Genotoxicity-Related Chemistry of Human Metabolites of Benzo[*ghi*]perylene (B[*ghi*]P) investigated using Electro-optical Arrays and DNA/Microsome Biocolloid Reactors with LC-MS/MS

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Supporting Information

1. Film Characterization of ECL Array. Film compositions used in the array were characterized by making the films on 9 MHz quartz crystal microbalance (QCM) gold-quartz resonators. Resonators were coated with 3-mercaptopropanoic acid before applying following layers. As shown in Fig. S1, the average change in QCM frequency (-ΔF) increased linearly with number of films for all three film types, indicative of stable and reproducible film growth.

Adsorbed mass/area (M/A) of each layer for dried films was obtained from the frequency change (ΔF) using the Sauerbrey equation:[1]

$$M\ (g/cm^2) = -\ \Delta F\ (Hz)/1.83\times10^8$$

Nominal thickness (*d*) was estimated using an expression confirmed by high-resolution electron microscopy.[2]

$$d\ (nm) = (-0.016\pm0.002)\ \Delta F\ (Hz)$$

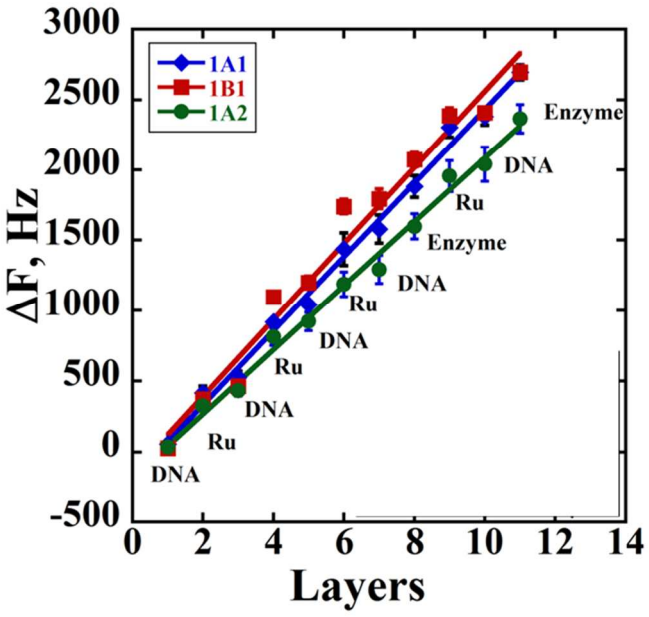

Figure S1. Quartz crystal microbalance frequency shifts for alternate adsorption on gold quartz resonators for RuPVP/DNA/enzymes film, Ru=RuPVP, enzyme sources are supersomes 1A1 (blue diamond), 1B1 (red square), and 1A2 (green dot).

The weight of each component and nominal film thickness calculated using equations above is shown in Table S1.

Table S1. Average composition of DNA/RuPVP/enzyme films from QCM.

Film (DNA/RuPVP/Enzyme)	DNA (μg cm^{-1})	RuPVP (μg cm^{-1})	Enzymes (μg cm^{-1})	Film Thickness (nm)
RuPVP/DNA/1A1	2.8±0.3	8.0±1.1	3.1±0.5	45.0±5.5
RuPVP/DNA/1B1	2.4±0.3	7.6±0.8	3.3±0.4	38.0±1.5
RuPVP/DNA/1A2	2.6±0.2	7.9±0.9	3.6±0.5	42.0±1.0

