Supporting Information

Identification and Toxicological Evaluation of Unsubstituted PAHs and Novel PAH Derivatives in Pavement Sealcoat Products

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Sample Collection

CT-1 product was collected from a 125 mL jar of commercially available product that was obtained from the commercial applicator during application.[1] CT-2 and AS products were collected from 5 gallon buckets after they were first stirred with a wooden stick, similar to paint stirrer. Each product was applied to a 12 square inches glass sheets that have been pre-cleaned with methanol. The application was done with a new natural bristle paint brush and each glass was dried in a dark room.

Sample Analysis

PAHs, MPAHs, and Hetero-PAHs were analyzed in electron impact ionization mode, and NPAHs and OPAHs were analyzed in electron capture negative ionization mode with a programmed temperature vaporization inlet (Gerstel, Germany) using previously described methods.[2,3] All analyses were run in selected ion monitoring mode with triplicate injections. Limits of detection ranged from 0.005 to 1.3 mg [PAH]/kg sealcoat.

GC/MS analyses for all analytes, except MW302-PAHs, were performed using an Agilent 6890 GC coupled with a 5973N mass selective detector and an Agilent DB-5MS (30 m × 0.25 mm I.D. × 0.25 μm film thickness) capillary column. For the MW302-PAHs, an Agilent DB-17MS (60 m × 0.25 mm I.D. × 0.25 μm film thickness) capillary column was used.[4,5] MW302-PAHs were also analyzed in electron impact ionization mode,[4] but with a slight modification to the oven programming that included a 45°C/min ramp to 200°C and a 15-min isothermal hold at 320°C.

58 **Quality assurance/quality control (QA/QC)**

59 Field replicates were obtained by collecting scrapes from five different spots in the
60 parking lot.[1] Instrument replicates were obtained by triplicate injection for every analyzed
61 sample. The results given in Table S1 are derived from the mean of the triplicate injections. Our
62 results were compared to results from Van Metre, et al. (2012) (Figure S1). The limit of
63 detection (LOD) was estimated for each compound using a signal to noise ratio of 3:1. The limit
64 of quantitation (LOQ) was estimated for each compound using a signal to noise ratio of 10:1.

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Sample	CT-1 (mg/kg)	CT-2 (mg/kg)	AS (mg/kg)	1.6 h (mg/kg)	1 d (mg/kg)	45 d (mg/kg)	149 d (mg/kg)	LOD (mg/kg)	LOQ (mg/kg)
NAP	1	130	1	1	4	1	0.5	0.005	0.02
ACY	4	5	<LOD	5	6	2	2	0.035	0.1
ACE	140	740	2.5	89	170	6	1	0.035	0.1
FLO	620	2,300	4.5	370	1,300	30	7	0.005	0.02
DBT	350	740	1	230	430	46	24	0.005	0.02
PHE	8,300	45,000	14	16,000	56,000	2,300	1,200	0.005	0.02
ANT	2,500	4,700	7	2,800	4,600	100	25	0.005	0.02
FLA	6,800	31,000	3	20,000	71,000	6,400	4,900	0.005	0.02
PYR	5,600	33,000	4	20,000	68,000	6,200	4,700	0.005	0.02
RET	10	5	2	10	9	4	<LOD	0.005	0.02
BcFlo	120	160	0.2	140	210	80	62	0.002	0.01
CPP	10	9	<LOD	3	4	2	7	0.005	0.02
BaA	2,400	2,800	0.8	4,000	15,000	1,300	770	0.005	0.02
CHR+TRI	1,600	1,300	3	3,100	3,700	1,500	1,400	0.003	0.01
BbF	2,700	2,500	2	5,400	4,600	2,100	2,300	0.005	0.02
BkF	1,100	900	0.04	2,000	1,900	890	940	0.005	0.02
Bj+eA	<LOD	2	<LOD	<LOD	<LOD	<LOD	<LOD	0.005	0.02
BeP	1,600	1,300	3	2,600	2,700	1,200	1,100	0.005	0.02
BaP	3,000	1,800	0.4	4,300	5,200	1,900	1,800	0.005	0.02
DBah+acA	71	30	<LOQ	40	52	19	27	0.005	0.02
IcdPY	1,500	970	0.7	1,000	2,300	610	750	0.005	0.02
BghiP	21	66	0.6	11	280	48	3	0.005	0.02
ANH	470	220	0.8	470	620	69	90	0.005	0.02
2-MNAP	8	140	4	8	9	<LOD	<LOD	0.035	0.1
1-MNAP	8	86	4	8	8	<LOD	<LOD	0.035	0.1
2,6-DMNAP	4	46	0.5	3	6	<LOD	<LOD	0.035	0.1
1,3-DMNAP	4	48	0.5	3	6	<LOD	<LOD	0.035	0.1

