**Note to readers with disabilities:** *EHP* strives to ensure that all journal content is accessible to all readers. However, some figures and Supplemental Material published in *EHP* articles may not conform to 508 standards due to the complexity of the information being presented. If you need assistance accessing journal content, please contact ehp508@niehs.nih.gov. Our staff will work with you to assess and meet your accessibility needs within 3 working days.

## Supplemental Material

**A Combined Morphometric and Statistical Approach to Assess Nonmonotonicity in the Developing Mammary Gland of Rats in the CLARITY-BPA Study**

Maël Montévil, Nicole Acevedo, Cheryl M. Schaeberle, Manushree Bharadwaj, Suzanne E. Fenton, and Ana M. Soto

## Table of Contents

**Figure S2.** *Simulated dose response with a=0.6 (without correlations).* The midline represents the median, the box represents the quartiles above and below the median and the whiskers represent the two other quartiles, excluding outliers. A: We represent a simulation with 10000 "animals" per group to show the shape of our simulated distribution. B: several iterations of our simulated distribution with the usual 10 animal per group.

**Figure S3.** *Effect of BPA on body weight and on mammary gland weight in PND21C.* Control: vehicle control, BPA: bisphenol A. Units: µg /kg body weight (bw)/day. The midline represents the median, the box represents the quartiles above and below the median and the whiskers represent the two other quartiles, excluding outliers. Number of animals per group n=8-10.

**Figure S4.** *Semiquantitative scoring of postnatal day 90 pilot (PND90P) glands.* Control: vehicle control, EE2: ethinyl estradiol, BPA: bisphenol A. Units: µg /kg body weight (bw)/day. A) PND90P animals from Fenton group in which the majority of animals were in estrus at necropsy (only females in estrus included; n=7, 10, 10, 4, 6, 4, 4; from left to right). * Indicates significantly accelerated gland development compared to vehicle controls (Kruskal Wallis; BPA 2.5 p=0.05, EE5 p=0.01). # Indicates increased gland proliferation that did not reach significance (Kruskal Wallis; BPA 25 p=0.09, EE0.5 p=0.1). B) PND90P animals that were cycling from both Fenton and Soto groups, with all estrous cycle stages at necropsy included except anestrus (n=12, 18, 14, 10, 12, 12, 15, from left to right). All animals in A were included in B analysis.

**Figure S5.** *Dimension 1 to 3 from PCA of PND21C animals with (top) and without (bottom) EE2 treatments.* Control: vehicle control, EE2: ethinyl estradiol, BPA: bisphenol A. Units: µg /kg body weight (bw)/day. We represent the average of each exposure group. Number of animals per group n=8-10.

**Figure S6.** *Comparison of the changes between consecutive doses for the 94 features in PND21C described in Table S 2.* Vehicle: vehicle control, BPA: bisphenol A. Units: µg /kg body weight (bw)/day. Largest consecutive changes meeting criterion B(0.5) for each observed feature in PND21C. All consecutive differences are normalized to a maximum of 1, in yellow. No data means that the criterion B(0.5) is not met for a given feature and consecutive concentration.

**Figure S7.** *Estimated type 1 error rates on data generated by simulation (0.05 in black, 0.01 in blue, 0.005 in red).* A, C; the different variables are not correlated by construction. B,D: the different variables are correlated with coefficients stemming from our data. A, B: Type 1 error rate as a function of the threshold for criterion B($p_{thr}$), with 20 variables. C, D: Type 1 error rate as a function of the number of features observed for $p_{thr}$ =0.5.

**Figure S8.** *Estimated type 2 error rates on data generated by simulation (0.05 in black, 0.01 in blue, 0.005 in red).* A, C, E: the different variables are not correlated by construction. B,D,F: the different variables are correlated with coefficients stemming from our data. A, B: type 2 error rate as a function of the threshold for criterion B($p_{thr}$), with 20 variables and a=0.6 which is an intermediate value. C,D: type 2 error rate as a function of a with N=20. E, F: type 2 error rate as a function of the number N of variables describing each individual with a=0.6, and $p_{thr}$=0.5.

**Figure S9.** *Graphical tests to assess the quality of the regressions in PND21 animals.* Control: vehicle control, BPA: bisphenol A. Units: µg /kg body weight (bw)/day.The method is provided by the lm method in cran R. The first graph, Residual versus Fitted assesses the presence of a pattern not taken into account by the model and homoscedasticity (i.e., that variance is constant). The second graph assesses the normality of residuals. The third graph is used to assess homoscedasticity. The fourth graph aims at assessing the presence of outliers. Last, the fifth graph displays a box plot of the data and the fitted model. The midline represents the median, the box represents the quartiles above and below the median and the whiskers represent the two other quartiles, excluding outliers. The features represented are A sd width 3D, B Thickness, C Fractal dimension in 3D, D Angle between beginning and end (here, the pattern does not fit the model completely), E Dim.3 resulting from PCA and F Aspect ratio.

**Figure S10.** *Graphical tests to assess the quality of the regressions in 90 day and 6 month animals.* The method is provided by the lm method in cran R. The first graph, Residual versus Fitted, assesses the presence of a pattern not taken into account by the model and homoscedasticity (i.e., that variance is constant). The second graph assesses the normality of residuals. The third graph is used to assess homoscedasticity. The fourth graph aims at assessing the presence of outliers. Last, the fifth graph displays a box plot of the data and the fitted model. The midline represents the median, the box represents the quartiles above and below the median and the whiskers represent the two other quartiles, excluding outliers. The features represented are A Mammary gland weight in PND90SD, B Density in area 3 in PND90CD, C Density in area 3 in 6MCD and D Density in area 3 in 6MSD.