2. Activation of PAHs by human supersomes.

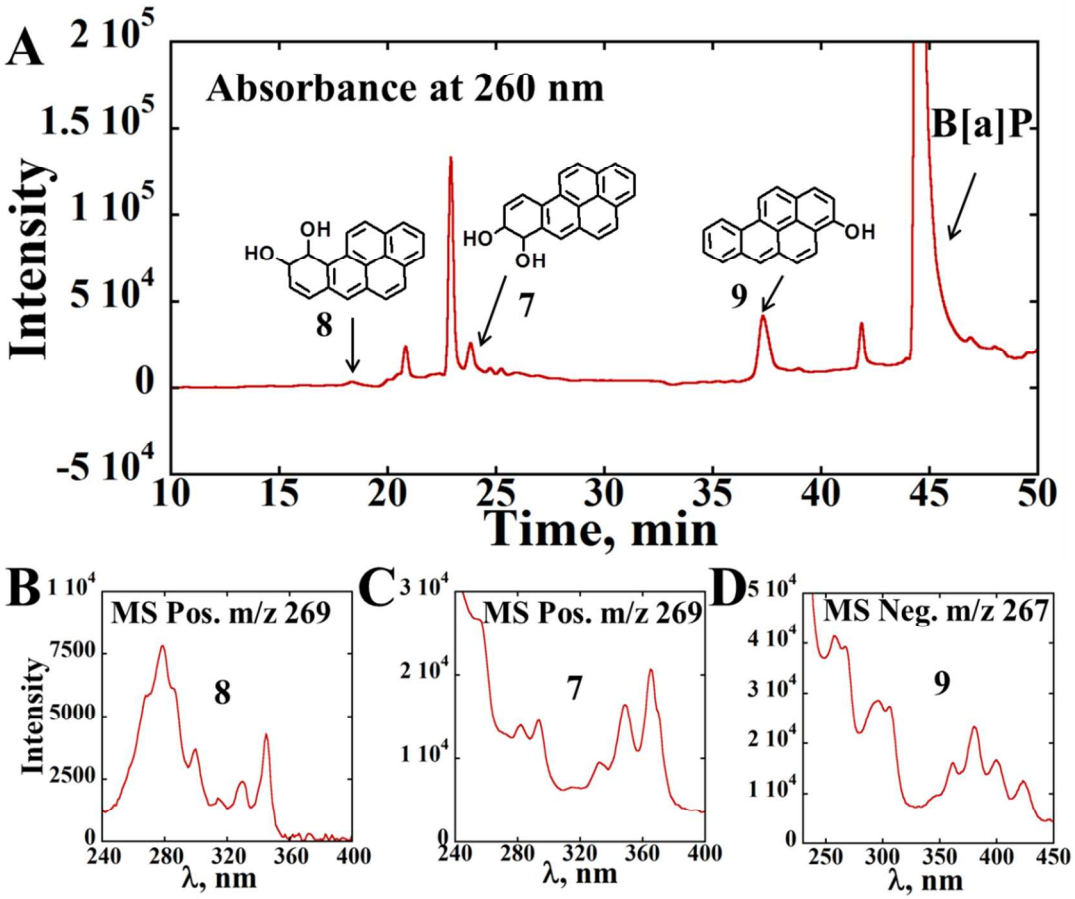

Figure S2. LC analysis of major B[*a*]P metabolites after incubations with biocolloid reactor particles with P450 1A1 supersome for 20 min. (A) representative LC chromatogram at an absorbance wavelength of 260 nm. Arrows represent peaks of 9,10-dihydroxy-9,10-dihydro B[*a*]P (B[*a*]P 9, 10-diol), 7,8-dihydroxy-7,8-dihydro B[*a*]P (B[*a*]P 7,8-diol) and 3-hydroxy B[*a*]P (3-OH B[*a*]P) with structures shown. The UV spectra of (B), B[*a*]P 9, 10-diol; (C), B[*a*]P 7,8-diol; (D), 3-OH B[*a*]P.