Sample	CT-1 (mg/kg)	CT-2 (mg/kg)	AS (mg/kg)	1.6 h (mg/kg)	1 d (mg/kg)	45 d (mg/kg)	149 d (mg/kg)	LOD (mg/kg)	LOQ (mg/kg)
2-MPHE	540	920	4	460	1,400	170	110	0.035	0.1
2-MANT	210	340	<LOD	110	250	18	<LOD	0.035	0.1
1-MPHE	320	500	1.4	270	480	92	62	0.035	0.1
3,6-DMPHE	46	63	3	44	59	18	15	0.006	0.02
1-MPYR	1,500	2,100	0.5	2,200	3,100	340	180	0.005	0.02
6-MCHR	58	46	2	48	76	27	22	0.005	0.02
N23bF	38	16	<LOD	50	36	25	32	0.035	0.1
DBae+bkF	370	120	<LOD	360	280	220	280	0.035	0.1
DBakF	200	68	<LOD	160	110	29	27	0.1	0.3
DBjlF	370	19	<LOD	650	490	350	500	0.05	0.2
DBalP	15	7	<LOD	13	8	1	1	0.05	0.2
N23kF	250	180	<LOD	460	310	77	130	0.5	1.7
N23eP	66	24	<LOD	72	54	41	47	0.035	0.1
DBaeP	160	46	<LOD	150	97	83	110	0.005	0.02
COR	120	36	<LOD	130	120	94	110	0.03	0.1
DBaiP	120	44	<LOD	80	61	38	44	0.05	0.2
DBahP	250	110	<LOD	69	41	25	29	0.1	0.3
2-MBF	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.004	0.01
THI	<LOD	4	<LOD	<LOD	<LOD	<LOD	<LOD	0.02	0.07
QUIN	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.008	0.03
IND	1	6	0.3	<LOD	<LOD	<LOD	<LOD	0.05	0.2
8-MQN	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.007	0.02
DBF	380	1,000	3	100	210	4	1	0.03	0.1
XAN	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.01	0.03
5,6-BQN	<LOD	7	<LOD	3	5	1	0.3	0.01	0.03
ACR	<LOD	1	<LOD	<LOD	<LOD	<LOD	<LOD	0.05	0.2
CARB	1,500	2,800	1	740	540	150	67	0.05	0.2
1-NN	<LOD	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	0.007	0.02
2-NN	<LOD	0.3	<LOD	<LOD	<LOD	<LOD	<LOD	0.007	0.02

Sample	CT-1 (mg/kg)	CT-2 (mg/kg)	AS (mg/kg)	1.6 h (mg/kg)	1 d (mg/kg)	45 d (mg/kg)	149 d (mg/kg)	LOD (mg/kg)	LOQ (mg/kg)
2-NBP	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.007	0.02
3-NBP	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.007	0.02
4-NBP	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.05	0.2
3-NBF	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.01	0.03
5-NAC	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.02	0.07
2-NFL	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.007	0.02
9-NAN	1	<LOD	<LOD	0.07	1	<LOD	<LOD	0.01	0.03
9-NPH	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.004	0.01
2-NBT	0.4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.05	0.2
3-NPH	<LOD	0.01	<LOD	<LOD	<LOD	<LOD	<LOD	0.001	0.003
2-NAN	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.002	0.01
2+3-NF	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.02	0.07
1-NP	<LOD	<LOD	<LOD	0.2	0.3	0.3	0.4	0.02	0.07
2-NP	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.2	0.05	0.2
2,8-DNDB	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.07	0.2
7-NBaA	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.007	0.02
1-NTR	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.007	0.02
6-NCH	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.02	0.07
3-NBENZ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.05	0.2
2-NTR	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.02	0.07
1,3-DNP	<LOD	0.4	<LOD	<LOD	0.3	0.2	<LOD	0.05	0.17
1,6-DNP	3	<LOD	<LOD	3	5	3	<LOD	0.05	0.2
1,8-DNP	4	10	<LOD	6	4	2	2	0.05	0.2
6-NBaP	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.07	0.2
9-FLU	1	11	1	2	3	1	5	0.007	0.02
PHD	0.5	0.1	<LOD	<LOD	0.4	<LOD	<LOD	0.05	0.2
ANQ	13	31	1	18	120	2	190	0.007	0.02
2-MANQ	1	3	<LOD	5	13	1	190	0.007	0.02
BFLN	9	13	0.025	52	78	96	115	0.007	0.02

Sample	CT-1 (mg/kg)	CT-2 (mg/kg)	AS (mg/kg)	1.6 h (mg/kg)	1 d (mg/kg)	45 d (mg/kg)	149 d (mg/kg)	LOD (mg/kg)	LOQ (mg/kg)
BEN	1	0.5	<LOD	0.3	0.4	0.04	<LOD	0.007	0.02
ACQN	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOD	1.3	4.3
BQN+PQN	6	10	<LOQ	170	120	190	420	0.007	0.02
BPYN	<LOD	0.25	<LOD	0.1	4	1	<LOD	0.007	0.02
1,6-BPQN	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.13	0.4

Table S1. Mean concentrations (dry weight, $n = 3$) and estimated LOD's and LOQ's of all the compounds detected in pavement sealcoat products and time point scrapes.

