Electronic Supplementary Material (ESI) for Toxicology Research.

Supplementary Information

EFFECTS OF MULTI-COMPONENT MIXTURES OF POLYAROMATIC HYDROCARBONS AND HEAVY METAL/LOID(S) ON Nrf2-ANTIOXIDANT RESPONSE ELEMENT (ARE) PATHWAY IN ARE REPORTER–HepG2 CELLS

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Table S2- Individual PAHs (benzo[a]pyrene (B[a]P), naphthalene (Nap), phenanthrene (Phe), and pyrene (Pyr)) and metal/loid(s) (arsenic (As), cadmium (Cd), lead (Pb)): Cytotoxicity and activation of Nrf2 antioxidant pathway response in ARE reporter-HepG2 cells.

Figure S1. Individual dose response of arsenic (As), cadmium (Cd) and lead (Pb) for activation of Nrf2 antioxidant pathway in ARE reporter-HepG2 cells.

Figure S2. Individual dose response of benzo[a]pyrene (B[a]P), naphthalene (Nap), phenanthrene (Phe), pyrene (Pyr), t-BHQ for activation of Nrf2 antioxidant pathway in ARE reporter-HepG2 cells.

Table S1. Concentrations of individual chemicals in the multi-component mixtures of PAHs and metal/loid(s) mixtures for activation of Nrf2 antioxidant response pathway assay in ARE reporter-HepG2 cells.

Binary mixtures	As (μM)	Cd (μM)	Pb (μM)	B[a]P (μM)	Nap (μM)	Phe (μM)	Pyr (μM)
Binary mixtures of heavy metal/loid(s)							
As + Cd	1.5	0.5					
As + Pb	1.5		3				
Cd + Pb		0.5	3				
Binary mixtures of B[a]P + heavy metal/loid(s)							
B[a]P + As	1.5			1.5			
B[a]P + Cd		0.5		1.5			
B[a]P + Pb		--	3	1.5			
Binary mixtures of B[a]P + other PAHs							
B[a]P + Nap				1.5	5		
B[a]P + Phe				1.5		5	
B[a]P + Pyr				1.5			5
Ternary mixture of heavy metal/loid(s)							
As + Cd + Pb	1.5	0.5	3				
Ternary mixtures of B[a]P + heavy metal/loid(s)							
B[aP + As + Cd	1.5	0.5		1.5			
B[aP + As + Pb	1.5		3	1.5			
B[aP + Cd + Pb		0.5	3	1.5			
Ternary mixtures of B[a]P + other PAHs							
B[a]P + Nap + Phe				1.5	5	5	
B[a]P + Nap + Pyr				1.5	5		5
B[a]P + Phe + Pyr				1.5		5	5
Quaternary mixtures of B[a]P + heavy metal/loid(s)							
B[a]P + As + Cd + Pb	1.5	0.5	3	1.5			
Quaternary mixtures of B[a]P + other PAHs							
B[a]P + Nap + Phe + Pyr				1.5	5	5	5
Seven-component mixtures							
PAHs + Heavy metal/loid(s)	1.5	0.5	3	1.5	5	5	5

