Supporting Information

Aryl hydrocarbon receptor activation and developmental toxicity in zebrafish in response to soil extracts containing unsubstituted and oxygenated PAHs

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14 pages with 6 tables and 3 figures.

Supporting Information

Table S1. Primers used for qRT-PCR analysis.

Gene	GenBank ID	Sequence
CYP1A	NM_131879	F: 5′-GCATTACGATACGTTCGATAAGGAC
		R: 5′-GCTCCGAATAGGTCATTGACGAT
CYP1B1	NM_001145708	F: 5′-CTGCATTGATTTCCGAGACGTG
		R: 5′-CACACTCCGTGTTGACAGC
CYP1C1	NM_001020610	F: 5′-AGTGGCACAGTCTACTTTGAGAG
		R: 5′-TCGTCCATCAGCACTCAG
CYP1C2	NM_001114849	F: 5′-GTGGTGGAGCACAGACTAAG
		R: 5′-TTCAGTATGAGCCTCAGTCAAAC
ahrra	NM_001035265	F: 5′-AGAACCAGCAGGACACACCA
		R: 5′-TGGTGGAAGCCCAGGTAATCA
ahrrb	NM_001033920	F: 5′-AGGACACTGATGAGGATGTTGTG
		R: 5′-GTAAGAAACGGAGTCAATTCAGGAG
ahr2	NM_131264	F: 5′-GGGAAGGTGGTTCTTGGCTAC
		R:5′-CTCCTGTCTTTATCATTCTGATGTGGTT
cox2b	NM_001025504	F: 5′-GGCTCATCCTTATTGGTGAGACTAT
		R: 5′-TCGGGATCAAACTTGAGCTTAAAATA
gstp1	NM_131734	F: 5′-CGACTTGAAAGCCACCTGTGTC
		R: 5′-CTGTCGTTTTTGCCATATGCAGC
gstp2	NM_001020513	F: 5′-CACTCTCACATACTTCGCTATC
		R: 5′-TTTGTCCGCCAGCAGGATCTT
hsp70	NM_131397	F: 5′-CAAAGGCAAATCCTCAGA
		R: 5′-CACAAAGTGGTTCACCATGC
*arnt2**	NM_131674	F: 5′-CACCTTTGGATCACATCTCATTG
		R: 5′-TCACCCTCCTTAGACGGACC
*l13**	NM_001020534	F: 5′-GCTAAGGACGGAGTGAACAAC
		R: 5′-GCACTCTCTTCTGCCAGTC

*The use of *arnt2* and *l13* as reference genes has been previously validated.[1-3]

Table S2. Results from statistical analysis of toxicity data describing developmental effects in response to soil extracts.

WOOD soil extracts*

	Mortality	Edema	-SB wo edema	
extract vs. DMSO	P value	P value	P value	*comments*
PAC 0.1	0.000	-	-	all dead in PAC
PAC 0.4	0.000	-	-	all dead in PAC
PAH 0.1	0.000	0.000	0.160	
PAH 0.4	0.000	-	-	all dead in PAH
Oxy-PAH 0.1	0.244	0.003	0.000	low mortality in OPAH
Oxy-PAH 0.4	0.000	0.000	-	all dead or edema in OPAH
high vs. low dose				
PAC	-	-	-	all dead in both doses
PAH	0.000	-	-	All dead in high dose
Oxy-PAH	0.000	0.017	-	all dead or edema in high dose
PAH vs. Oxy-PAH				
0.1	0.000	0.000	0.000	97% dead or edema in PAH, 20% in OPAH but 60% -SB
0.4	0.000	0.000	-	all dead in PAH, all dead or edema in OPAH
PAH vs. BP**				
0.1 vs. 4	0.000	0.000	-	97% dead or edema in PAH, no dead & 4% edema in BP
0.1 vs. 20	0.000	0.000	-	97% dead or edema in PAH, no dead & 4% edema in BP
0.1 vs. 100	0.001	0.000	-	97% dead or edema in PAH, 14% in BP
0.1 vs. 500	0.129	0.001	-	97% dead or edema in PAH, 32% in BP
0.4 vs. 4	0.000	-	-	all dead in PAH, no dead in BP
0.4 vs. 20	0.000	-	-	all dead in PAH, no dead in BP
0.4 vs. 100	0.000	-	-	all dead in PAH, 14% in BP
0.4 vs. 500	0.000	-	-	all dead in PAH, 32% in BP
* soil extracts corresponding to 0.1 or 0.4 mg soil/ml				
** BP concentrations given as ng/ml				