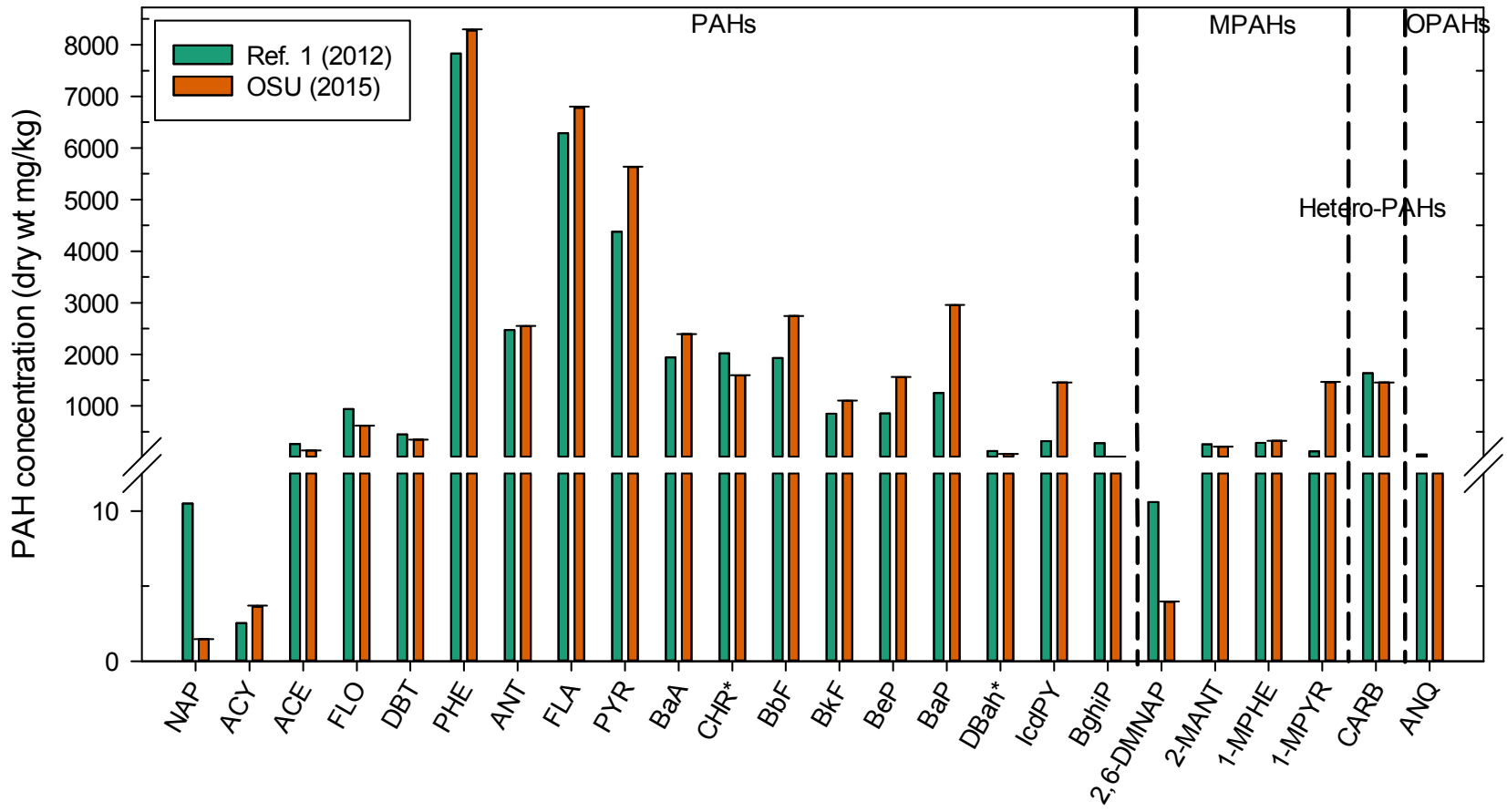

Figure S1. Comparison of PAH and PAH derivatives concentrations in the CT-1 product by prior publication[1] and those measured for this study. Only compounds that were detected in both studies were included here. CHR and DBahA in prior publication[1] were compared to CHR+TRI and DBah+acA concentrations in this study, respectively. Error bars represented standard errors of triplicate injections.

