------------|-------------|--------|
| Protein                | 17.7        | 18.8   |
| Carbohydrate           | 60.1        | 63.9   |
| Fat                    | 7.2         | 17.2   |

Kcal/g 3.8
**Supplemental Material, Table 2:** Components of BPA-$d_6$ diet (100mg/kg feed weight) from Harlan-Teklad

| Diet Component                                               | g/kg   |
|--------------------------------------------------------------|--------|
| Casein                                                       | 200.0  |
| L-Cystine                                                    | 3.0    |
| Corn Starch                                                  | 397.386|
| Maltodextrin                                                 | 132.0  |
| Sucrose                                                      | 90.0   |
| Corn Oil                                                     | 70.0   |
| Cellulose                                                    | 35.0   |
| Mineral Mix, AIN-93G-MX (94046)                              | 35.0   |
| Vitamin Mix, AIN-93-VX (94047)                               | 10.0   |
| Choline Bitartrate                                           | 2.5    |
| TBHQ, Antioxidant                                            | 0.014  |
| BPA-$d_6$ Mixture (1% in Sucrose)                            | 10.0   |
| Pink Food Color                                              | 0.1    |

**Selected Nutrient Information**

|                           | % by Weight | % kcal |
|---------------------------|-------------|--------|
| Protein                   | 17.7        | 18.8   |
| Carbohydrate              | 60.0        | 63.9   |
| Fat                       | 7.2         | 17.3   |

Kcal/g 3.8
**Supplemental Material, Table 3:** Definition of the different pharmacokinetic parameters computed by using a non-compartmental analysis

| Pharmacokinetic Parameter | Definition |
|---------------------------|------------|
| AUC\_\%Extrap\_obs        | Percentage of AUCINF\_obs that is due to extrapolation from T\_last to infinity; extrapolation done with lambda\_z; AUC: Area under the curve |
| AUCINF\_obs               | AUC from time of dosing (0) to infinity; extrapolation with the last quantifiable (i.e. above LOQ) concentration divided by the terminal slope (lambda\_z) |
| AUCLast                   | AUC from time of dosing (0) to the time of the last quantifiable concentration |
| Cl\_F\_obs                | Apparent total serum clearance for extravascular administration (or oral clearance) calculated from AUCINF\_obs |
| Cl\_F\_last               | Apparent total serum clearance for extravascular administration (or oral clearance) calculated from AUCLast |
| Cl\_last                  | Concentration observed at T\_last |
| Cmax                      | Maximal serum BPA concentration |
| HL\_Lambda\_z             | Terminal half-life (ln(2)/terminal slope); best fit option of WinNonlin |
| MRTINF\_obs               | Mean Residence Time (MRT) extrapolated to infinity using the last quantifiable serum concentration for extrapolation |
| MRT\_last                 | Mean Residence Time (MRT) from time of dosing to the last quantifiable serum BPA concentration |
| T\_last                   | Time of last quantifiable serum concentration |
| Tmax                      | Time of maximal serum BPA concentration |

| Additional output | Definition |
|-------------------|------------|
| Corr\_XY          | Correlation between time (X) and log concentration (Y) for the points used in estimation of the terminal slope (lambda\_z) |
| SE\_AUCLast       | Standard error (SE) associated with AUCLast estimate for sparse data analysis in WinNonlin (mouse data) |
| SE\_Cmax          | Standard error (SE) associated with Cmax estimate for sparse data analysis in WinNonlin (mouse data) |
**Supplemental Material, Table 4:** Pharmacokinetic parameters of unconjugated and total BPA-$d_6$ obtained by a non compartmental data analysis in mice, BPA-$d_6$ dose of 20 mg/kg by oral route of exposure