GAS soil extracts*

	Mortality	Edema	-SB wo edema	
extract vs. DMSO	P value	P value	P value	*comments*
PAC 0.4	0.497	0.001	0.000	low mortality in PAC
PAC 0.8	0.065	0.000	0.050	low mortality in PAC
PAH 0.4	-	0.078	0.102	no dead, low effects on edema
PAH 0.8	-	0.255	1.000	no dead, low effects on edema
Oxy-PAH 0.4	-	0.276	0.439	no dead, low effects on edema
Oxy-PAH 0.8	-	0.255	1.000	no dead, low effects on edema
high vs. low dose				
PAC	0.379	0.000	0.000	low mortality in both doses

PAH	-	1.000	0.094	no dead, low effects on edema
Oxy-PAH	-	1.000	0.335	no dead, low effects on edema
PAH vs. Oxy-PAH				
0.4	-	1.000	0.492	no dead, low effects on edema
0.8	-	1.000	1.000	no dead, low effects on edema
PAH vs. BP**				
0.4 vs. 4	-	1.000	0.000	no dead, 0% & 17% -SB in PAH vs. BP
0.4 vs. 20	-	1.000	0.000	no dead, 0% & 58% -SB in PAH vs. BP
0.4 vs. 100	0.229	1.000	0.000	no dead in PAH, 7% in BP
0.4 vs. 500	0.013	0.389	0.000	no dead in PAH, 18% in BP
0.8 vs. 4	-	1.000	0.001	no dead, 9% & 17% -SB in PAH vs. BP
0.8 vs. 20	-	1.000	0.001	no dead, 9% & 58% -SB in PAH vs. BP
0.8 vs. 100	0.491	1.000	0.000	no dead in PAH, 7% in BP
0.8 vs. 500	0.020	0.182	0.002	no dead in PAH, 18% in BP
* extracts corresponding to 0.4 or 0.8 mg soil/ml				
** BP concentrations given as ng/ml				

COKE soil extracts*

	Mortality	**Edema**	**-SB wo edema**	
extract vs. DMSO	**P value**	**P value**	**P value**	***comments***
PAC 0.1	0.000	0.000	0.006	45% dead or edema in PAC
PAC 0.4	0.000	0.005	-	all dead or edema in PAC
PAH 0.1	-	0.244	0.004	no dead and low edema in PAH
PAH 0.4	0.000	0.000	0.011	57% dead or edema in PAH
Oxy-PAH 0.1	0.013	0.006	0.033	low mortality and edema in OPAH
Oxy-PAH 0.4	0.000	0.000	0.256	74% dead or edema in OPAH
high vs. low dose				
PAC	0.000	0.097	-	all dead or edema in high dose
PAH	0.000	0.001	0.852	
Oxy-PAH	0.001	0.098	0.417	
PAH vs. Oxy-PAH				
0.1	0.013	0.205	0.453	no dead in PAH, 10% in OPAH
0.4	0.028	0.836	0.162	30% dead in PAH, 50% in OPAH
PAH vs. BP**				
0.1 vs. 4	-	1.000	0.098	no dead and low edema in both
0.1 vs. 20	-	1.000	0.155	no dead and low edema in both
0.1 vs. 100	0.092	0.641	0.067	no dead in PAH, 7% in BP
0.1 vs. 500	0.001	0.088	0.227	no dead in PAH, 18% in BP
0.4 vs. 4	0.001	0.032	0.095	30% dead in PAH, no in BP
0.4 vs. 20	0.001	0.018	0.093	30% dead in PAH, no in BP
0.4 vs. 100	0.027	0.046	0.037	30% dead in PAH, 7% in BP
0.4 vs. 500	0.598	0.405	0.141	30% dead in PAH, 18% in BP

* soil extracts corresponding to 0.1 or 0.4 mg soil/ml
** BP concentrations given as ng/ml

BP**

	Mortality	Edema	-SB wo edema	
BP vs. DMSO	**P value**	**P value**	**P value**	***comments***
BP 4	-	0.453	0.004	no dead
BP 20	-	0.463	0.004	no dead
BP 100	0.237	0.237	0.001	
BP 500	0.015	0.036	0.008	

** BP concentrations given as ng/ml

Table S3. Results from statistical analysis of toxicity data investigating the role of Ah receptor in mediating developmental effects in response to soil extracts.