Compounds Group	Compound	Assigned RPF Value (Draft U.S. EPA RPF)[6]	B[*a*]P$_{eq}$ concentration (mg/kg)			Assigned PEF Value (Health Canada PEF)[7]	B[*a*]P$_{eq}$ concentration (mg/kg)		
			CT-1	CT-2	AS		CT-1	CT-2	AS
PAHs	ANH	0.4	190	86	0.8	0.1	47	22	0.2
	ANT	0	0	0	0	NV	NV	NV	NV
	BaA	0.2	478	570	0.4	0.1	239	285	0.2
	BaP	1	2,950	1,850	0.9	1	2,950	1,850	0.9
	BbF	0.8	2,190	2,000	2.1	0.1	274	249	0.2
	BcFlo	20	2,370	3,100	25	NV	NV	NV	NV
	BghiP	0.009	0.2	0.6	0.01	0.01	0.2	0.7	0.006
	Bj+eA	60 (from Benz[j]aceanthrylene)	0	119	0	NV	NV	NV	NV
	BkF	0.03	33	27	0.002	0.1	110	90	0.004
	CHR+TRI	0.1 (from Chrysene)	160	128	0.4	0.01 (from Chrysene)	16	13	0.03
	CPP	0.4	4	3.6	0	0.1	1	0.9	0
	FLA	0.08	540	2,490	0.6	0.001	6.8	31	0.003
	DBah+acA	10 (from Dibenzo[a,h]anthracene)	710	304	0	1 (from Dibenzo[a,h]anthracene)	71	30	0
	IcdPY	0.07	102	68	0.06	0.1	146	97	0.07
	PHE	0	0	0	0	0.001	8.3	45	0.02
	PYR	0	0	0	0	NV	NV	NV	NV
		SUM	9,730	10,750	30.3	***SUM***	3,870	2,710	1.9
MW302-PAHs	DBaeF+bkF	0.9 (from Dibenzo[a,e]fluoranthene)	332	109	0	1 (from Dibenzo[a,e]fluoranthene)	370	121	0
	DBaeP	0.4	63	18	0	1	160	46	0
	DBahP	0.9	220	97	0	1	246	107	0
	DBaiP	0.6	74	26	0	1	125	44	0
	DBalP	30	461	200	0	100	1,540	667	0
	N23eP	0.3	20	7	0	NV	NV	NV	NV
		SUM	1,170	457	0	***SUM***	2,430	985	0
		TOTAL	***10,900***	***15,320***	***30.3***	***TOTAL***	***6,310***	***3,700***	***1.9***

74 **Table S2.** List of PAHs and MW302-PAHs with their corresponding draft RPF and PEF values. NV stands for 'No Value'.

Compounds Group	Compound	Assigned RPF Value (Draft U.S. EPA RPF)[6]	B[*a*]P$_{eq}$ concentration (mg/kg)				Assigned PEF Value (Health Canada PEF)[7]	B[*a*]P$_{eq}$ concentration (mg/kg)			
			1.6 h	**1 d**	**45 d**	**149 d**		**1.6 h**	**1 d**	**45 d**	**149 d**
PAHs	ANH	0.4	1,130	1,840	40	10	0.1	47	62	7	9
	ANT	0	0	0	0	0	NV	NV	NV	NV	NV
	BaA	0.2	805	3,080	250	150	0.1	403	1,540	125	77
	BaP	1	4,300	5,170	1,900	1,800	1	4,300	5,170	1,900	1,800
	BbF	0.8	4,340	3,710	1,650	1,850	0.1	543	464	206	231
	BcFlo	20	2,730	4,180	1,600	1,240	NV	NV	NV	NV	NV
	BghiP	0.009	0.1	2.5	0.4	0.03	0.01	0.1	2.7	0.5	0.03
	Bj+eA	60 (from Benz[j]aceanthrylene)	0	0	0	0	NV	NV	NV	NV	NV
	BkF	0.03	60	60	27	28	0.1	197	187	89	94
	CHR+TRI	0.1 (from Chrysene)	310	370	150	140	0.01 (from Chrysene)	31	37	15	14
	CPP	0.4	1.2	1.5	0.7	2.6	0.1	0.3	0.4	0.2	0.7
	FLA	0.08	1,570	5,650	510	390	0.001	20	71	6	5
	DBah+acA	10 (from Dibenzo[a,h]anthracene)	400	515	190	270	1 (from Dibenzo[a,h]anthracene)	40	52	19	27
	IcdPY	0.07	70	160	40	50	0.1	101	225	61	75
	PHE	0	0	0	0	0	0.001	16	56	2.3	1.2
	PYR	0	0	0	0	0	NV	NV	NV	NV	NV
		SUM	15,700	24,700	6,370	5,960	***SUM***	8,440	12,050	4,040	3,600
MW302-PAHs	DBaeF+bkF	0.9 (from Dibenzo[a,e]fluoranthene)	325	250	195	250	1 (from Dibenzo[a,e]fluoranthene)	361	280	216	282
	DBaeP	0.4	60	40	33	43	1	146	97	83	109
	DBahP	0.9	63	37	23	26	1	69	41	25	29
	DBaiP	0.6	48	37	23	26	1	80	61	38	44
	DBalP	30	401	225	39	31	100	1,340	751	130	104
	N23eP	0.3	22	16	12	14	NV	NV	NV	NV	NV
		SUM	917	607	325	395	***SUM***	2,000	1,230	493	568
		TOTAL	***16,600***	***25,300***	***6,690***	***6,350***	***TOTAL***	***10,440***	***13,280***	***4,530***	***4,170***

Table S3. List of PAHs and MW302-PAHs with their corresponding draft RPF and PEF values. NV stands for 'No Value'.