| Parameter                  | Units     | Estimate for Unconjugated BPA-$d_6$ | Estimate for Total BPA-$d_6$ |
|---------------------------|-----------|-------------------------------------|-----------------------------|
| Rsq                       |           | 0.9946                              | 0.9683                      |
| Rsq_adjusted              |           | 0.9893                              | 0.9366                      |
| Corr_XY                   |           | -0.9973                             | -0.9840                     |
| No_points_lambda_         |           | 3.0000                              | 3.0000                      |
| Lambda_z                 | 1/hr      | 0.1356                              | 0.1758                      |
| Lambda_z_lower           | hr        | 6.0000                              | 6.0000                      |
| Lambda_z_upper           | hr        | 24.0000                             | 24.0000                     |
| HL_Lambda_z              | hr        | 5.1109                              | 3.9435                      |
| Tlag                     | hr        | 0.0000                              | 0.0000                      |
| Tmax                     | hr        | 1.0000                              | 6.0000                      |
| Cmax                     | ng/mL     | 21.0000                             | 1636.5000                   |
| SE_Cmax                  | ng/mL     | 3.9038                              | 642.6338                    |
| Tlast                    | hr        | 24.0000                             | 24.0000                     |
| Clast                    | ng/mL     | 1.2533                              | 80.1000                     |
| AUClast                  | hr*ng/mL  | 200.9942                            | 21979.3583                  |
| SE_AUClast               | hr*ng/mL  | 20.6535                             | 3813.4673                   |
| AUCall                   | hr*ng/mL  | 200.9942                            | 21979.3583                  |
| SE_AUCall                | hr*ng/mL  | 20.6535                             | 3813.4673                   |
| AUCINF_obs               | hr*ng/mL  | 210.2355                            | 22435.0636                  |
| AUCINF_D_obs             | hr*kg*ng/mL/n | 0.0000 | 0.0006 |
| AUC_%Extrap_obs          | %         | 4.3957                              | 2.0312                      |
| Vz_F_obs                 | mL/kg     | 701443.2838                         | 8984.5299                   |
| Cl_F_obs                 | mL/hr/kg  | 95131.4064                          | 1579.2244                   |
| AUCINF_pred              | hr*ng/mL  | 210.5049                            | 22478.8676                  |
| AUCINF_D_pred            | hr*kg*ng/mL/n | 0.0000 | 0.0006 |
| AUC_%Extrap_pred         | %         | 4.5181                              | 2.2221                      |
| Vz_F_pred                | mL/kg     | 700545.6892                         | 8967.0220                   |
| Cl_F_pred                | mL/hr/kg  | 95009.6725                          | 1576.1470                   |
| AUMClast                 | hr*hr*ng/mL | 1462.5800 | 175837.4333 |
| AUMCINF_obs              | hr*hr*ng/mL | 1752.5126 | 189366.9594 |
| Parameter                        | Unit       | Observed | Predicted |
|---------------------------------|------------|----------|-----------|
| AUMC_%Extrap_ob                 | %          | 16.5438  | 16.9443   |
| AUMCINF_pred                    | hr*hr*ng/mL| 1760.9636| 190667.4665|
| MRTlast                         | hr         | 7.2767   | 8.0001    |
| MRTINF_obs                      | hr         | 8.3359   | 8.4407    |
| MRTINF_pred                     | hr         | 8.3654   | 8.4821    |
**Supplemental Material, Table 5:** Pharmacokinetic parameters of unconjugated and total BPA-\textsubscript{d\textsubscript{6}} obtained by a non compartmental data analysis in mice exposed to BPA-\textsubscript{d\textsubscript{6}} through the diet