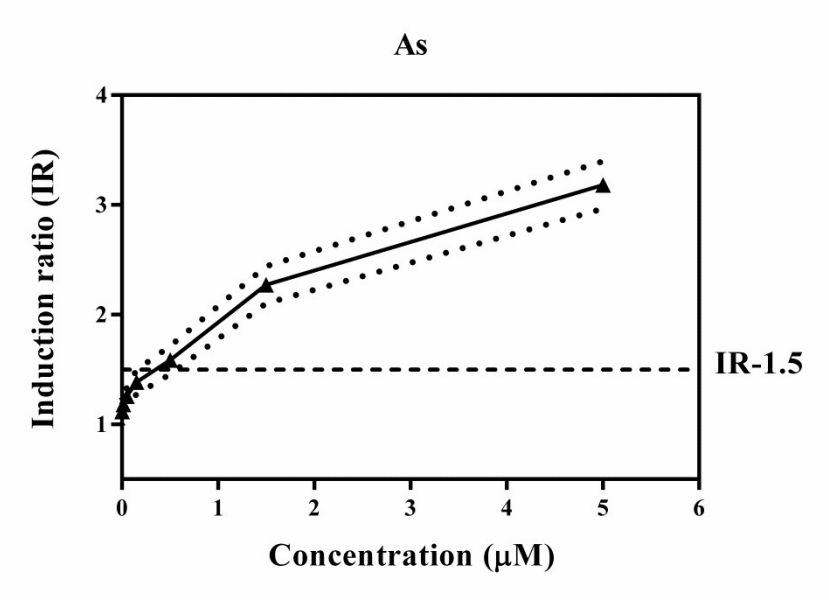
Chemicals were mixed at 1:1 ratio using a fixed concentration ratio based on their individual $EC_{IR1.5}$ value and diluted in 1: 3 serial dilution for seven times and dose response study was carried out. For ternary and quaternary mixtures of B[a]P + PAHs, mixtures were serially diluted for 6 times and dose response study was carried out. The chemical mixtures and vehicle control were treated in triplicate for each experiment. Three independent experiments were conducted for all each mixtures except for quaternary mixtures of B[a]P + other PAHs and seven-component mixtures, two independent experiments was carried out.

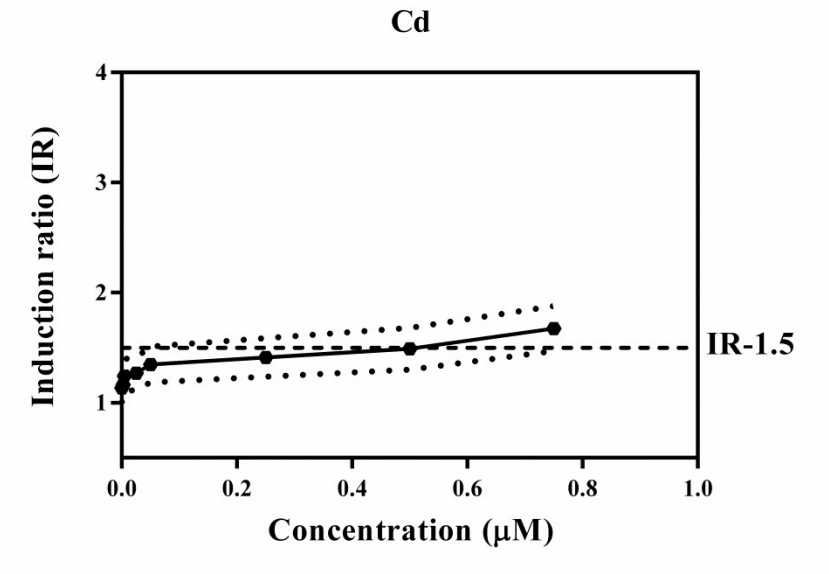
Table S2: Individual PAHs (benzo[a]pyrene (B[a]P), naphthalene (Nap), phenanthrene (Phe), and pyrene (Pyr)) and metal/loid(s) (arsenic (As), cadmium (Cd), lead (Pb)): Cytotoxicity and activation of Nrf2 antioxidant pathway response in ARE reporter-HepG2 cells.

	Cytotoxicity	**Nrf2 antioxidant pathway induction**
Chemical	**IC_{50} (μM)**	**$EC_{IR1.5}$ (μM)**
As	72 (65 – 79)	1.09 ± 0.23
Cd	3.36 (2.9 – 4)	0.58 ±0.24
Pb	108 (96 – 122)	3.10 ± 0.57
B[a]P*	Maximum of 30% reduction in cell viability at 25 μM	0.93 ± 0.27
Nap	N.D.	7.10 ± 2.60
Phe	N.D.	6.40 ± 1.60
Pyr	N.D.	7.20 ± 2.50

Cytotoxicity was determined using MTS assay. Chemicals were exposed to ARE reporter-HepG2 cells for 24 h. IC_{50} – Cytotoxicity expressed as concentration causing 50% inhibition of cell growth; *- reduction in cell viability at >12.5 μM and max. of 30% reduction in cell viability was observed at 25 μM. N.D. = Toxicity was not detected up to maximum soluble concentration in culture medium.