WOOD soil extracts*

	Mortality	Edema	-SB wo edema	
CT-MO vs. NI	P value	P value	P value	*comments*
DMSO	-	-	1.000	no dead or edema
PAC 0.1	-	-	-	all dead
PAH 0.1	0.779	0.761	1.000	
Oxy-PAH 0.1	1.000	0.731	0.103	
AHR2-MO vs. NI				
DMSO	-	-	1.000	no dead or edema
PAC 0.1	0.000	0.000	-	all dead in NI, no dead but all edema in AHR2-MO
PAH 0.1	0.001	0.000	0.238	67% dead in NI, no dead but 97% edema in AHR2-MO
Oxy-PAH 0.1	1.000	0.024	0.000	20% edema in NI, no edema but 97% -SB in AHR2-MO
extract vs. DMSO: NI				
PAC 0.1	0.000	-	1.000	all dead in PAC, no in DMSO
PAH 0.1	0.000	0.005	1.000	93% dead or edema in PAH, no in DMSO
Oxy-PAH 0.1	0.492	0.024	0.000	27% dead or edema in OPAH, no in DMSO
extract vs. DMSO: AHR2-MO				
PAC 0.1	-	0.000	-	no dead but all edema in PAC, no in DMSO
PAH 0.1	-	0.000	-	no dead but 97% edema in PAH, no in DMSO
Oxy-PAH 0.1	0.492	-	0.000	no edema but 97% -SB in OPAH, no in DMSO
* soil extracts corresponding to 0.1 mg soil/ml				

GAS soil extracts*

	Mortality	Edema	-SB wo edema	
CT-MO vs. NI	P value	P value	P value	*comments*
DMSO	0.485	-	0.674	low mortality, no edema and low -SB
PAC 0.8	1.000	0.773	0.754	
AHR2-MO vs. NI				
DMSO	1.000	-	0.409	low mortality, no edema and low -SB
PAC 0.8	0.235	0.396	0.252	0 / 40% mortality and edema in AHR2-MO, 12 / 56% in NI
extract vs. DMSO: NI				
PAC 0.8	0.067	0.000	0.099	12 / 56% mortality and edema in PAC, no in DMSO
extract vs. DMSO: AHR2-MO				
PAC 0.8	1.000	0.000	0.000	40% / 52% edema and -SB in PAC, 0% / 5% in DMSO
* soil extracts corresponding to 0.8 mg soil/ml				

COKE soil extracts*

	Mortality	Edema	-SB wo edema	

CT-MO vs. NI	<u>**P value**</u>	<u>**P value**</u>	<u>**P value**</u>	***comments***
DMSO	-	-	0.442	no dead or edema
PAC 0.4	0.468	1.000	0.119	
PAH 0.4	1.000	0.799	0.856	
Oxy-PAH 0.4	0.360	0.314	0.841	
AHR2-MO vs. NI				
DMSO	-	-	0.058	no dead or edema
PAC 0.4	0.000	0.002	0.000	3 / 72% mortality and edema in AHR2-MO, 50 / 50% in NI
PAH 0.4	0.096	0.813	0.855	20% dead or edema in AHR2-MO, 33% in NI
Oxy-PAH 0.4	0.000	0.000	0.304	5% dead or edema in AHR2-MO, 67% in NI
extract vs. DMSO: NI				
PAC 0.4	0.000	0.000	0.058	all dead or edema in PAC, no in DMSO
PAH 0.4	0.000	0.001	0.000	33% dead or edema in PAH, no in DMSO
Oxy-PAH 0.4	0.000	0.000	0.002	67% dead or edema in OPAH, no in DMSO
extract vs. DMSO: AHR2-MO				
PAC 0.4	0.497	0.000	0.000	3% dead and 72% edema in PAC, no in DMSO
PAH 0.4	0.119	0.001	0.001	7% dead and 13% edema in PAH, no in DMSO
Oxy-PAH 0.4	1.000	0.496	0.002	1.5% dead and 4% edema in OPAH, no in DMSO
* soil extracts corresponding to 0.4 mg soil/ml				

Table S4. Levels (µg/g soil) of PAHs and oxy-PAHs in the soil extracts.