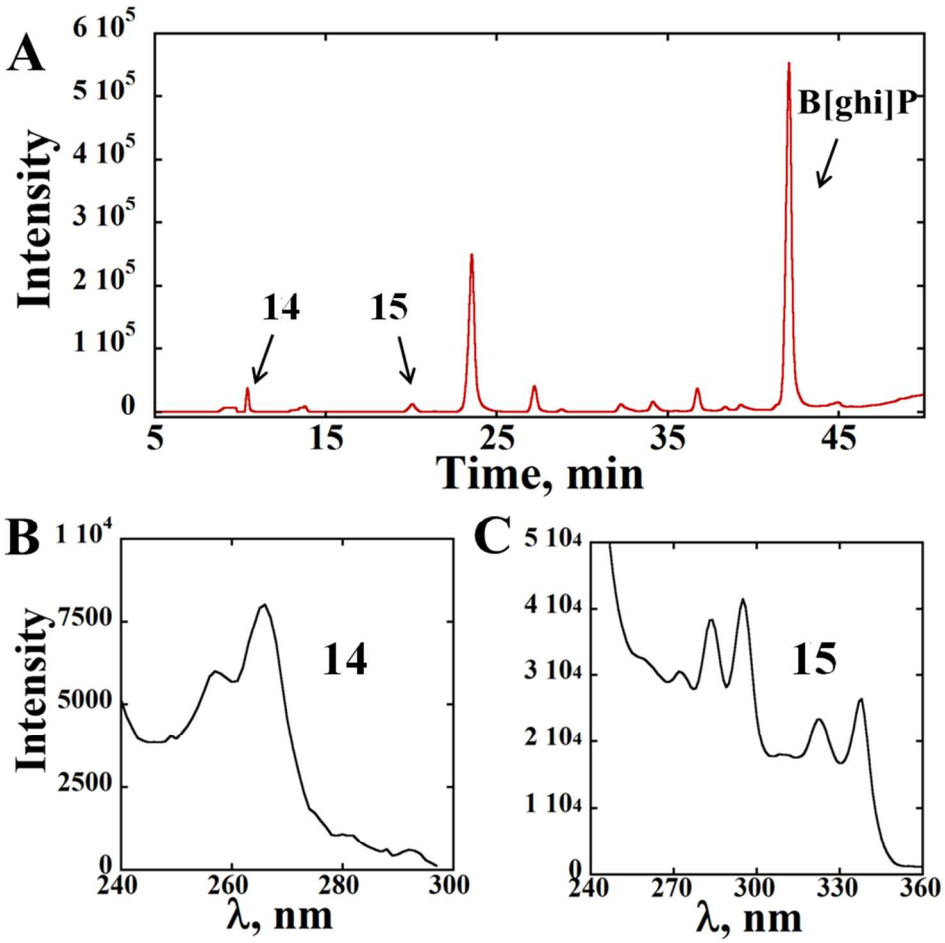

Figure S3. Liquid chromatogramic results for B[*ghi*]P metabolism analysis after a 20-min reaction with P450 1A1 supersomes. (A) A representative chromatogram monitored at UV absorbance of 260 nm; arrows represent compounds **14** and **15,** and B[*ghi*]P. (B) and (C) UV spectra of compounds **14** and **15**.

3. Synthesis of B[*ghi*]P 3,4-oxide. The formation of B[*ghi*]P 3,4-oxide was confirmed by the appearance of two doublets of H3 and H4 at 4.95 and 5.02 ppm in the proton NMR spectrum and a UV spectrum characteristic of the chromophore of 3,4-dihydro B[*ghi*]P. The amount of B[*ghi*]P 3, 4-oxide in the mixture was determined by integration of the H3/H4 doublets compared to the intensity of the aromatic protons in the 1H NMR spectrum with B[*ghi*]P 3,4 oxide content percentage of ~ 40%