Nrf2 antioxidant pathway induction by PAHs and metal/loid(s) was determined using the luciferase assay system. Chemicals were exposed to ARE reporter-HepG2 cells for 24 h. $EC_{IR1.5}$ – Concentration that results in 1.5 fold of induction in luciferase assay. Values are expressed as mean ± SD (n=9 from three independent experiments)


As
Induction ratio (IR)
4
3
2
1
0
1
2
3
4
5
6
IR-1.5
Concentration (μM)


Cd
Induction ratio (IR)
4
3
2
1
0.0
0.2
0.4
0.6
0.8
1.0
IR-1.5
Concentration (μM)

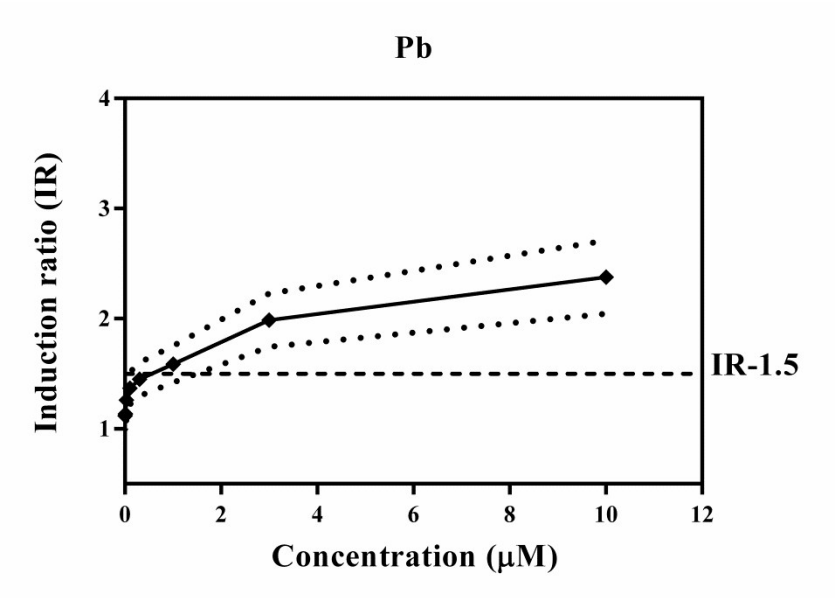
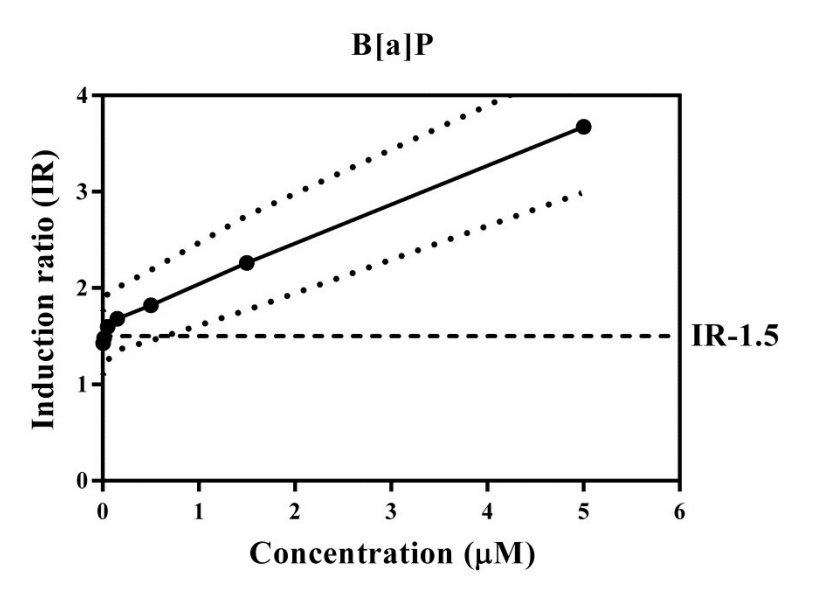
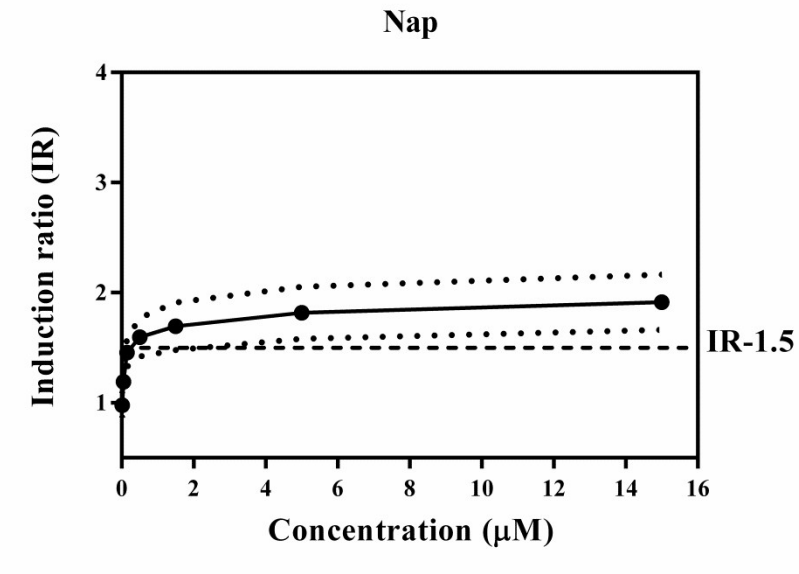
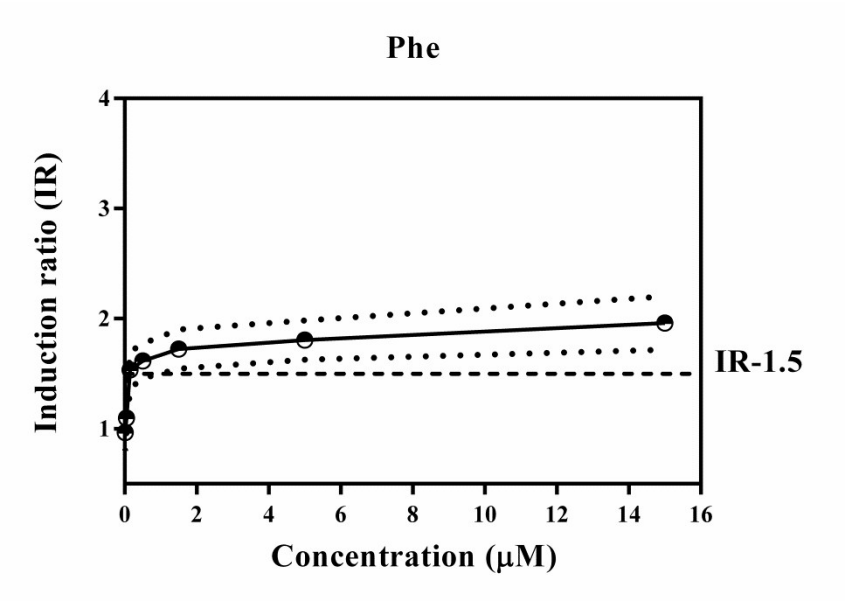
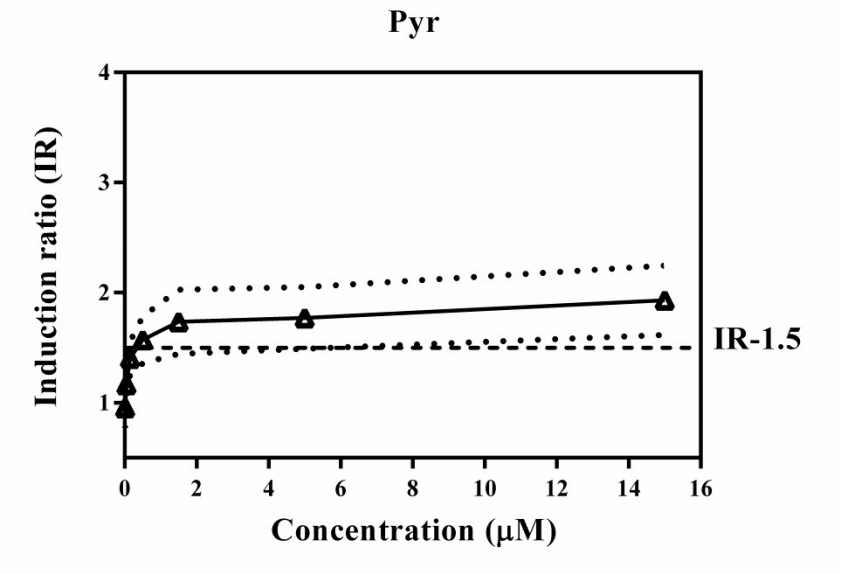