| Parameter       | Units      | Estimate for Unconjugated BPA-\textsubscript{d\textsubscript{6}} | Estimate for Total BPA-\textsubscript{d\textsubscript{6}} | Estimate for Unconjugated BPA-\textsubscript{d\textsubscript{6}} | Estimate for Total BPA-\textsubscript{d\textsubscript{6}} |
|-----------------|------------|---------------------------------------------------------------|---------------------------------------------------------|---------------------------------------------------------------|---------------------------------------------------------|
| Tmax            | h          | 6.0000                                                       | 6.0000                                                  | 6.0000                                                        | 6.0000                                                  |
| Cmax            | ng/mL      | 18.8150                                                     | 802.2500                                               | 28.9460                                                        | 1234.2300                                               |
| SE_Cmax         | ng/mL      | 4.3763                                                       | 126.5717                                               | 6.7327                                                        | 194.7239                                               |
| Cmax_D          | kg*ng/mL/ng| 0.0000                                                       | 0.0000                                                  | 0.0000                                                        | 0.0001                                                  |
| Tlast           | h          | 24.0000                                                      | 24.0000                                                 | 24.0000                                                        | 24.0000                                                 |
| Clast           | ng/mL      | 6.9417                                                       | 193.9333                                               | 10.6782                                                        | 296.8600                                                |
| AUClast         | h*ng/mL    | 147.8358                                                     | 11547.3200                                             | 227.4235                                                        | 17755.3500                                              |
| SE_AUClast      | h*ng/mL    | 26.7339                                                      | 1219.5527                                              | 41.1317                                                        | 1874.6794                                               |
| AUCall          | h*ng/mL    | 147.8358                                                     | 11547.3200                                             | 227.4235                                                        | 17755.3500                                              |
| SE_AUCall       | h*ng/mL    | 26.7339                                                      | 1219.5527                                              | 41.1317                                                        | 1874.6794                                               |
| AUCINF\_obs    | h*ng/mL    | 359.5439                                                     | 13867.3357                                             | 553.0144                                                        | 21293.9150                                              |
| AUCINF\_D\_obs | h*kg*ng/mL/ng | 0.0000                                                       | 0.0004                                                  | 0.0000                                                        | 0.0016                                                  |
| AUC\_%Extrap\_obs | %       | 58.8824                                                      | 16.7301                                                | 58.8757                                                        | 16.6177                                                  |
| Vz\_F\_obs    | mL/kg      | 1102719.5094                                                | 30564.4501                                             | 716774.4759                                                    | 7277.1835                                                |
| Cl\_F\_obs    | mL/h/kg    | 36156.9223                                                   | 2554.9248                                              | 23507.5252                                                      | 610.5030                                                  |
| AUCINF\_pred  | h*ng/mL    | 312.0034                                                     | 13993.5203                                             | 479.9031                                                        | 21487.2824                                               |
| AUCINF\_D\_pred | h*kg*ng/mL/ng | 0.0000                                                       | 0.0004                                                  | 0.0000                                                        | 0.0017                                                  |
| AUC\_%Extrap\_pred | %       | 52.6172                                                      | 17.4810                                                | 52.6105                                                        | 17.3681                                                  |
| Vz\_F\_pred   | mL/kg      | 1270742.7590                                                | 30288.8394                                             | 825972.2273                                                    | 7211.6950                                                |
| Cl\_F\_pred   | mL/h/kg    | 41666.2141                                                   | 2531.8861                                              | 27088.8036                                                      | 605.0090                                                  |
| AUMClast       | h*ng/mL    | 1830.9650                                                    | 123052.8533                                            | 2816.6625                                                       | 189078.0700                                              |
| AUMCINF\_obs  | h*ng/mL    | 13368.6633                                                   | 206487.4734                                            | 20558.5290                                                      | 316183.2496                                              |
| AUMC\_%Extrap\_obs | %       | 86.3041                                                      | 40.4066                                                | 86.2993                                                        | 40.1998                                                  |
| AUMCINF\_pred | h*ng/mL    | 10777.7943                                                   | 211025.4451                                            | 16574.5991                                                      | 323129.0041                                              |
| AUMC\_%Extrap\_pred | %       | 83.0117                                                      | 41.6881                                                | 83.0062                                                        | 41.4853                                                  |
| MRTlast        | h          | 12.3851                                                      | 10.6564                                                | 12.3851                                                        | 10.6491                                                  |
| MRTINF\_obs   | h          | 37.1823                                                      | 14.8902                                                | 37.1754                                                        | 14.8485                                                  |
| MRTINF\_pred  | h          | 34.5438                                                      | 15.0802                                                | 34.5374                                                        | 15.0382                                                  |
**Supplemental Material, Table 6:** Pharmacokinetic parameters of unconjugated BPA-\(d_6\)