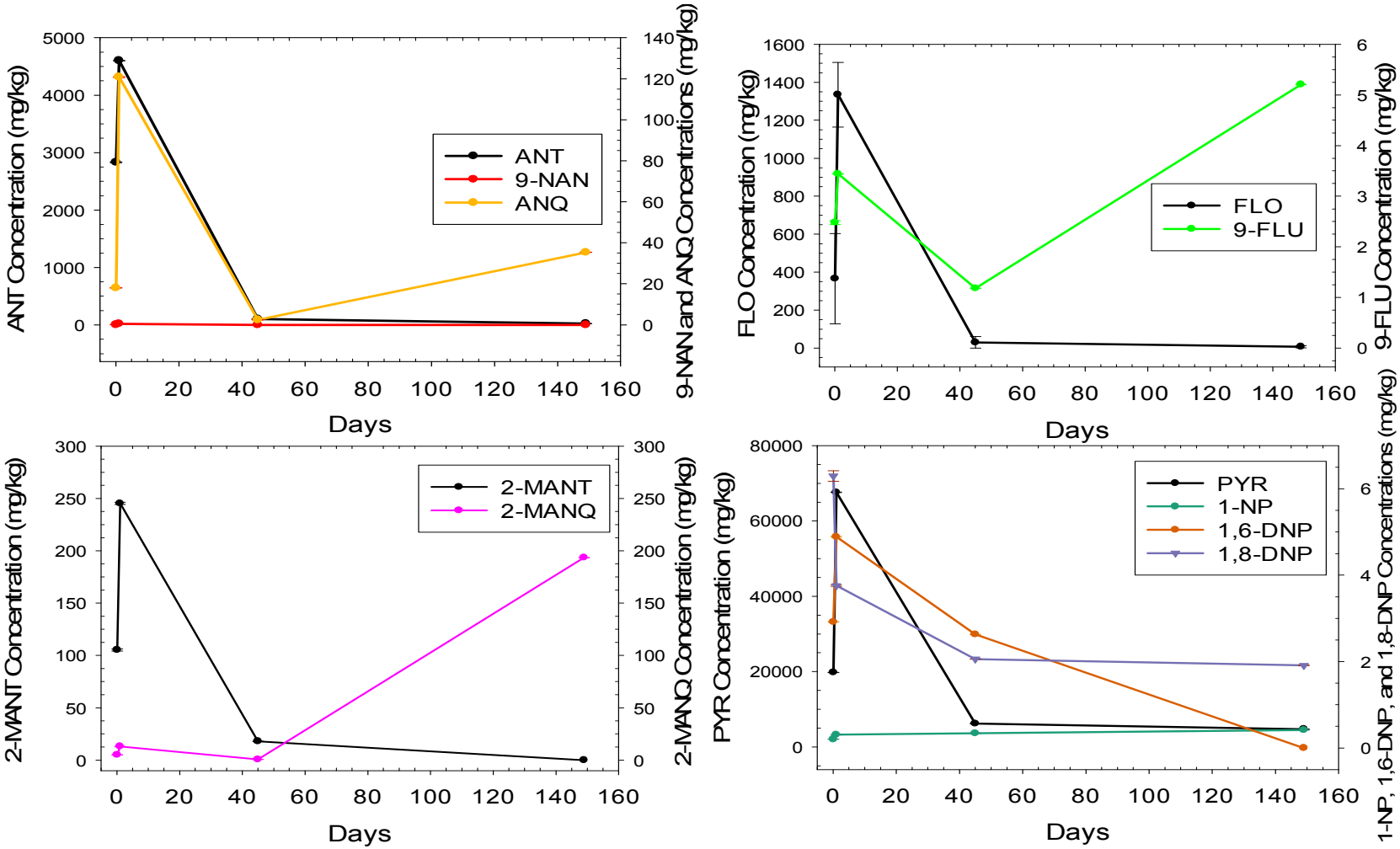

Figure S2. Concentration of PAHs and MPAHs and their Nitro- and Oxy-PAHs derivative products post pavement sealcoat application.

