Supplementary Information

Mixture Effects of Benzene, Toluene, Ethylbenzene and Xylenes to Lung Carcinoma Cells via a Hanging Drop Air Exposure System

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Table S1. GCMS operation specification from Liu et al.[1] Reprinted from *J. Hazard. Mater.*, 261, Liu, F. F., Peng, C., Escher, B. I., Fantino, E., Giles, C., Were, S., Duffy, L. and Ng, J. C. , Hanging drop: An *in vitro* air toxic exposure model using human lung cells in 2D and 3D structures., 701-710, Copyright 2013, with permission from Elsevier.

GC-MS parameter	Descriptions
Oven Temperature	40°C for 1 min, then ramped at 10°C/min to 120°C
Carrier Gas	Helium at 1 mL/min
Operation mode	SIM (selected ion monitoring)
Mass Spectrometer Detector	78, 77 and 51 m/z
Injection method	10 µL samples were injected directly through the septum using a 50 µL air tight Hamilton syringe
Inlet temperature	250°C
Injection mode	splitless mode for 0.5 min with split flow 50 mL/min

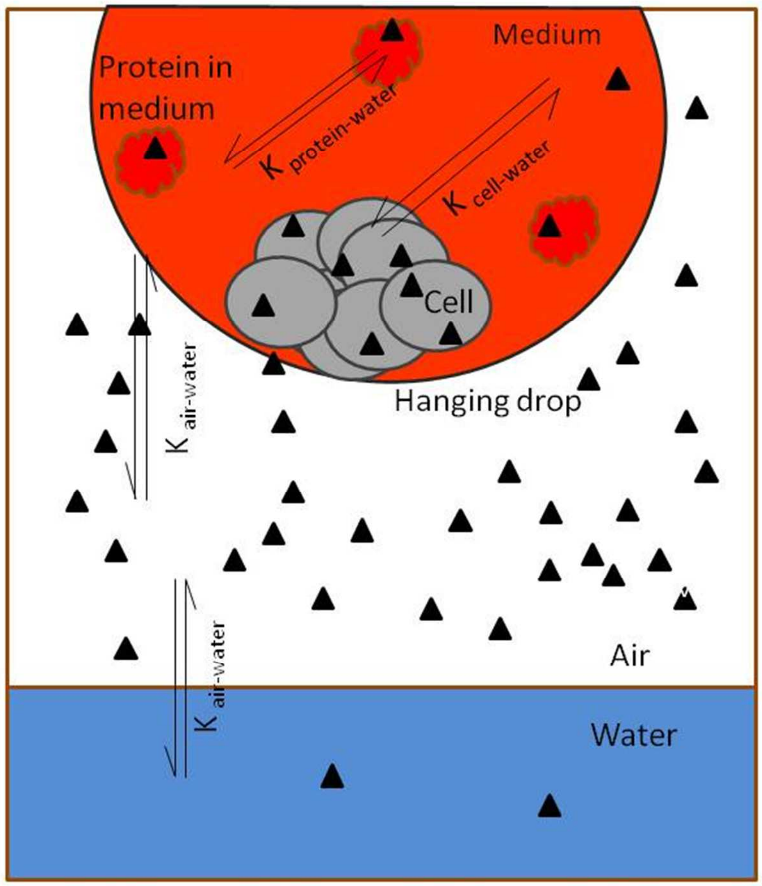

Figure S1. Distribution of benzene, toluene, ethylbenzene and xylenes (BTEX) within a hanging drop air exposure system (not up to scale, the hanging drop is only 20 µL and the volatile organic analysis vials can be 20 mL or 40 mL and black triangles represent BTEX molecules in the system).

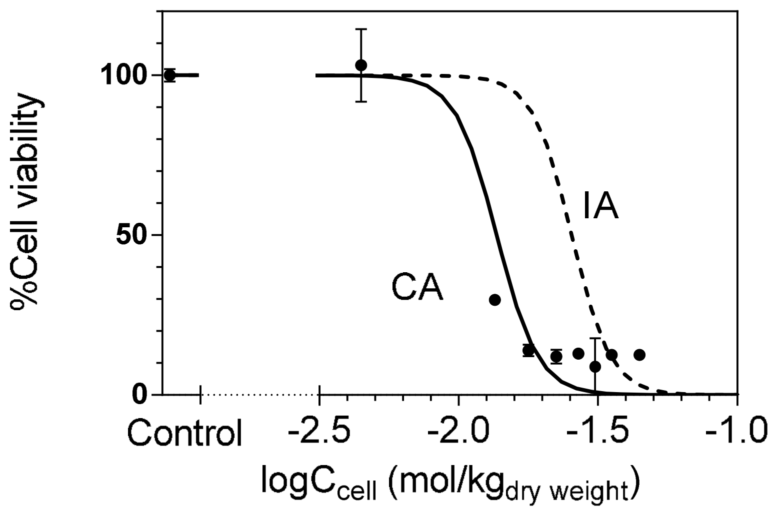

Figure S2. Concentration addition and independent action modeling for double toluene mixture 1 h exposure. Index of prediction quality (IPQ) for CA = 0.20.

