

Prenatal Developmental Toxicity Testing of Petroleum Substances Using the Zebrafish Embryotoxicity Test

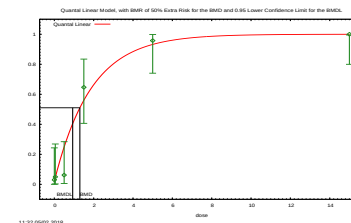
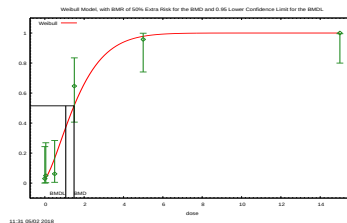
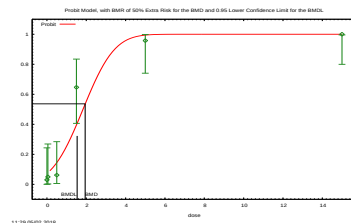
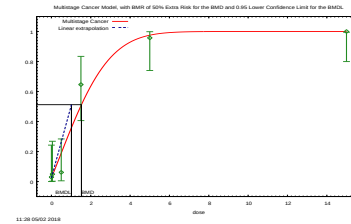
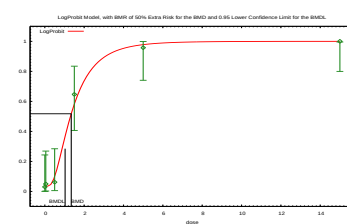
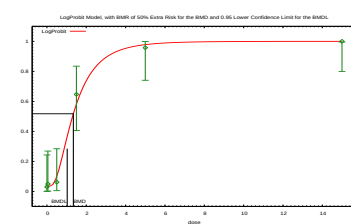
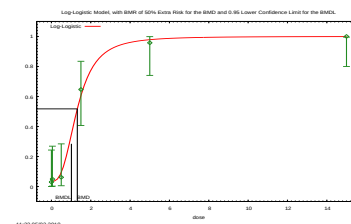
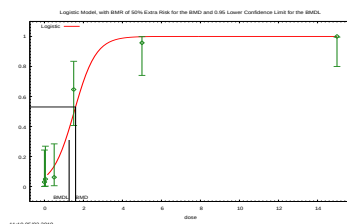
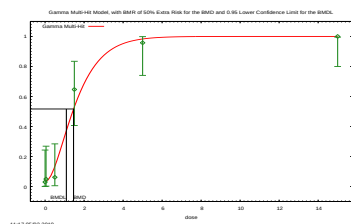
Supplementary Data

Appendix A: Results from concentration-response modeling of the data on zebrafish embryo test (ZET) of 9 petroleum substances (PS) extracts

Tab. A1: Results from a BMD analysis of the data on ZET of sample #034 – heavy fuel oil (HFO)

The table presents the benchmark concentration for 50% effect on differentiation (BMC50) and the 95% benchmark dose lower confidence limit (BMCL50) values for a BMR of 10% extra risk with characteristics of the model fit. The selected model (BMC/BMCL < 3, lowest AIC) is given in bold.