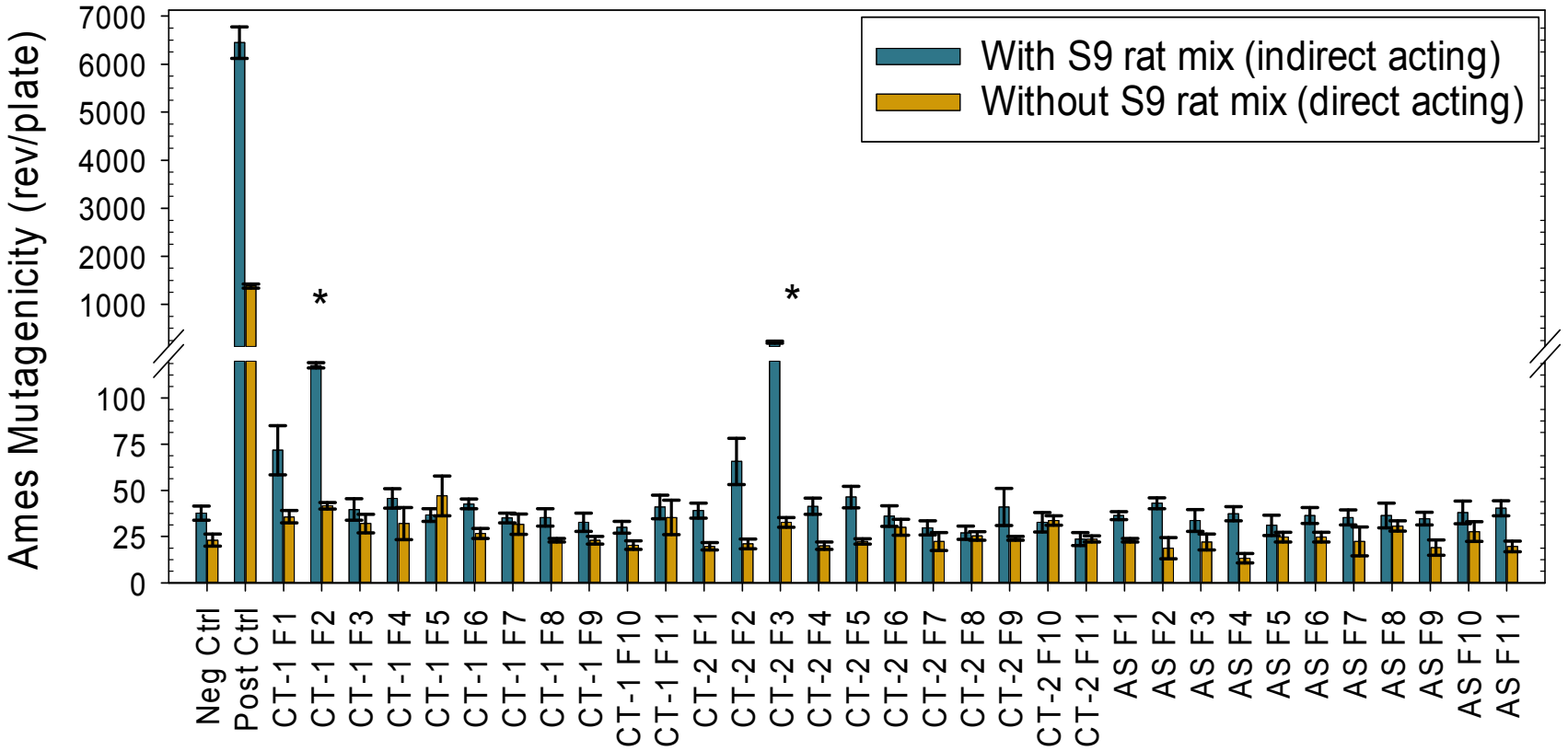

Figure S3. Mutagenicity data of F1-F11 for the pavement sealcoat products analyzed at 1:100 dilution. The blue shade illustrates the indirect acting mutagenicity and the light orange shade illustrates the direct acting mutagenicity. * denotes significance from negative control ($p < 0.05$). Error bars represent one standard deviation.