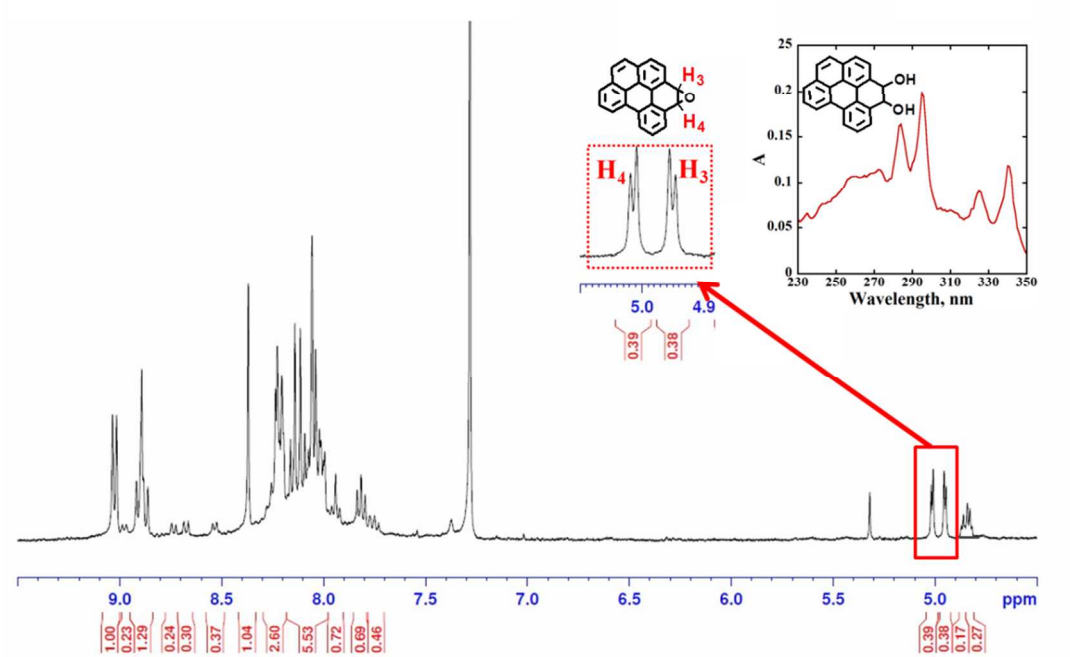

Figure S4. 1H NMR spectrum of products of B[*ghi*]P DMDO reaction. Inset is the UV chromatogram corresponded to hydrolyzed product of synthesized B[*ghi*]P 3,4 oxide.

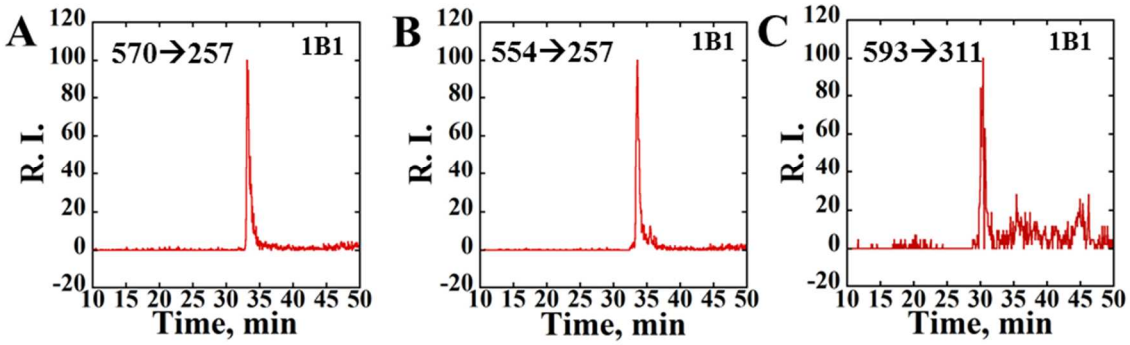

Figure S5. LC-MS/MS analysis of reactions of magnetic biocolloid reactors with B[*a*]P and B[*ghi*]P using 1B1 supersomes. (A) and (B) Representative SRM chromatogram with mass transition m/z 570→257 and m/z 570→257 indicating the formation of BPDE-dG and BPDE-dA adducts after 20 min reaction followed by enzyme hydrolysis using supersomes 1B1. (C) Representative SRM chromatogram with mass transition m/z 593→311 indicating the formation of B[*ghi*]P 3,4,11,12-bisoxides-dG adducts after 20 min reaction followed by enzyme hydrolysis using supersomes 1B1.