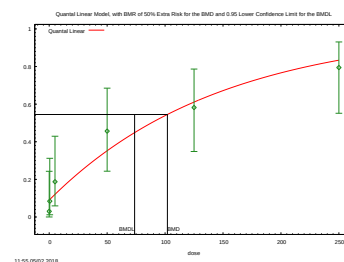
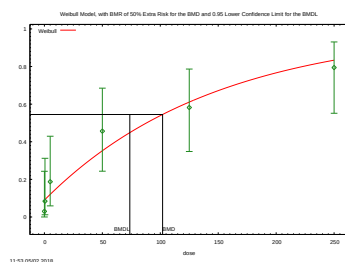
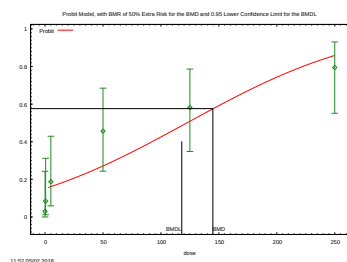
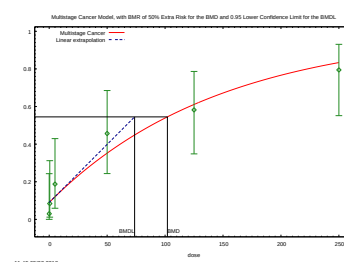
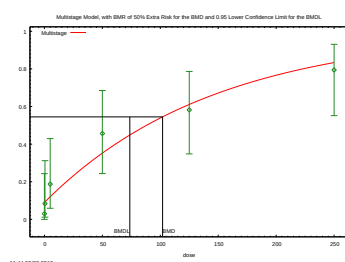
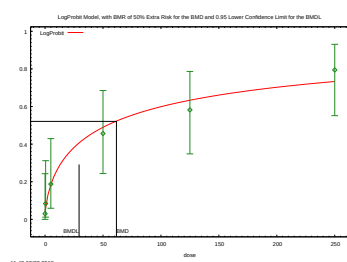
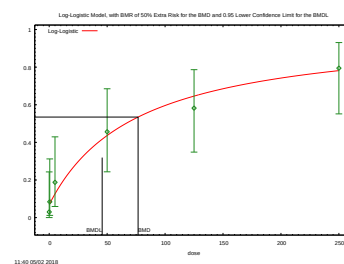
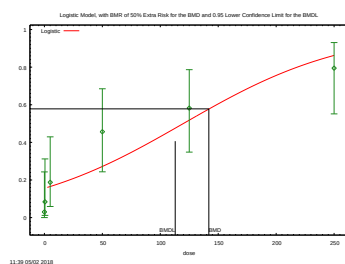
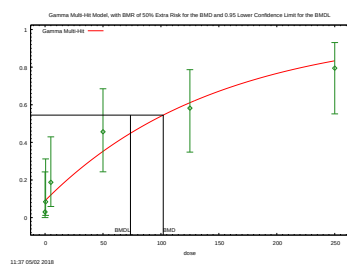
Model type	Risk type	BMFR	Restricted model	No of parameters	p-value (goodness of fit)	AIC	BMC50 (µg raw material/ml)	BMCL50 (µg raw material/ml)	BMC/BMCL
Gamma	Extra	0.1	yes	2	0.4041	64.42	1.46	1.09	1.34
Logistic	Extra	0.1	no	2	0.0004	66.88	1.60	1.27	1.26
LogLogistic	Extra	0.1	yes	2	0.813	62.43	1.32	1.02	1.29
LogProbit	Extra	0.1	no	2	0.7557	62.68	1.34	1.03	1.30
Multistage	Extra	0.1	no	3	0.7557	62.68	1.34	1.03	1.30
Multistage Cancer	Extra	0.1	yes	2	0.2537	66.18	1.53	1.02	1.50
Probit	Extra	0.1	yes	2	0.0189	68.88	1.94	1.54	1.27
Weibull	Extra	0.1	yes	2	0.3375	65.24	1.49	1.06	1.40
Quantal Linear	Extra	0.1	na	2	0.3192	65.44	1.30	0.93	1.39



Tab. A2: Results from a BMD analysis of the data on ZET of sample #097 – distillate aromatic extract (DAE)

The table presents the benchmark concentration for 50% effect on differentiation (BMC50) and the 95% benchmark dose lower confidence limit (BMCL50) values for a BMR of 10% extra risk with characteristics of the model fit. The selected model (BMC/BMCL < 3, lowest AIC) is given in bold.