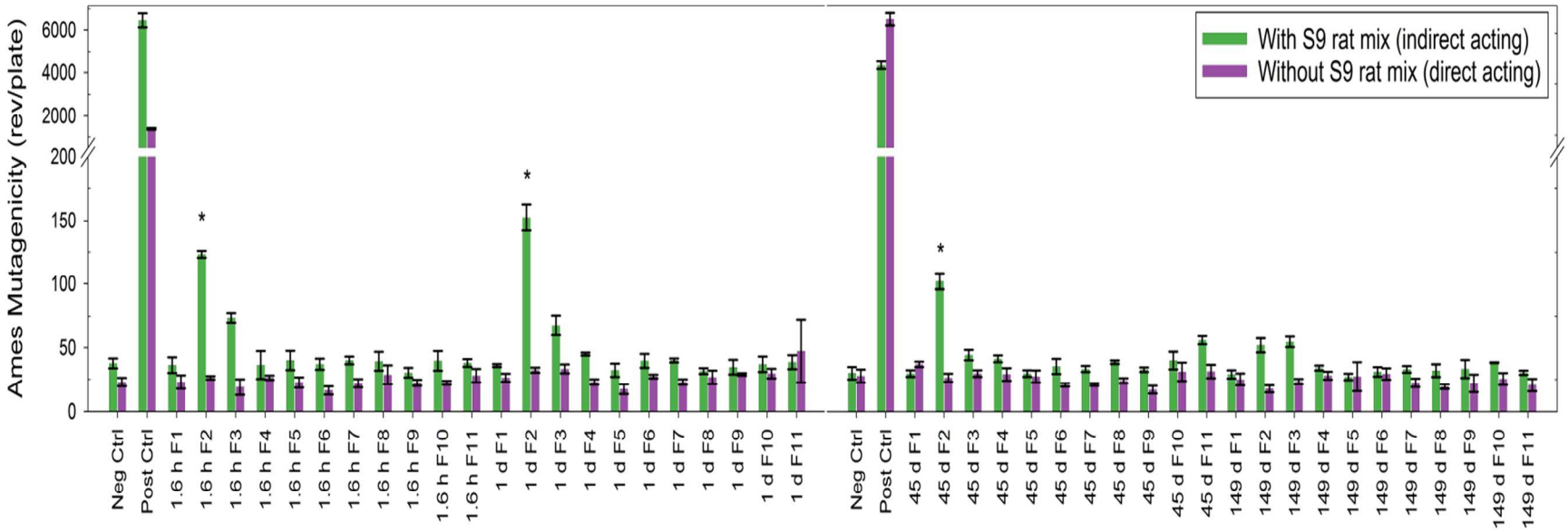

Figure S4. Mutagenicity data of F1-F11 for time point scrapes analyzed at 1:100 dilution. The green shade illustrates the indirect acting mutagenicity and the purple illustrates the direct acting mutagenicity. * denotes significance from negative control ($p < 0.05$). Error bars represent one standard deviation.

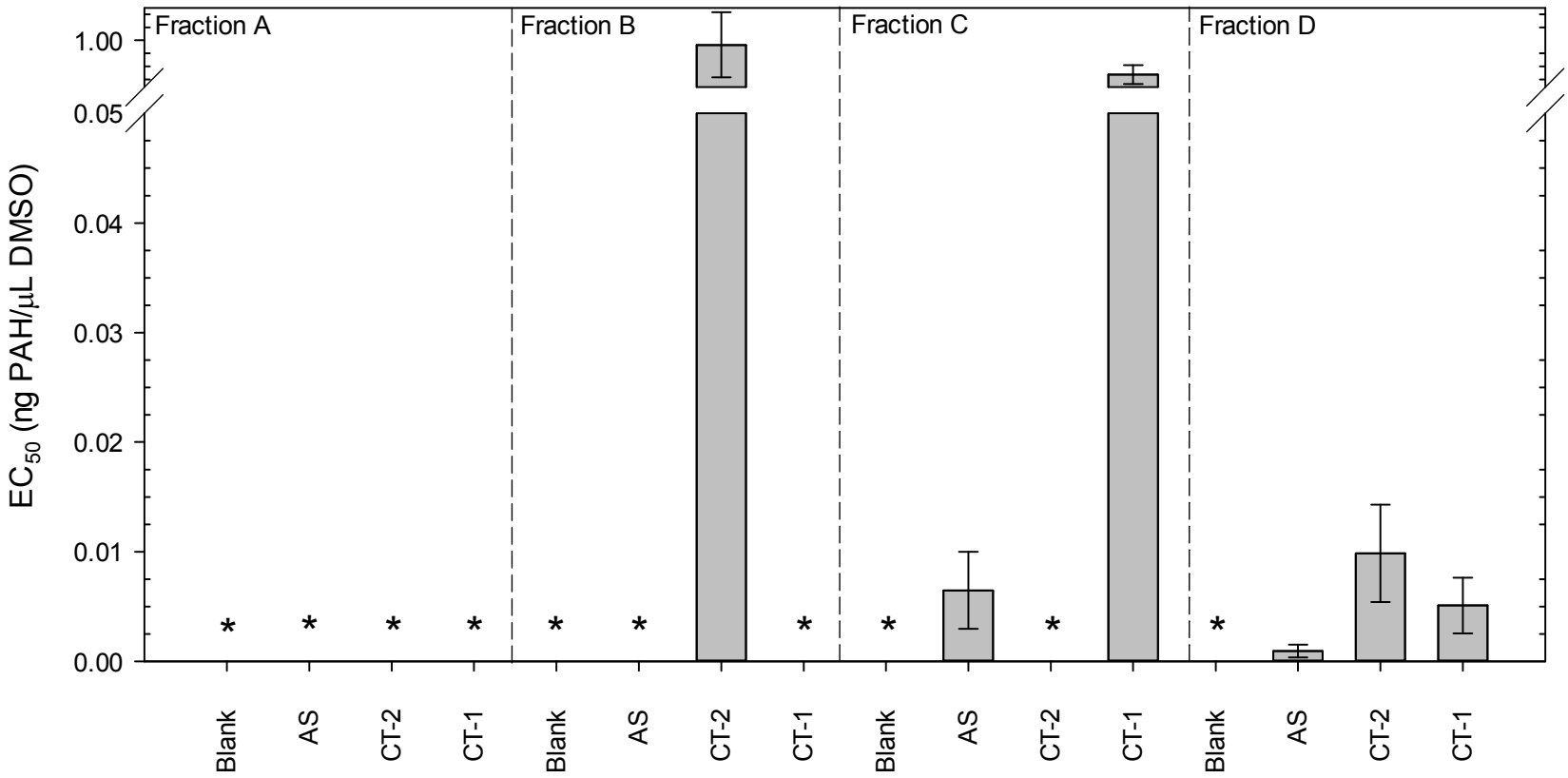

Figure S5. EC_{50} concentration of pavement sealcoat products calculated based on the lowest effect level (LEL) of "any effect" in the zebrafish developmental toxicity test. Error bars represent standard errors and * denotes no toxicity observed (i.e. no calculated EC_{50}).