4. Reaction of nucleophiles (Nu) with active BPDE carbenium ion and B[*ghi*]P 3,4 oxide.

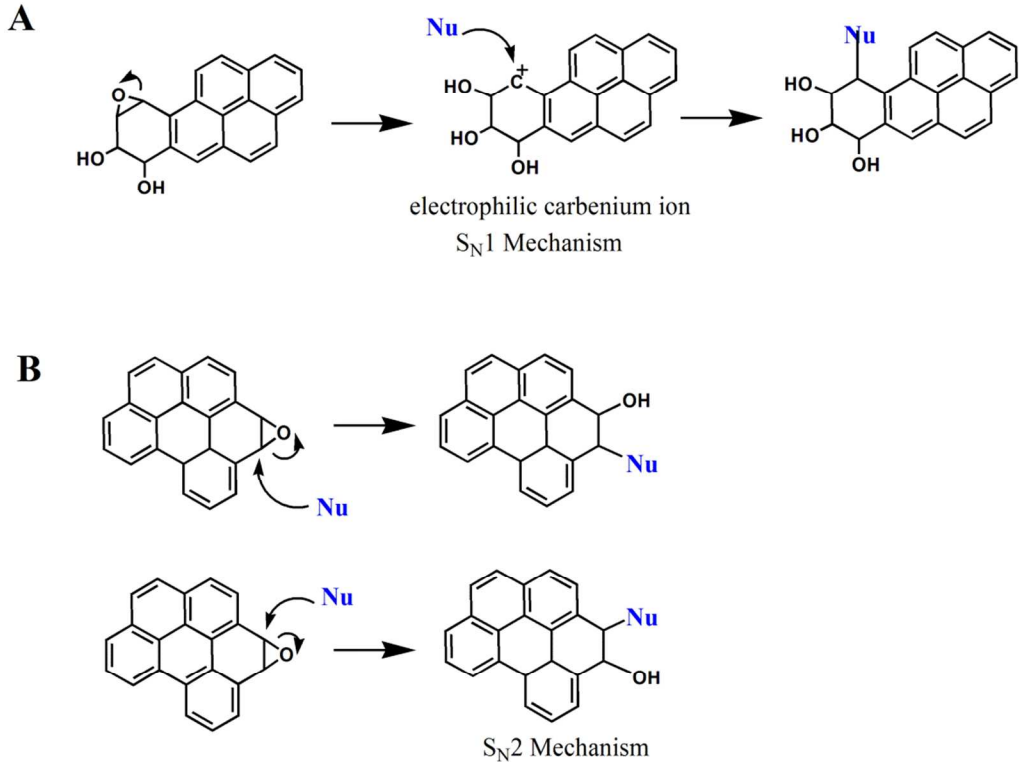

Figure S6. (A) Formation of highly reactive BPDE carbenium ions which is followed by SN1 reaction mechanism to form DNA adducts. (B) Formation of B[*ghi*]P 3,4-oxide which is followed by SN2 reaction mechanisms to form DNA adducts. Nu stands for nucleophiles such as DNA bases.

References

(1) Lvov, Y. M., Lu, Z., Schenkman, J. B., Zu, X., and Rusling, J. F. (1998) Direct Electrochemistry of Myoglobin and Cytochrome P450cam in Alternate Layer-by-Layer Films with DNA and Other Polyions. *J. Am. Chem. Soc. 120*, 4073-4080.

(2) Lvov, Y., Ariga, K., Ichinose, I., and Kunitake, T. (1995) Assembly of Multicomponent Protein Films by Means of Electrostatic Layer-by-Layer Adsorption. *J. Am. Chem. Soc. 117*, 6117-6123.