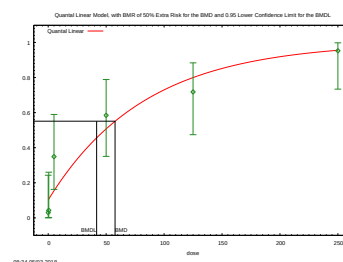
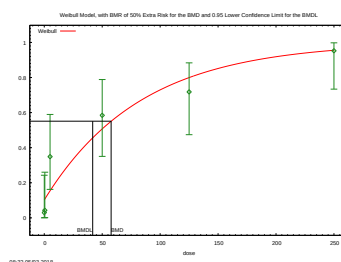
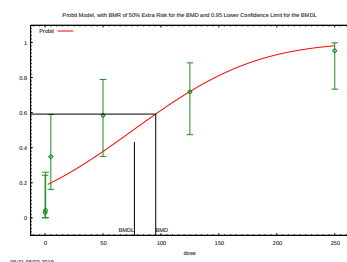
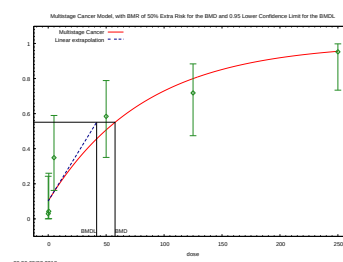
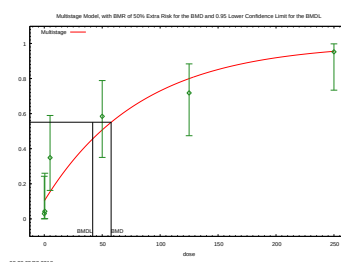
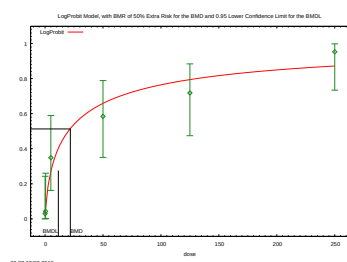
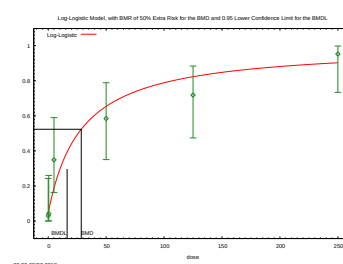
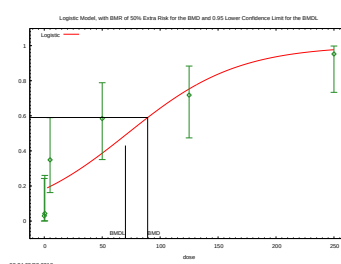
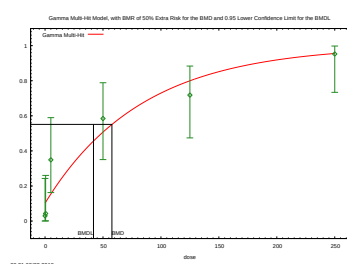
Model type	Risk type	BMFR	Restricted model	No of parameters	p-value (goodness of fit)	AIC	BMC50 (µg raw material/ml)	BMCL50 (µg raw material/ml)	BMC/ BMCL
Gamma	Extra	0.1	yes	2	0.5585	118.43	102.01	73.71	1.38
Logistic	Extra	0.1	no	2	0.0989	123.85	141.90	112.88	1.26
LogLogistic	Extra	0.1	yes	2	0.8238	116.94	76.71	45.71	1.68
LogProbit	Extra	0.1	no	2	0.8238	118.23	61.60	29.36	2.10
Multistage	Extra	0.1	no	3	0.5585	118.43	102.01	73.71	1.38
Multistage Cancer	Extra	0.1	yes	2	0.5585	118.43	102.01	73.71	1.38
Probit	Extra	0.1	yes	2	0.1039	123.66	144.81	117.99	1.23
Weibull	Extra	0.1	yes	2	0.5585	118.43	102.01	73.71	1.38
Quantal Linear	Extra	0.1	na	2	0.5585	118.43	102.01	73.71	1.38



Tab. A3: Results from a BMD analysis of the data on ZET of sample #098 – distillate aromatic extract (DAE)

The table presents the benchmark concentration for 50% effect on differentiation (BMC50) and the 95% benchmark dose lower confidence limit (BMCL50) values for a BMR of 10% extra risk with characteristics of the model fit. The selected model (BMC/BMCL < 3, lowest AIC) is given in bold.