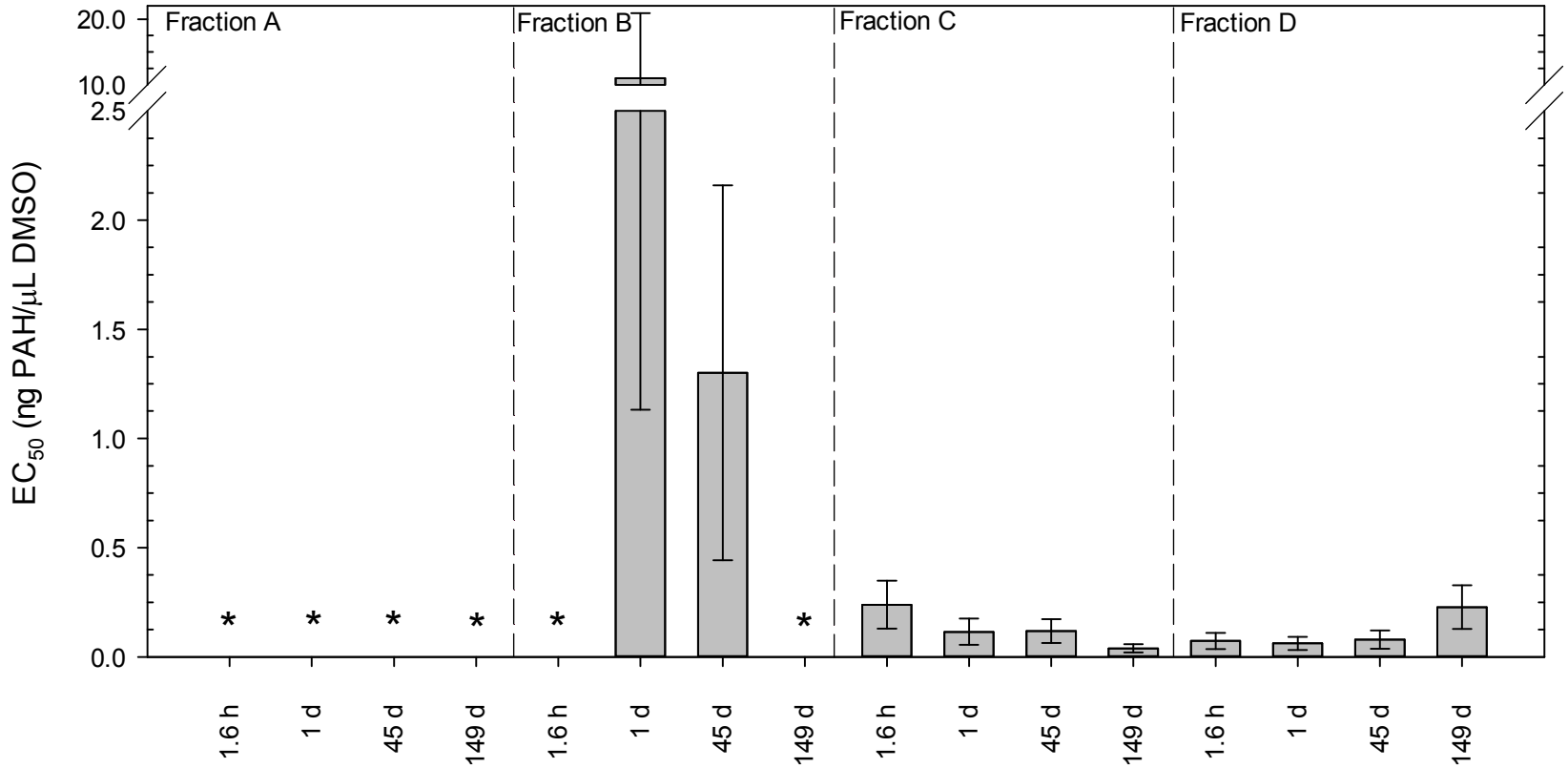

Figure S6. EC_{50} concentration of CT-1 time point scrapes calculated based on the lowest effect level (LEL) of "any effect" in the zebrafish developmental toxicity test. Error bars represent standard errors and * denotes no toxicity observed (i.e. no calculated EC_{50}).

Reference

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