

PAH-CALUX, an optimized bioassay for AhR-mediated hazard
identification of polycyclic aromatic hydrocarbons (PAHs) as
individual compounds and in complex mixtures;

Supplementary Data

Pieterse B, Felzel E, Winter R, van der Burg B, Brouwer A

6 pages, 2 figures, and 6 tables.

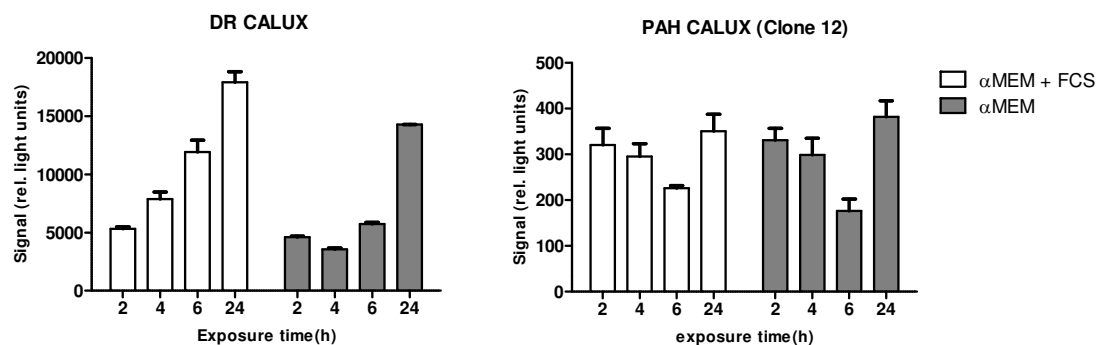


Figure S1. Background signals in time for the DR CALUX and the PAH CALUX (clone 12) cell lines cultured in α MED medium with or without 10% fetal calf serum (FCS). The exposure time indicates the time after addition of 100 μ l of the respective medium to cells that had already been cultured for 24 hours in 100 μ l of α MED with 10% FCS. Error bars indicate standard error from the mean (SEM).

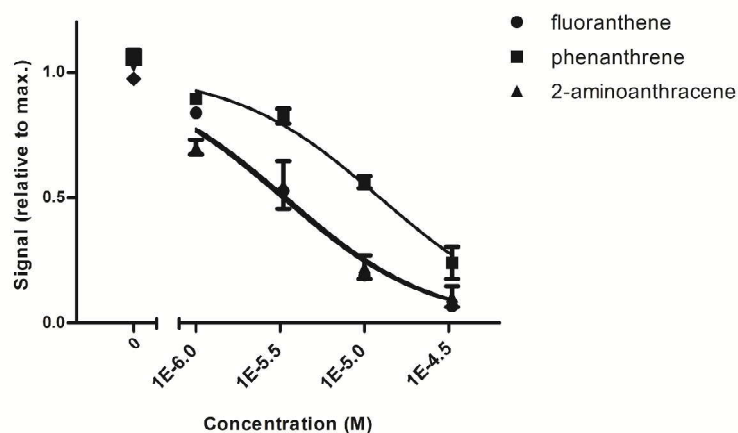


Figure S2. Antagonistic effect of fluoranthene, phenanthrene, and 2-aminoanthracene on the PAH CALUX cell line (Clone 12) tested by co-incubation with a fixed concentration of 3E-9M benzo[*a*]pyrene. Fluoranthene and 2-aminoanthracene curves are partially overlapping. The signal in response to 3E-9 benzo[*a*]pyrene was taken as maximum. Error bars indicate standard error from the mean (SEM). Co-incubation of the same PAHs with an excess amount of 1E-7M benzo[*a*]pyrene confirmed that the observed decreases in signal are not due to cytotoxic effects (results not shown).

