

Serra et al.:

Hazard Assessment of Air Pollutants: The Transforming Ability of Complex Pollutant Mixtures in the Bhas 42 Cell Model

Supplementary Data

Tab. S1: Bhas 42 CTA protocol

Bhas 42 CTA Protocol	OECD Bhas 42 CTA GD definition	Seeding density	Chemical Treatment	Proposed definition
Schedule 1	Initiation assay	Low (4 x 10 ³ cell/well)	72 h, from Day 1 to Day 4 after seeding	Acute exposure assay
Schedule 2	Promotion assay	High (14 x 10 ³ cells/well)	10 days, from Day 4 to Day 14 after seeding, repeated at each medium change (3 times)	Late repeated exposure assay

Tab. S2: Complete list of PAHs and NPAHs measured in the PM extracts

The chemicals are ordered by IARC classification.

Chemical name	CAS number	IARC classification	References	Autumn 2012		Summer 2013	
				PM ₁ (ng/m ³)	PM _{2.5} (ng/m ³)	PM ₁ (ng/m ³)	PM _{2.5} (ng/m ³)
PAHs							
Benzo[a]pyrene	50-32-8	1	1,2,3	0.062	0.173	0.030	0.002
Cyclopenta[cd]pyrene	27208-37-3	2A	1,2	0.030	0.024	0.011	0.010
Dibenz[a,c][a,h]anthracene	[a,h] 53-70-3 + [a,c] 215-58-7	2A + 3	1,2	0.079	0,078	0,002	0,003
Dibenzo[a,i]pyrene	191-30-0	2A	1,2	0.036	0.034	0.002	< LOD
Naphthalene	91-20-3	2B	4	< LOD	< LOD	< LOD	< LOD
Benzo[a]anthracene	56-55-3	2B	1,2	0.153	0.150	0.002	0.010
Chrysene	218-01-9	2B	2	0.260	0.257	0.020	0.018
Benzo[k]fluoranthene	207-08-9	2B	2	0.288	0.257	0.009	0.009
Indeno[1,2,3-cd]pyrene	193-39-5	2B	1,2	0.320	0.310	0.010	0.012
Dibenzo[a,i]pyrene	189-55-9	2B	2	0.004	0.002	< LOD	< LOD
Dibenzo[a,h]pyrene	189-64-0	2B	1,2	< LOD	< LOD	< LOD	< LOD
Benzo(b+j)fluoranthene	205-99-2 + 205-82-3	2B + 2B	2	0.811	0.889	0.041	0.040
Acenaphthene	83-32-9	3	2	< LOD	< LOD	< LOD	< LOD
Fluorene	86-73-7	3	1,2	0.002	< LOD	< LOD	< LOD
Phenanthrene	85-01-8	3	1,2	0.061	0.033	0.032	0.022
Anthracene	120-12-7	3	1,2	0.001	0.002	< LOD	< LOD
Fluoranthene	206-44-0	3	1,2	0.139	0.130	0.037	0.032
Pyrene	129-00-0	3	1,2	0.149	0.128	0.034	0.038
Benzo[e]pyrene	192-97-2	3	1,2	0.404	0.394	0.022	0.023
Perylene	198-55-0	3	1,2	0.019	0.033	< LOD	< LOD
Benzo[ghi]perylene	191-24-2	3	1,2	0.558	0.521	0.018	0.022
Dibenzo[a,e]fluoranthene	5385-75-1	3	1,2	0.047	0.045	< LOD	< LOD
Dibenzo[a,e]pyrene	192-65-4	3	1,2	0.035	0.040	< LOD	< LOD
Acenaphthylene	208-96-8	-	-	< LOD	< LOD	< LOD	< LOD
NPAHs							
1-Nitropyrene	5522-43-0	2A	1,5,6	0.026	0.027	< LOD	< LOD
6-Nitrochrysene	7496-02-8	2A	1,5,6	< LOD	< LOD	< LOD	< LOD
2-Nitrofluorene	607-57-8	2B	5,6	< LOD	< LOD	< LOD	< LOD
3-Nitrobenzanthrone	17117-34-9	2B	5	< LOD	< LOD	< LOD	< LOD
1,6-Dinitropyrene	42397-64-8	2B	5,6	< LOD	< LOD	< LOD	< LOD