	WOOD[4]	GAS	COKE[4]
PAHs			
Naphthalene	2.7	1.1	7.1
2-Methylnaphthalene	2.1	0.58	6.3
1-Methylnaphthalene	1.0	0.36	3.2
Biphenyl	0.24	0.14	7.4
2,6-Dimethylnaphthalene	1.4	0.28	8.3
Acenaphthylene	2.2	0.75	2.9
Acenaphthene	28	0.33	10
2,3,5-Trimethylnaphthalene	4.3	0.15	2.1
Fluorene	20	1.1	17
Phenanthrene	29	12	71
Anthracene	38	2.5	12
1-Methylphenanthrene	27	1.2	2.8
Fluoranthene	665	21	57
Pyrene	391	16	37
Benzo[*a*]anthracene	96	12	24
Chrysene	113	14	26
Benzo[*b*]fluoranthene	58	16	34
Benzo[*k*]fluoranthene	21	6.4	11
Benzo[*e*]pyrene	22	8.6	16
Benzo[*a*]pyrene	16	10	18
Perylene	4.6	2.6	5.2
Dibenz[*a.h*]anthracene	2.4	2.3	3.8
Indeno[*cd*]pyrene	7.9	7.9	15
Benzo[*ghi*]perylene	5.7	7.1	13
Total PAHs	**1559**	**144**	**410**
oxy-PAHs			
1-Indanone	0.38	0.12	0.11
1-Acenaphthenone	1.8	0.37	0.30
9-Fluorenone	16	2.9	48
Methyl-9-fluorenone	6.4	1.3	23
Anthracene-9.10-dione	15	3	6.4
4H-Cyclopenta[*def*]phenanthrenone	134	2.2	5.3
2-Methylanthracene-9.10-dione	4.9	0.96	0.75
4-Oxapyrene-5-one	2.7	0.4	0.18
Benzo[*a*]fluorenone	21	4.3	10
7H-Benz[*de*]anthracene-7-one	4.1	1.1	2.8
Benz[*a*]anthracene-7.12-dione	7.6	1.4	0.71
Naphthacene-5.12-dione	17	1.1	0.41
Benzo[*cd*]pyrenone	22	3.2	8.8
Total oxy-PAHs	**253**	**22**	**107**
Total PACs	**1811**	**166**	**517**

Table S5. Toxic equivalency factors (TEFs) and levels (pg/g soil) of dioxin like compounds (DLCs), including toxic PCDDs, PCDFs and dioxin like PCBs, in the soils. The calculated total toxic equivalents (TEQs; pg/g soil) are also given.

	TEF[5]	COKE	GAS	WOOD
Dioxins				
2378 TeCDD	1	<0.2	0.34	<0.2
12378 PeCDD	1	0.23	2.6	0.52
123478 HxCDD	0.5	0.13	2.2	0.41
123678 HxCDD	0.01	0.58	11	4.3
123789 HxCDD	0.01	0.34	4.7	1.3
1234678 HpCDD	0.001	4.3	75	34
OCDD	<0.0001	29	402	138
Furans				
2378 TeCDF	0.05	0.76	7.6	<0.2
12378 PeCDFa	0.05	0.39	11	0.53
23478 PeCDF	0.5	0.70	6.4	0.68
123478 HxCDFb	0.1	0.36	21	2.3
123678 HxCDF	0.1	0.34	7.4	1.7
234678 HxCDF	0.1	0.50	2.4	0.66
123789 HxCDF	0.1	0.51	3.7	2.9
1234678 HpCDF	0.01	22	55	103
1234789 HpCDF	0.01	0.3	3.6	1.4
OCDF	<0.0001	15	84	58
Non-*ortho* PCBs				
PCB # 81	0.0005	0.87	2.8	4
PCB# 77	0.0001	10	71	17
PCB # 126	0.005	3.1	7.2	2.8
PCB # 169	0.00005	<0.5	1.2	<0.5
Mono-*ortho* PCBs				
PCB # 105	<0.000005	136	266	49
PCB # 114	<0.000005	13	10	6.7
PCB # 118	<0.000005	268	715	164
PCB # 123	<0.000005	<1	<1	<1
PCB # 156	<0.000005	55	189	<1
PCB # 157	<0.000005	11	43	29
PCB # 167	<0.000005	31	127	19
PCB # 189	<0.000005	8.6	49	6.2
∑DLCs		613.8	2183.5	650.0
TEQ ($\Sigma[C_i]\times TEF_i$)		1.3	13	3.2

Table S6. B[a]P content and B[a]P_{eq} levels of the soil extract concentrations used in the study. B[a]P_{eq} levels were calculated by using TEF values[6] and PAH content as shown in SI Table S4.