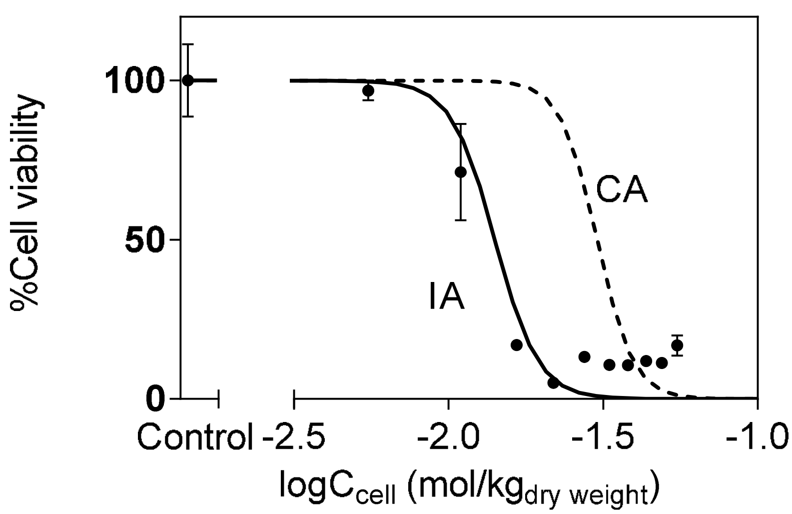

Figure S3. Concentration addition and independent action modeling for double ethylbenzene mixture for 1 h exposure. IPQ for CA = 0.10.

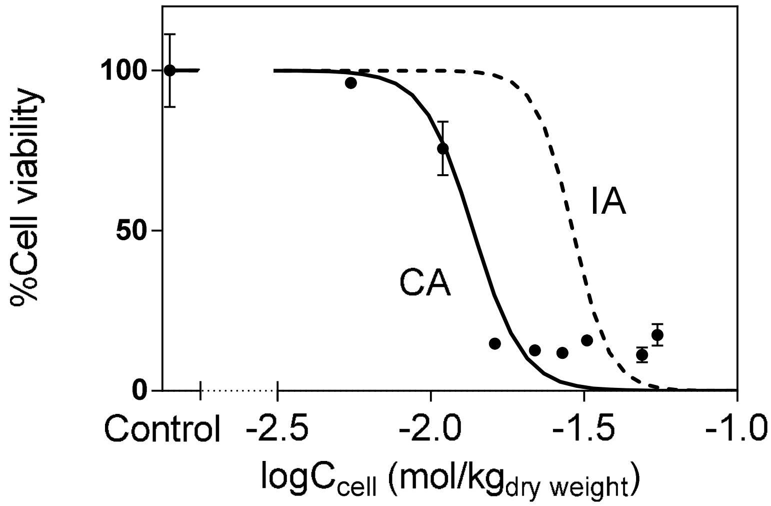

Figure S4. Concentration addition and independent action modeling for double m-xylene mixture for 1 h exposure. IPQ for CA = 0.06.