obtained by compartmental data analysis in mice, BPA-\(d_6\) dose of 20 mg/kg by oral bolus route

| Parameter | Units       | Estimate     | Std Error     | CV%  |
|-----------|-------------|--------------|---------------|------|
| V_F       | mL/kg       | 873624.901968 | 115632.597853 | 13.24|
| Initial   | l/hr        | 3.299308     | 5.038292      | 152.71|
| Terminal  | l/hr        | 0.108765     | 0.018661      | 17.16 |
| AUC       | hr*ng/mL    | 210.483104   | 22.448839     | 10.67 |
| Initial t\(\frac{1}{2}\) | hr         | 0.210089     | 0.320501      | 152.56|
| Terminal t\(\frac{1}{2}\) | hr         | 6.372909     | 1.092303      | 17.14 |
| Tmax      | hr          | 1.069499     | 1.183828      | 110.69|
| Cmax      | ng/mL       | 20.379160    | 2.671239      | 13.11 |
**Supplemental Material, Table 7:** Deconvolution results giving input rate (mg/h) for step of 0.084 h (5.04 min), corresponding cumulative input (mg) and in percentage of the total input rate

| Time (h) | Input Rate (mg/h) | Cumulative Input (mg) | Fraction Input |
|---------|-------------------|-----------------------|----------------|
| 0.00    | 0.000000          | 0.000000              | 0.000000       |
| 0.08    | 0.000000          | 0.000000              | 0.000000       |
| 0.17    | 0.000000          | 0.000000              | 0.000000       |
| 0.25    | 0.000000          | 0.000000              | 0.000000       |
| 0.34    | 0.000000          | 0.000000              | 0.000000       |
| 0.42    | 0.000000          | 0.000000              | 0.000000       |
| 0.50    | 0.000000          | 0.000000              | 0.000000       |
| 0.59    | 0.000000          | 0.000000              | 0.000000       |
| 0.67    | 0.000000          | 0.000000              | 0.000000       |
| 0.76    | 0.000000          | 0.000000              | 0.000000       |
| 0.84    | 0.000000          | 0.000000              | 0.000000       |
| 0.92    | 0.000000          | 0.000000              | 0.000000       |
| 1.01    | 0.000164          | 0.000000              | 0.000000       |
| 1.09    | 0.021896          | 0.000682              | 0.00044        |
| 1.18    | 0.074875          | 0.004551              | 0.00292        |
| 1.26    | 0.152720          | 0.013951              | 0.00894        |
| 1.34    | 0.250350          | 0.030751              | 0.00197        |
| 1.43    | 0.363730          | 0.056435              | 0.00361        |
| 1.51    | 0.489640          | 0.092185              | 0.00590        |
| 1.60    | 0.625520          | 0.138940              | 0.00890        |
| 1.68    | 0.769330          | 0.197460              | 0.01265        |
| 1.76    | 0.919460          | 0.268320              | 0.01719        |
| 1.85    | 1.074600          | 0.352010              | 0.02255        |
| 1.93    | 1.233800          | 0.448900              | 0.02876        |
| 2.02    | 1.396100          | 0.559300              | 0.03583        |
| 2.10    | 1.561000          | 0.683430              | 0.04379        |