1,8-Dinitropyrene	42397-65-9	2B	1,5,6	< LOD	< LOD	< LOD	0.022
9-Nitroanthracene	602-60-8	3	1,7	< LOD	< LOD	< LOD	< LOD
3-Nitrofluoranthene	892-21-7	3	1,7	0.356	0.334	< LOD	< LOD
7-Nitrobenz[a]anthracene	20268-51-3	3	6	0.029	0.026	< LOD	< LOD
6-Nitrobenzo[a]pyrene	63041-90-7	3	1, 6	< LOD	< LOD	< LOD	< LOD
9-Nitrophenanthrene	954-46-1	na	na	< LOD	< LOD	< LOD	< LOD

LOD, Limit of Detection; na, not available

References

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Tab. S3: Effects of the organic extracts of PM₁ and PM_{2.5} samples collected in autumn or summer on the transformation rate of Bhas 42 cells

Treatment		CG ^a				Foci/well ^b				
Autumn 2012										
Acute exposure assay										
Untreated cells		104	0.67	±	0.50		104	0.67	±	0.50
DMSO 0.5% ^c		100	0.67	±	0.50		100	0.67	±	0.50
3-MCA 1 µg/ml (0.5% DMSO) ^d		65	10.00	±	2.97 _↓		65	10.00	±	2.97 _↓
urban site (m ³ /well)										
	PM₁					PM_{2.5}				
	2	88	3.17	±	1.33	2	79	2.17	±	1.83
	4	76	5.50	±	1.22*	4	66	4.67	±	2.42*
	6	55	7.17	±	2.56*	6	53	5.17	±	3.60*
	8	45	6.50	±	2.88*	8	43	6.17	±	2.48*
Late repeated exposure assay										
Untreated cells		104	1.00	±	0.50		104	1.00	±	0.50
DMSO 0.5% ^c		100	1.67	±	0.52		100	1.67	±	0.52
TPA 50 ng/ml (0.5% DMSO) ^d		107	5.83	±	1.94 _↓		107	5.83	±	1.94 _↓
urban site (m ³ /well)										
						PM_{2.5}				
	2	95	3.83	±	1.72					
	4	97	8.17	±	2.32*					
	6	85	10.00	±	1.90*					
	8	91	13.17	±	4.12*					
Summer 2013										
Acute exposure assay										
Untreated cells		97	0.17	±	0.50		97	0.17	±	0.50
DMSO 0.5% ^c		100	0.67	±	0.52		100	0.67	±	0.52
3-MCA 1 µg/ml (0.5% DMSO) ^d		56	6.67	±	3.27 _↓		56	6.67	±	3.27 _↓
urban site (m ³ /well)										
	PM₁					PM_{2.5}				
	2	91	0.67	±	0.52	2	97	1.33	±	1.37
	4	92	1.00	±	1.55	4	91	2.17	±	1.60
	6	89	1.83	±	0.75	6	96	2.17	±	1.17
	8	82	2.17	±	1.17	8	91	0.83	±	0.98
Late repeated exposure assay										
Untreated cells		107	0.50	±	0.50		107	0.50	±	0.50
DMSO 0.5% ^c		100	0.83	±	0.75		100	0.83	±	0.75
TPA 50 ng/ml (0.5% DMSO) ^d		172	6.00	±	2.28 _↓		172	6.00	±	2.28 _↓
urban site (m ³ /well)										
	PM₁					PM_{2.5}				
	2	99	2.33	±	0.82	2	96	3.67	±	3.01
	4	100	4.67	±	1.86*	4	97	4.17	±	1.17*
	6	94	8.33	±	3.14*	6	99	5.00	±	2.19*
	8	103	12.50	±	2.26*	8	97	7.00	±	3.22*

^a % of cell growth compared to that of solvent control; ^b average number of transformed foci/well ± SD; ^c Solvent control: final solvent concentration of the working culture media in parentheses; ^d Solvent control for the positive control: final solvent concentration of the working culture media in parentheses; _↓ p<0.05; one-sided t-test. vs corresponding solvent control; * p<0.05; one-sided Dunnett test vs corresponding solvent control.