Soil extracts (mg soil/ml)	B[a]P (µg/ml)	B[a]P_{eq} (µg/ml)
WOOD		
0.1	0.0016	0.0064
0.4	0.0064	0.0256
GAS		
0.4	0.0040	0.0068
0.8	0.0080	0.0136
COKE		
0.1	0.0018	0.0032
0.4	0.0072	0.0127

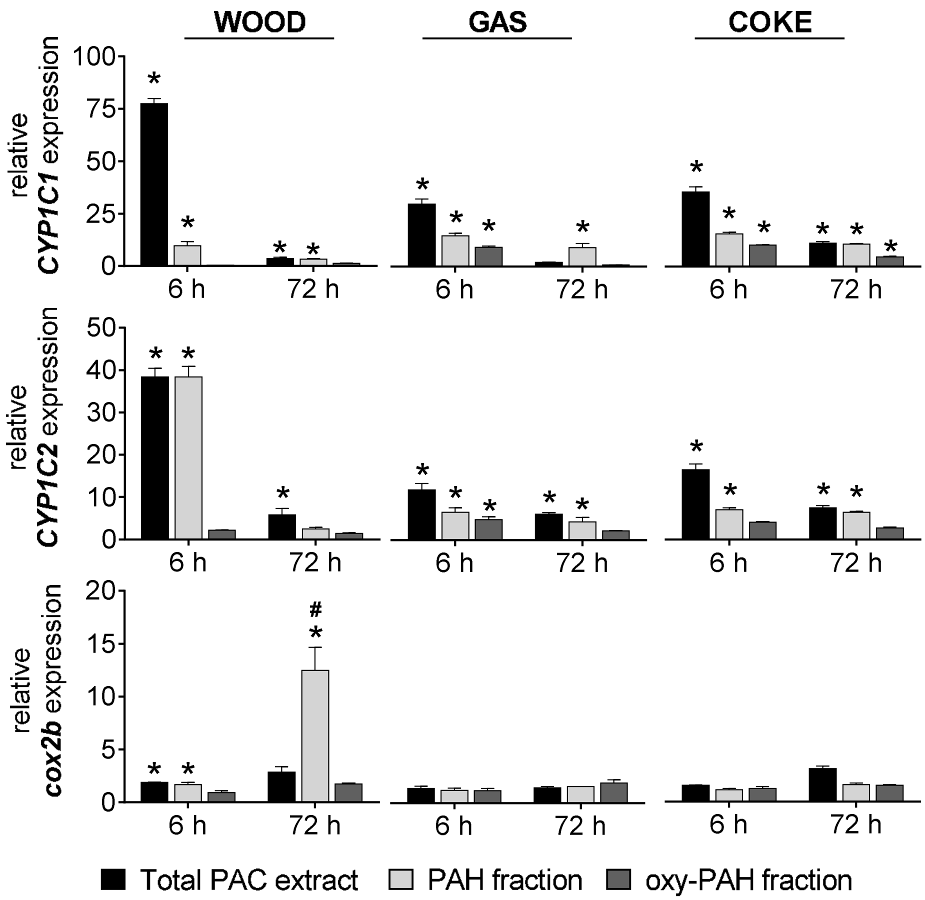

Figure S1. PAH and oxy-PAH soil extracts are potent inducers of Ah receptor signaling in zebrafish embryos. QRT-PCR analysis of Ah receptor downstream genes after exposure to the WOOD, GAS or COKE soil extracts or their PAH and oxy-PAH containing fractions corresponding to 0.1 mg soil/ml for 6 and 72 h. *$p<0.05$ compared to vehicle DMSO control (DMSO = 1), $^{\#}p<0.05$ compared to 6 h. $n \geq 3$, with 10 pooled embryos in each sample