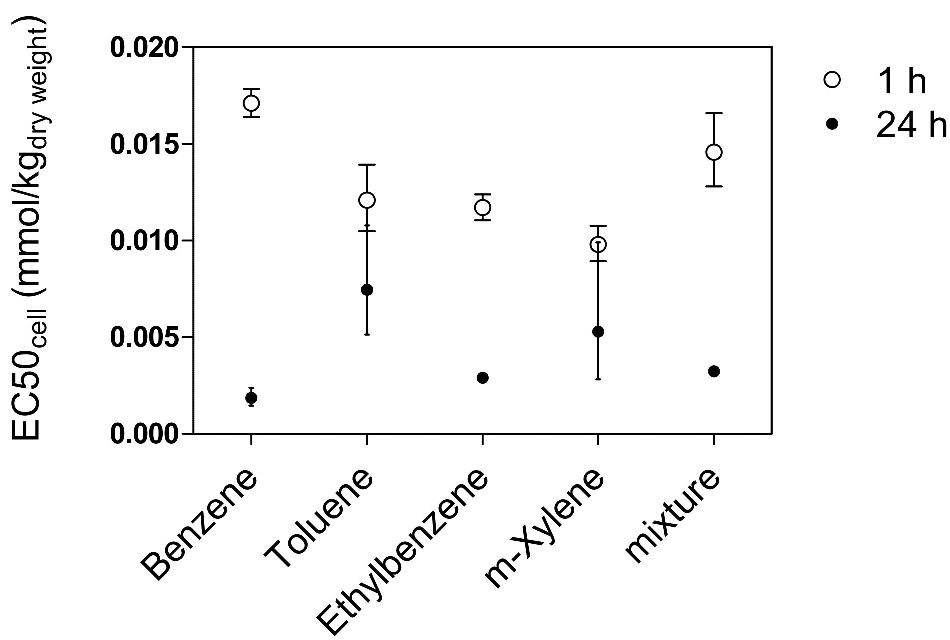

Figure S5. Changes in EC_{50} values of A549 cells exposed to BTE*m*X for 1 h and 24 h. The decrease in benzene concentration of 30% over 24 h period was not shown in the graph as the changes are gradual which may or may not be the same for all BTEX component. *P*-xylene and *o*-xylene were not included in this test due to the lowest injection volume limitation.

Calculation of air BTEX concentration

Air benzene was introduced into VOA vials as liquid form using 10μL air tight Hamilton syringes and hence the benzene concentration in ppm was calculated as below according to Bankand et al. [2]:

$$Air\ ppm\ concentration = \left\{ \frac{10^6 W / MW}{V / V_m} \right\}$$

$$V_m = 24.45 \left(\frac{760}{P}\right)\left(\frac{t + 273.15}{296.15}\right)$$

Vm = molecular volume in a sealed bottle at 37°C= 24.45

W = weight of volatile liquid introduced in grams

MW = molecular weight

Since

Benzene Density= 0.879g/cm^3 =8.79 x10^{-4}g/μL, and molecular weight = 78.1 g/mol;

Toluene Density=0.8661 g/cm$^{3=}$ 8.661 x10^{-4}g/μL, and molecular weight = 92.1g/mol;

Ethylbenzene Density=0.867 g/cm$^{3=}$ 8.67 x10^{-4}g/μL, and molecular weight = 106.2 g/mol;

Xylene Density=0.865 g/cm$^{3=}$ 8.65 x10^{-4}g/μL, and molecular weight = 106.2 g/mol.

Hence in the sealed container, the Pa may change however the Vm will not change

1μL of benzene in 20mL vial $= \dfrac{10\text{E}6 \text{ x}8.79 \text{ x}10\text{E}{-4} / 78.1}{0.02 / 24.45}$

1μL of benzene in 20mL vial = 13758.994 (ppm) of benzene

1μL of Toluene in 20mL vial $= \frac{10E6 \text{ x } 8.661 \text{ x}10E-4 / 92.1}{0.02 / 24.45}$

$= 11496.279$ (ppm) of toluene

1μL of ethylbenzene in 20mL vial $= \frac{10E6 \text{ x}8.67\text{x}10E-4 / 106.2}{0.02 / 24.45}$

$= 9980.297$ (ppm) of ethylbenzene

1μL of m-xylene in 20mL vial $= \frac{10E6 \text{ x } 8.65\text{x}10E-4 / 106.2}{0.02 / 24.45}$

$= 9957.274$ (ppm) of m-xylene

1μL of p-xylene in 20mL vial $= \frac{10E6 \text{ x } 8.65 \text{ x}10E-4 / 106.2}{0.02 / 24.45}$

$= 9957.274$ (ppm) of p-xylene

Reference:

(1) Liu, F. F., Peng, C., Escher, B. I., Fantino, E., Giles, C., Were, S., Duffy, L. and Ng, J. C. (2013) Hanging drop: An *in vitro* air toxic exposure model using human lung cells in 2D and 3D structures. *J. Hazard. Mater. 261*, 701-710.

(2) Bakand, S., Winder, C., Khalil, C. and Hayes, A. (2006) A novel *in vitro* exposure technique for toxicity testing of selected volatile organic compounds. *J. Environ. Monit. 8*, 100-105.