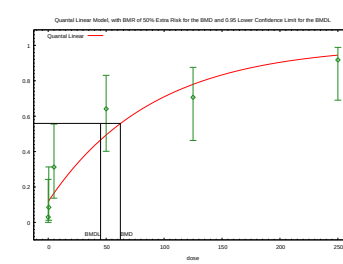
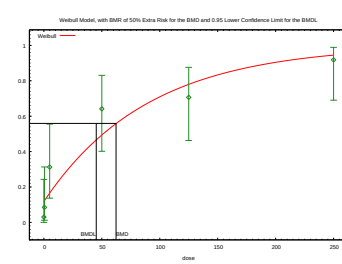
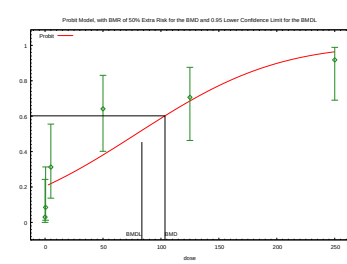
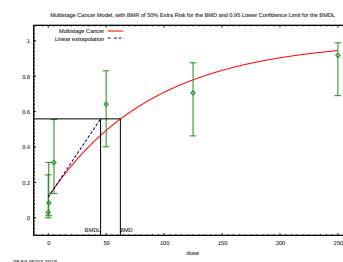
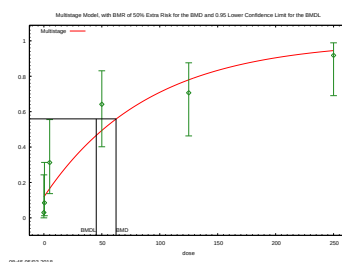
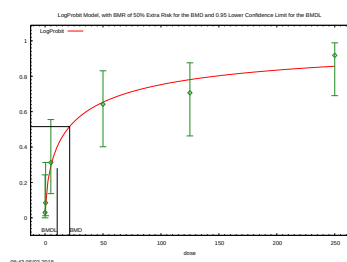
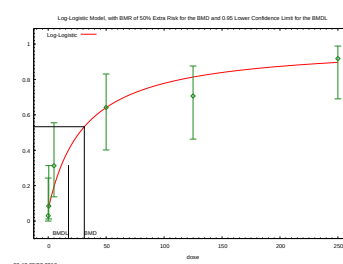
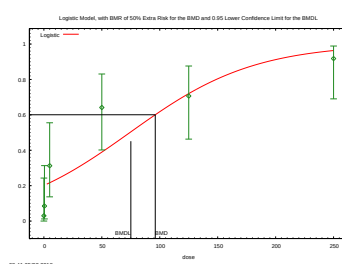
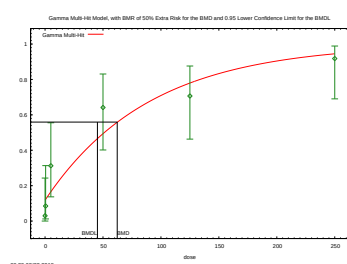
Model type	Risk type	BMFR	Restricted model	No of parameters	p-value (goodness of fit)	AIC	BMC50 (µg raw material/ml)	BMCL50 (µg raw material/ml)	BMC/ BMCL
Gamma	Extra	0.1	yes	2	0.0583	109.39	57.70	41.79	1.38
Logistic	Extra	0.1	no	2	0.0136	115.12	89.23	70.05	1.27
LogLogistic	Extra	0.1	yes	2	0.1907	106.52	28.52	16.32	1.75
LogProbit	Extra	0.1	no	2	0.3678	106.37	21.82	11.47	1.90
Multistage	Extra	0.1	no	3	0.0583	109.39	57.70	41.79	1.38
Multistage Cancer	Extra	0.1	yes	2	0.0583	109.39	57.70	41.79	1.38
Probit	Extra	0.1	yes	2	0.011	115.56	95.51	77.10	1.24
Weibull	Extra	0.1	yes	2	0.0583	109.39	57.70	41.79	1.38
Quantal Linear	Extra	0.1	na	2	0.0583	109.39	57.70	41.79	1.38



Tab. A4: Results from a BMD analysis of the data on ZET of sample #099 – distillate aromatic extract (DAE)

The table presents the benchmark concentration for 50% effect on differentiation (BMC50) and the 95% benchmark dose lower confidence limit (BMCL50) values for a BMR of 10% extra risk with characteristics of the model fit. The selected model (BMC/BMCL < 3, lowest AIC) is given in bold.