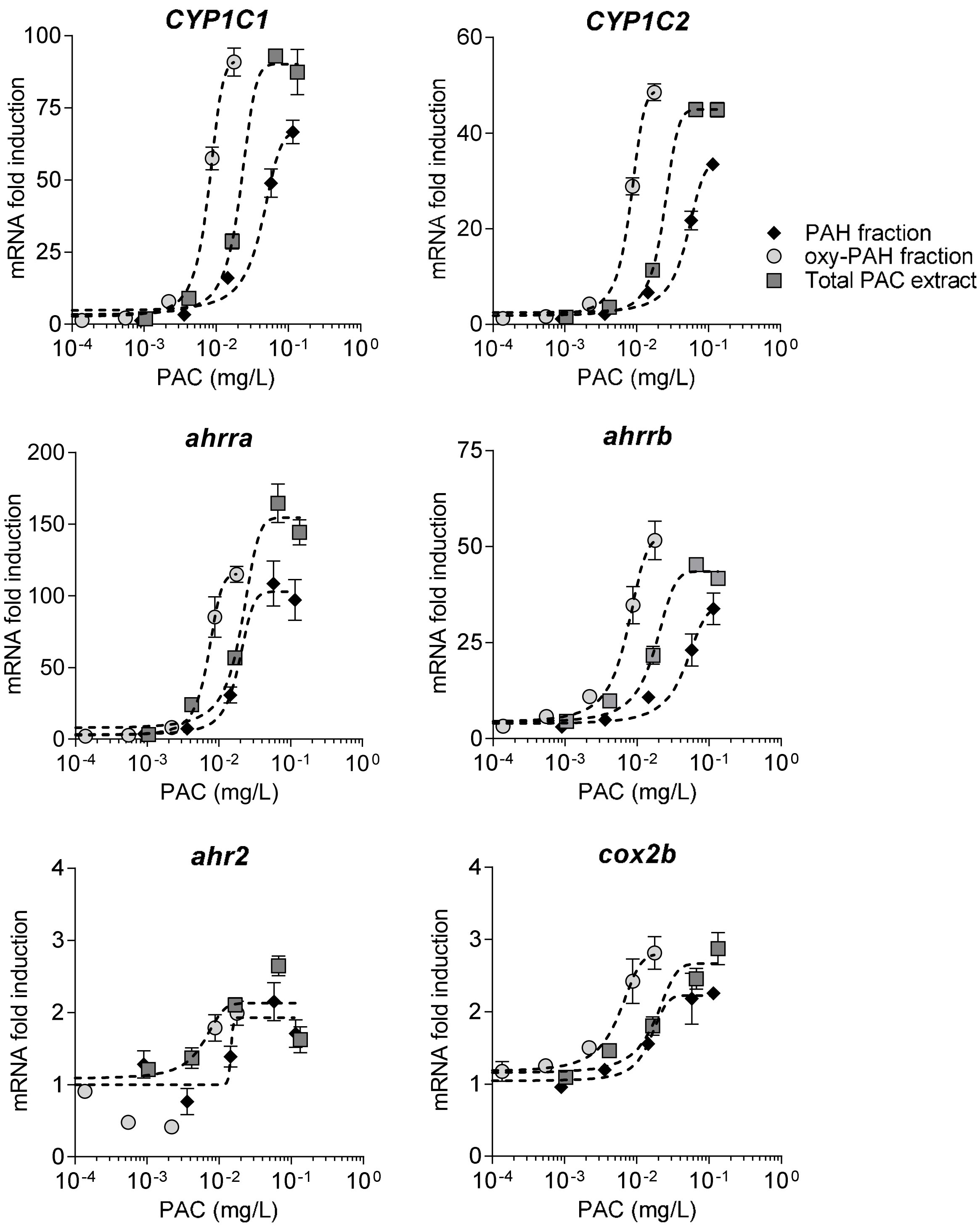

Figure S2. PAH and oxy-PAH soil extracts are potent activators of Ah receptor signaling. Dose response curves of relative mRNA levels in 1 dpf zebrafish in response to PAC-containing soil extract (or fractions) from a gas works site after exposure for 6 h compared to DMSO vehicle control (DMSO = 1). n≥3, with 10 pooled embryos in each sample.

Figure S3. Typical phenotypes of 5 dpf zebrafish embryos exposed to soil extract. Zebrafish exposed to soil extract corresponding to 0.1 mg soil/ml for 4 days (left). Observed malformations included edemas, lack of inflated swim-bladder, tail shortening, small eyes and cranio-facial malformations. DMSO-treated control is shown to the right.

References

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