PAH	Industrial soil Sweden 1 (1)	Industrial soil Sweden 2 (1)	Industrial soil France (2)	Industrial soil Germany (2)	Industrial soil Portugal (2)	Roadside India (3)	Urban soil United Kingdom (4)
naphtalene	5.4E-05	5.1E-04	1.7E-03	2.9E-03	4.2E-03	2.6E-03	1.5E-04
acenaphtylene	3.7E-05	1.8E-04	0.0E+00	1.8E-04	7.6E-04	9.5E-04	0.0E+00
acenaphthene	4.7E-04	6.0E-04	1.6E-04	5.7E-04	1.8E-03	9.6E-04	3.4E-04
fluorene	3.1E-04	9.4E-04	5.0E-04	5.0E-04	1.7E-03	8.9E-04	3.2E-04
phenanthrene	4.2E-04	3.7E-03	2.7E-03	1.6E-03	3.0E-03	5.5E-04	1.7E-03
anthracene	5.5E-04	6.2E-04	2.5E-03	8.2E-04	8.2E-04	1.8E-03	3.1E-04
fluoranthene	8.5E-03	2.6E-03	3.5E-03	2.8E-03	1.9E-03	2.0E-03	3.9E-03
pyrene	5.0E-03	1.7E-03	1.7E-03	1.8E-03	1.5E-03	8.5E-04	2.0E-03
chrysene	1.1E-03	1.0E-03	6.7E-04	9.6E-04	5.2E-04	4.3E-03	1.6E-03
benz[a]anthracene	1.3E-03	9.7E-04	9.8E-04	1.1E-03	6.1E-04	0.0E+00	1.6E-03
benzo[b]fluoranthene	5.9E-04	1.2E-03	5.4E-04	8.7E-04	5.2E-04	1.6E-03	1.5E-03
benzo[k]fluoranthene	2.1E-04	4.0E-04	3.9E-04	5.8E-04	2.3E-04	3.7E-04	0.0E+00
benzo[a]pyrene	1.6E-04	6.6E-04	7.3E-04	8.7E-04	5.3E-04	4.8E-04	9.5E-04
dibenz[a,h]anthracene	2.2E-05	1.3E-04	4.5E-04	1.4E-04	6.5E-05	0.0E+00	0.0E+00
indeno[1,2,3-cd]pyrene	7.4E-05	5.0E-04	4.3E-04	6.6E-04	5.7E-04	0.0E+00	0.0E+00
benzo[ghi]perylene	5.3E-05	4.3E-04	4.5E-04	7.1E-04	4.6E-04	9.5E-04	9.8E-04

Table S1. Concentrations of PAHs (M) in reconstituted mixtures. Concentrations are in identical molar ratios as the established concentrations in various soil.

PAH	Industrial soil Sweden 1 (1)	Industrial soil Sweden 2 (1)	Industrial soil France (1)	Industrial soil Germany (2)	Industrial soil Portugal (2)	Roadside India (3)	Urban soil United Kingdom (4)
naphtalene	2.8E-08	2.6E-07	8.4E-07	1.5E-06	2.1E-06	1.3E-06	7.6E-08
acenaphtylene	2.2E-08	1.1E-07	0.0E+00	1.1E-07	4.6E-07	5.7E-07	0.0E+00
acenaphthene	2.9E-07	3.6E-07	9.8E-08	3.5E-07	1.1E-06	5.9E-07	2.1E-07
fluorene	2.0E-07	6.2E-07	3.3E-07	3.3E-07	1.1E-06	5.9E-07	2.1E-07
phenanthrene	3.0E-07	2.6E-06	1.9E-06	1.1E-06	2.1E-06	3.9E-07	1.2E-06
anthracene	3.9E-06	4.4E-06	1.7E-05	5.8E-06	5.8E-06	1.2E-05	2.2E-06
fluoranthene	6.8E-06	2.1E-06	2.8E-06	2.2E-06	1.5E-06	1.6E-06	3.1E-06
pyrene	4.0E-06	1.3E-06	1.3E-06	1.4E-06	1.2E-06	6.8E-07	1.6E-06
chrysene	9.8E-06	9.5E-06	6.0E-06	8.7E-06	4.7E-06	3.9E-05	1.4E-05
benz[a]anthracene	1.2E-04	8.7E-05	8.9E-05	9.7E-05	5.5E-05	0.0E+00	1.4E-04
benzo[b]fluoranthene	5.9E-05	1.2E-04	5.4E-05	8.7E-05	5.2E-05	1.6E-04	1.5E-04
benzo[k]fluoranthene	2.1E-05	4.0E-05	3.9E-05	5.8E-05	2.3E-05	3.7E-05	0.0E+00
benzo[a]pyrene	1.6E-04	6.6E-04	7.3E-04	8.7E-04	5.3E-04	4.8E-04	9.5E-04
dibenz[a,h]anthracene	1.2E-04	6.9E-04	2.5E-03	7.5E-04	3.6E-04	0.0E+00	0.0E+00
indeno[1,2,3-cd]pyrene	8.1E-06	5.5E-05	4.8E-05	7.3E-05	6.2E-05	0.0E+00	0.0E+00
benzo[ghi]perylene	5.8E-07	4.7E-06	4.9E-06	7.7E-06	5.0E-06	1.0E-05	1.1E-05
Total	5.2E-04	1.7E-03	3.5E-03	2.0E-03	1.1E-03	7.4E-04	1.3E-03