| 2.18    | 1.727800          | 0.821500              | 0.05263        |
| 2.27    | 1.896300          | 0.973650              | 0.06238        |
| 2.35    | 2.066100          | 1.140000              | 0.07304        |
| 2.44    | 2.236900          | 1.320700              | 0.08462        |
| 2.52    | 2.408500          | 1.515700              | 0.09711        |
| 2.60    | 2.580700          | 1.725100              | 0.11054        |
| 2.69    | 2.753400          | 1.949100              | 0.12489        |
| 2.77    | 2.926600          | 2.187600              | 0.14017        |
| 2.86    | 3.100000          | 2.440600              | 0.15638        |
| 2.94    | 3.273800          | 2.708200              | 0.17353        |
| 3.02    | 3.447700          | 2.990400              | 0.19161        |
| 3.11    | 3.621800          | 3.287200              | 0.21062        |
| 3.19    | 3.796000          | 3.598600              | 0.23058        |
| 3.27    | 3.970400          | 3.924700              | 0.25147        |
| 3.36    | 4.144800          | 4.265400              | 0.27330        |
| X   | Y     | Z     | A     |
|-----|-------|-------|-------|
| 3.44| 4.319300 | 4.620800 | 0.296070 |
| 3.53| 4.493800 | 4.990800 | 0.319780 |
| 3.61| 4.668400 | 5.375500 | 0.344430 |
| 3.69| 4.843000 | 5.774800 | 0.370020 |
| 3.78| 5.017600 | 6.188800 | 0.396540 |
| 3.86| 5.192200 | 6.617400 | 0.424010 |
| 3.95| 5.366900 | 7.060800 | 0.452410 |
| 4.03| 5.535200 | 7.518700 | 0.481750 |
| 4.11| 5.632400 | 7.988100 | 0.511830 |
| 4.20| 5.656300 | 8.462500 | 0.542230 |
| 4.28| 5.621800 | 8.936400 | 0.572590 |
| 4.37| 5.540800 | 9.405400 | 0.602640 |
| 4.45| 5.422800 | 9.865900 | 0.632150 |
| 4.53| 5.275300 | 10.315000 | 0.660940 |
| 4.62| 5.104500 | 10.751000 | 0.688870 |
| 4.70| 4.915000 | 11.172000 | 0.715830 |
| 4.79| 4.710700 | 11.576000 | 0.741740 |
| 4.87| 4.494600 | 11.963000 | 0.766500 |
| 4.95| 4.269100 | 12.331000 | 0.790080 |
| 5.04| 4.036100 | 12.679000 | 0.812430 |
| 5.12| 3.797200 | 13.008000 | 0.833500 |
| 5.21| 3.553500 | 13.317000 | 0.853280 |
| 5.29| 3.306100 | 13.605000 | 0.871730 |
| 5.37| 3.055700 | 13.872000 | 0.888850 |
| 5.46| 2.802900 | 14.118000 | 0.904610 |
| 5.54| 2.548200 | 14.434000 | 0.919010 |
| 5.63| 2.292000 | 14.546000 | 0.932030 |
| 5.71| 2.034500 | 14.728000 | 0.943670 |
| 5.79| 1.776200 | 14.888000 | 0.953920 |
| 5.88| 1.517000 | 15.026000 | 0.962780 |
| 5.96| 1.257300 | 15.142000 | 0.970240 |
| 6.05| 1.005600 | 15.237000 | 0.976320 |
| 6.13| 0.800240 | 15.313000 | 0.981150 |
| 6.21| 0.636810 | 15.373000 | 0.985000 |
| 6.30| 0.506760 | 15.421000 | 0.988060 |
| 6.38| 0.403270 | 15.459000 | 0.990500 |
| 6.47| 0.320910 | 15.489000 | 0.992440 |
| 6.55| 0.255370 | 15.513000 | 0.993990 |
| 6.63| 0.203220 | 15.532000 | 0.995210 |
| 6.72| 0.161720 | 15.547000 | 0.996190 |
| 6.80| 0.128690 | 15.560000 | 0.996970 |
| 6.89| 0.102410 | 15.569000 | 0.997590 |
| 6.97| 0.081493 | 15.577000 | 0.998080 |
| 7.05| 0.064850 | 15.583000 | 0.998470 |