Tab. S4: Cell transformation induced by PAHs and NPAHs in Bhas 42 cells

CAS number	Chemical name	Initiation schedule		Promotion schedule		Reference
		Evaluation	LTC * (ng/ml)	Evaluation	LTC * (ng/ml)	
91-20-3	Naphthalene	-	> 10,000	-	> 10,000	Asada et al., 2005
208-96-7	Acenaphthilene	-	> 10,000	?	10,000	Asada et al., 2005
85-01-8	Phenanthrene	-	> 10,000	-	> 10,000	Asada et al., 2005
		-	> 150,000	-	> 60,000	Sakai et al., 2010
120-12-7	Anthracene	-	> 10,000	-	> 10,000	Asada et al., 2005
		-	> 10,000	-	> 10,000	Tanaka et al., 2009
		-	100,000	-	> 100,000	Sakai et al., 2010
		-	> 50,000	?	2,500 (+ 1 of 3)	Sakai et al., 2011
129-00-0	Pyrene	-	> 100,000	+	2,000	Asada et al., 2005
		+	50,000	+	10,000	Tanaka et al., 2009
56-55-3	Benzo(a)anthracene	+	500	+	200	Asada et al., 2005
		+	313	+	313	Tanaka et al., 2009
		+	1,000	+	1,000	Sakai et al., 2010**
218-01-9	Chrysene	+	200	+	50	Asada et al., 2005
				+	230	Misaki et al., 2016
205-99-2	Benzo(b)fluoranthene	na	na	+	25	Misaki et al., 2016
192-97-2	Benzo(e)pyrene	-	> 10,000	+	500	Asada et al., 2005
		na	na	+	250 < LTC < 2,500	Misaki et al., 2016
50-32-8	Benzo(a)pyrene	+	500	-	> 100	Asada et al., 2005
		+	10	-	> 100	Tanaka et al., 2009
		+	10	-	> 250	Sakai et al., 2010
		na	na	-	> 250	Misaki et al., 2016**
198-55-0	Perylene	+	1,000	+	100	Asada et al., 2005
53-70-3	Dibenzo(ah)anthracene	+	30	-	> 1,000	Sakai et al., 2010
		+	10	-	> 10,000	Sakai et al., 2011
191-24-2	Benzo(g,h,i)perylene	-	> 10,000	+	50	Asada et al., 2005
191-30-0	Dibenzo[a,l]pyrene	na	na	-	> 30 **	Misaki et al., 2016
892-21-7	3-Nitrofluoranthene	na	na	+	25 < LTC < 250	Misaki et al., 2016
5522-43-0	1-Nitropyrene	na	na	+	25 < LTC < 250	Misaki et al., 2016
7496-02-8	6-Nitrochrysene	na	na	+	25	Misaki et al., 2016
17117-34-9	3-Nitrobenzanthrone	na	na	+	25	Misaki et al., 2016
42397-64-8	1,6-Dinitropyrene	na	na	+	3 < LTC < 30	Misaki et al., 2016
42397-65-9	1,8-Dinitropyrene	na	na	+	3 < LTC < 30	Misaki et al., 2016

* LTC, Lowest Transforming Concentration in Bhas 42 CTA experiments. The values reported in Table S4 were extrapolated from the literature figures, however it was not always possible to extract the lowest concentration able to induce the cell transformation. In some cases a range including the lowest dose inducing Bhas 42 cell transformation was reported, in others the data was reported as "less than [conc]", because that value represented the lowest tested concentration and a NOEL (No Observed Effect Level) was not experimentally observed. Due to these uncertainties, the concentrations reported in Table S4 are more accurately described as the "lowest transforming concentration" (LTC), rather than the LOEL (Lowest Observed Effect Level).
na, not available; ** highly cytotoxic (<10% survival)

References

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