Table S2. Toxic equivalences in the synthetic mixtures expressed per PAH in M BEQ (benzo[a]pyrene equivalences), based on TEF values according to Nisbet and Lagoy (5). See Material and Methods section for the calculations that were performed.

PAH	Industrial soil Sweden 1	Industrial soil Sweden 2	Industrial soil France	Industrial soil Germany	Industrial soil Portugal	Roadside India	Urban soil United Kingdom
naphtalene	0	0	0	0	0	0	0
acenaphtylene	0	0	0	0	0	0	0
acenaphthene	0	0	0	0	0	0	0
fluorene	0	0	0	0	0	0	0
phenanthrene	0	0	0	0	0	0	0
anthracene	0	0	0	0	0	0	0
fluoranthene	0	0	0	0	0	0	0
pyrene	0	0	0	0	0	0	0
chrysene	8.7E-04	8.4E-04	5.3E-04	7.7E-04	4.2E-04	3.4E-03	1.3E-03
benz[a]anthracene	3.8E-04	2.9E-04	3.0E-04	3.2E-04	1.8E-04	0.0E+00	4.8E-04
benzo[b]fluoranthene	3.0E-03	6.2E-03	2.7E-03	4.4E-03	2.6E-03	8.0E-03	7.7E-03
benzo[k]fluoranthene	7.9E-04	1.5E-03	1.4E-03	2.1E-03	8.5E-04	1.4E-03	0
benzo[a]pyrene	1.6E-04	6.6E-04	7.3E-04	8.7E-04	5.3E-04	4.8E-04	9.5E-04
dibenz[a,h]anthracene	2.9E-05	1.6E-04	5.8E-04	1.8E-04	8.4E-05	0	0
indeno[1,2,3-cd]pyrene	9.6E-05	6.5E-04	5.6E-04	8.6E-04	7.4E-04	0	0
benzo[ghi]perylene	0	0	0	0	0	0	0
Total	5.3E-03	1.0E-02	6.8E-03	9.5E-03	5.4E-03	1.3E-02	1.0E-02

Table S3. Toxic equivalences in the synthetic mixtures expressed per PAH in M BEQ, based on REP values derived from PAH CALUX analysis. See Material and Methods section for the calculations that were performed.

PAH	Sewage sludge (LGC9182)	River sediment (LGC6288)	Industrial soil (BCR524)
naphtalene	2.6E-06	1.7E-06	
acenaphtylene			
acenaphthene	6.5E-07	4.5E-07	
fluorene	1.1E-06	7.2E-07	
phenanthrene	5.8E-06	5.8E-06	
anthracene	9.6E-07	2.0E-06	
fluoranthene	9.0E-06	8.9E-06	
pyrene	7.6E-06	7.3E-06	8.6E-04
chrysene	3.7E-06	3.6E-06	
benz[a]anthracene	2.9E-06	3.6E-06	9.9E-05
benzo[b]fluoranthene	3.8E-06	3.3E-06	5.4E-05
benzo[k]fluoranthene	1.8E-06	2.0E-06	2.5E-05
benzo[a]pyrene	2.3E-06	2.6E-06	3.4E-05
dibenz[a,h]anthracene		4.7E-07	
indeno[1,2,3-cd]pyrene	2.1E-06	1.3E-06	1.8E-05
benzo[ghi]perylene	2.2E-06	1.3E-06	

Table S4. Concentrations of PAHs (mol / kg) in reference samples as determined by chemical analysis and reported by the suppliers. Empty cells indicate that for the respective PAH no concentration was reported by the supplier.