Figure S1. Individual dose response of arsenic (As), cadmium (Cd) and lead (Pb) for activation of Nrf2 antioxidant pathway in ARE reporter-HepG2 cells after 24 h exposure. The experimental data are from three independent experiments in triplicate for each exposure concentration. Values are expressed as mean and dashed line indicates 95% confidence interval. IR- induction ratio


B[a]P
Induction ratio (IR)
Concentration (μM)
IR-1.5
0
1
2
3
4
5
6


Nap
Induction ratio (IR)
Concentration (μM)
IR-1.5
0
2
4
6
8
10
12
14
16


Phe
Induction ratio (IR)
4
3
2
1
0
2
4
6
8
10
12
14
16
Concentration (μM)
IR-1.5


Pyr
Induction ratio (IR)
4
3
2
1
0
2
4
6
8
10
12
14
16
Concentration (μM)
IR-1.5

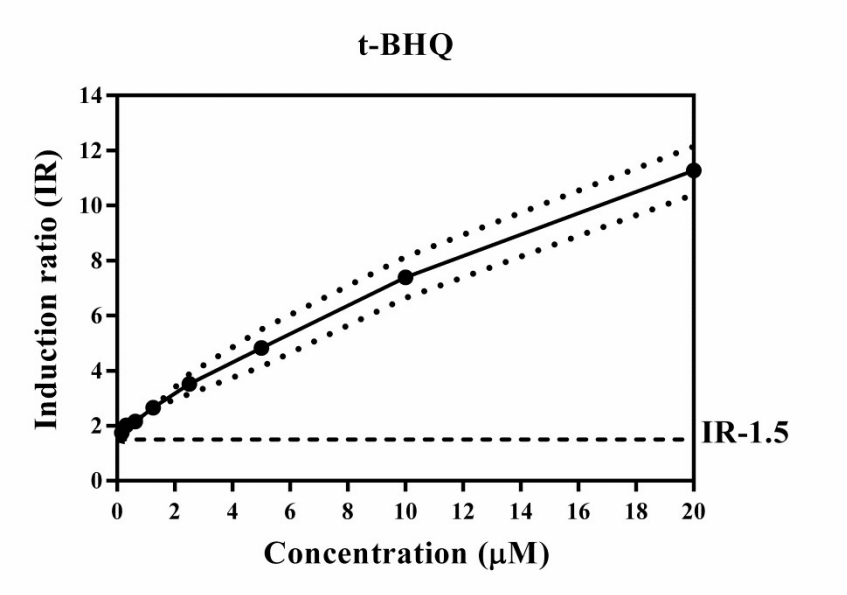


Figure S2. Individual dose response of benzo[a]pyrene (B[a]P), naphthalene (Nap), phenanthrene (Phe), pyrene (Pyr), tert-butylhydroquinone (t-BHQ) for activation of Nrf2 antioxidant pathway in ARE reporter-HepG2 cells after 24 h exposure. The experimental data are from three independent experiments in triplicate for each exposure concentration. Values are expressed as mean and dashed line indicates 95% confidence interval. IR- induction ratio