| 7.14| 0.051606 | 15.588000 | 0.998780 |
| 7.22| 0.041067 | 15.592000 | 0.999030 |
|   |      |      |      |
|---|------|------|------|
| 7.31 | 0.032680 | 15.595000 | 0.999230 |
| 7.39 | 0.026006 | 15.597000 | 0.999390 |
| 7.47 | 0.020695 | 15.599000 | 0.999510 |
| 7.56 | 0.016468 | 15.601000 | 0.999610 |
| 7.64 | 0.013105 | 15.602000 | 0.999690 |
| 7.73 | 0.010429 | 15.603000 | 0.999750 |
| 7.81 | 0.008299 | 15.604000 | 0.999800 |
| 7.89 | 0.006604 | 15.604000 | 0.999840 |
| 7.98 | 0.005255 | 15.605000 | 0.999880 |
| 8.06 | 0.004182 | 15.605000 | 0.999900 |
| 8.15 | 0.003328 | 15.606000 | 0.999920 |
| 8.23 | 0.002648 | 15.606000 | 0.999940 |
| 8.31 | 0.002108 | 15.606000 | 0.999950 |
| 8.40 | 0.001677 | 15.606000 | 0.999960 |
| 8.48 | 0.001335 | 15.606000 | 0.999970 |
| 8.56 | 0.001062 | 15.606000 | 0.999980 |
| 8.65 | 0.000845 | 15.607000 | 0.999980 |
| 8.73 | 0.000673 | 15.607000 | 0.999980 |
| 8.82 | 0.000535 | 15.607000 | 0.999990 |
| 8.90 | 0.000426 | 15.607000 | 0.999990 |
| 8.98 | 0.000339 | 15.607000 | 0.999990 |
| 9.07 | 0.000270 | 15.607000 | 0.999990 |
| 9.15 | 0.000215 | 15.607000 | 0.999990 |
| 9.24 | 0.000171 | 15.607000 | 1.000000 |
| 9.32 | 0.000136 | 15.607000 | 1.000000 |
| 9.40 | 0.000108 | 15.607000 | 1.000000 |
| 9.49 | 0.000086 | 15.607000 | 1.000000 |
| 9.57 | 0.000068 | 15.607000 | 1.000000 |
| 9.66 | 0.000055 | 15.607000 | 1.000000 |
| 9.74 | 0.000043 | 15.607000 | 1.000000 |
| 9.82 | 0.000035 | 15.607000 | 1.000000 |
| 9.91 | 0.000027 | 15.607000 | 1.000000 |
| 9.99 | 0.000022 | 15.607000 | 1.000000 |
| 10.08 | 0.000017 | 15.607000 | 1.000000 |
| 10.16 | 0.000014 | 15.607000 | 1.000000 |
| 10.24 | 0.000011 | 15.607000 | 1.000000 |
| 10.33 | 0.000009 | 15.607000 | 1.000000 |
| 10.41 | 0.000007 | 15.607000 | 1.000000 |
| 10.50 | 0.000006 | 15.607000 | 1.000000 |
| 10.58 | 0.000004 | 15.607000 | 1.000000 |
| 10.66 | 0.000004 | 15.607000 | 1.000000 |
| 10.75 | 0.000003 | 15.607000 | 1.000000 |
| 10.83 | 0.000002 | 15.607000 | 1.000000 |
| 10.92 | 0.000002 | 15.607000 | 1.000000 |
| 11.00 | 0.000001 | 15.607000 | 1.000000 |
**Supplemental Material, Figure 1:** Semi-logarithmic plot of observed (○) and fitted (−) unconjugated BPA-\(d_6\) concentrations versus time (h) after oral bolus at 20 mg/kg BW. Pooled data were fitted by using the so-called Bateman equation and weighted by the inverse of the observed value (1/Yobs).
Supplemental Material, Figure 2: Time versus weighted residual pooled serum concentrations of BPA-\(d_6\) for a monocompartmental model without lag-time. Visual inspection of the graph indicates appropriate scatter of residuals (no bias, homoscedasticity), and thus, the goodness of the fit of the model.
Supplemental Material, Figure 3: Curve representing the mean unconjugated BPA-$d_6$ serum concentration profile (ng/mL) between 0 and 11 h after the beginning of the meal obtained by a smoothing procedure (not a modelling).
Supplemental Material, Figure 4: Input rate (mg/h) versus time (h) of BPA-$d_6$ in mice fed with the supplemented BPA-$d_6$ diet.