PAH	Sewage sludge (LGC9182)	River sediment (LGC6288)	Industrial soil (BCR524)
naphthalene	1.3E-09	8.7E-10	
acenaphthylene			
acenaphthene	4.0E-10	2.8E-10	
fluorene	7.5E-10	4.8E-10	
phenanthrene	4.1E-09	4.1E-09	
anthracene	6.7E-09	1.4E-08	
fluoranthene	7.2E-09	7.1E-09	
pyrene	6.1E-09	5.9E-09	6.9E-07
chrysene	3.3E-08	3.3E-08	
benz[a]anthracene	2.6E-07	3.3E-07	8.9E-06
benzo[b]fluoranthene	3.8E-07	3.3E-07	5.4E-06
benzo[k]fluoranthene	1.8E-07	2.0E-07	2.5E-06
benzo[a]pyrene	2.3E-06	2.6E-06	3.4E-05
dibenz[a,h]anthracene		2.6E-06	
indeno[1,2,3-cd]pyrene	2.3E-07	1.5E-07	2.0E-06
benzo[ghi]perylene	2.5E-08	1.4E-08	
Total	3.5E-06	6.2E-06	5.4E-05

Table S5. Toxic equivalences expressed per PAH in mol BEQ / kg, based on TEF values according to Nisbet and Lagoy (5). See Material and Methods section for the calculations that were performed. Empty cells indicate that for the respective PAH no concentration was reported by the supplier.

PAH	Sewage sludge (LGC9182)	River sediment (LGC6288)	Industrial soil (BCR524)
naphthalene	0	0	
acenaphthylene			
acenaphthene	0	0	
fluorene	0	0	
phenanthrene	0	0	
anthracene	0	0	
fluoranthene	0	0	
pyrene	0	0	0
chrysene	2.9E-06	2.9E-06	
benz[a]anthracene	8.7E-07	1.1E-06	3.0E-05
benzo[b]fluoranthene	1.9E-05	1.6E-05	2.7E-04
benzo[k]fluoranthene	6.6E-06	7.3E-06	9.1E-05
benzo[a]pyrene	2.3E-06	2.6E-06	3.4E-05
dibenz[a,h]anthracene		6.1E-07	
indeno[1,2,3-cd]pyrene	2.7E-06	1.7E-06	2.4E-05
benzo[ghi]perylene	0	0	
Total	3.4E-05	3.3E-05	4.5E-04

Table S6. Toxic equivalences expressed per PAH in mol BEQ / kg, based on REP values derived from PAH CALUX analysis. See Material and Methods section for the calculations that were performed. Empty cells indicate that for the respective PAH no concentration was reported by the supplier.

References

- ¹ Mattsson, A.; Lundstedt, S.; Stenius, U. Exposure of HepG2 cells to low levels of PAH-containing extracts from contaminated soils results in unpredictable genotoxic stress responses. *Env. Mol. Mut.* **2009**, 50, 337-348.
- ² Roos P.H. Differential induction of CYP1A1 in duodenum, liver and kidney of rats after oral intake of soil containing polycyclic aromatic hydrocarbons. *Arch. Toxicol.* **2002**, 76(2), 75-82.

³ Masih, A.; Taneje, A. Polycyclic aromatic hydrocarbons (PAHs) concentrations and related carcinogenic potencies in soil at a semi-arid region of India. *Chemosphere*, **2006**, 65, 449-456.

⁴ Jones, K.C.; Stratford, J.A.; Waterhouse, K.S.; Vogt, NB. Organic contaminants in Welsh soils: polynuclear aromatic hydrocarbons. *Env. Sci. Technol.* **1989**, 23, 540-550.

⁵ Nisbet, I.C.T.; LaGoy P.K. Toxic equivalence factors (TEF) for polycyclic aromatic hydrocarbons (PAHs). *Regul. Toxicol. Pharmacol.* **1992**, 16, 290-300.