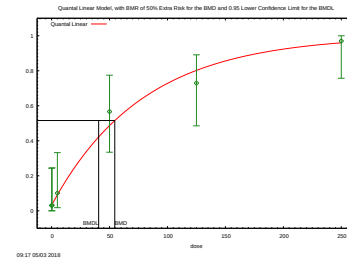
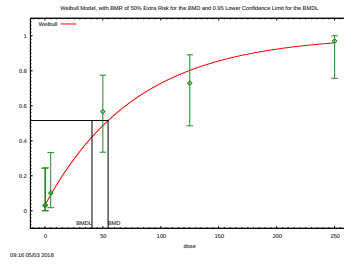
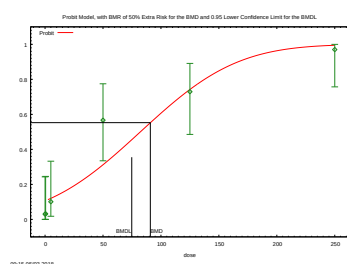
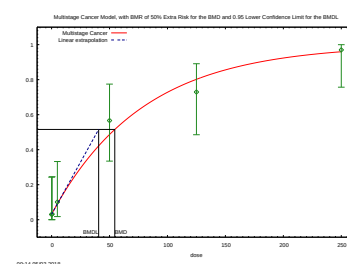
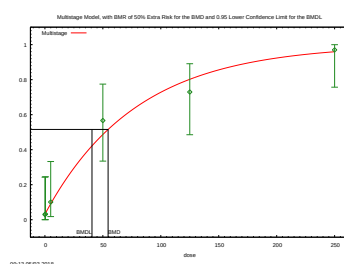
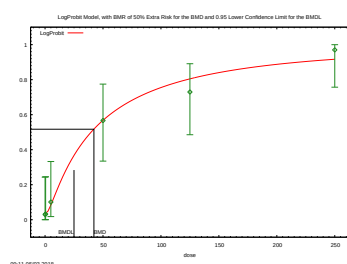
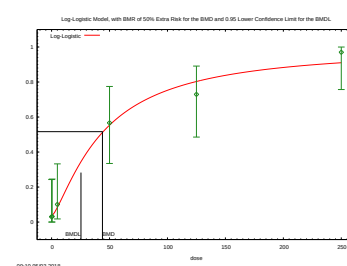
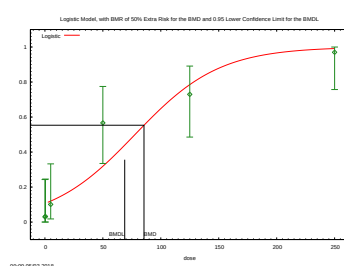
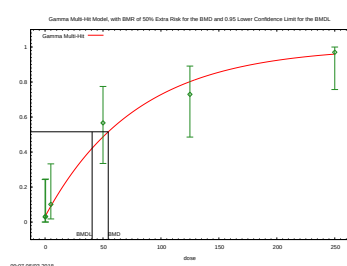
Model type	Risk type	BMFR	Restricted model	No of parameters	p-value (goodness of fit)	AIC	BMC50 (µg raw material/ml)	BMCL50 (µg raw material/ml)	BMC/ BMCL
Gamma	Extra	0.1	yes	2	0.1106	115.08	62.28	45.19	1.38
Logistic	Extra	0.1	no	2	0.0114	121.91	96.12	75.05	1.28
LogLogistic	Extra	0.1	yes	2	0.4349	111.05	31.16	17.51	1.78
LogProbit	Extra	0.1	no	2	0.7279	110.88	21.23	10.47	2.03
Multistage	Extra	0.1	no	3	0.1106	115.08	62.28	45.19	1.38
Multistage Cancer	Extra	0.1	yes	2	0.1106	115.08	62.28	45.19	1.38
Probit	Extra	0.1	yes	2	0.009	122.46	103.50	83.55	1.24
Weibull	Extra	0.1	yes	2	0.1106	115.08	62.28	45.19	1.38
Quantal Linear	Extra	0.1	na	2	0.1106	115.08	62.28	45.19	1.38



Tab. A5: Results from a BMD analysis of the data on ZET of sample #171 – gas oil (GO)

The table presents the benchmark concentration for 50% effect on differentiation (BMC50) and the 95% benchmark dose lower confidence limit (BMCL50) values for a BMR of 10% extra risk with characteristics of the model fit. The selected model (BMC/BMCL < 3, lowest AIC) is given in bold.

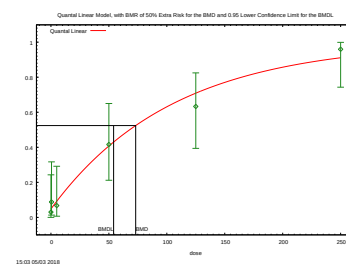
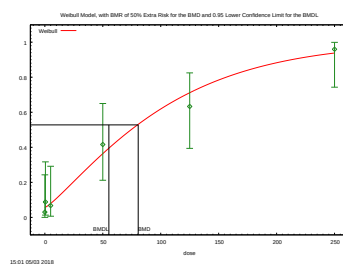
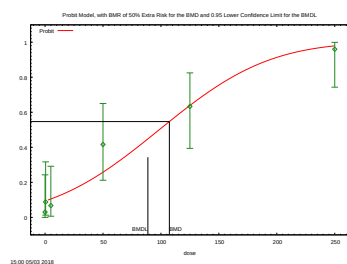
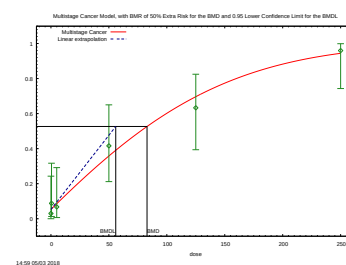
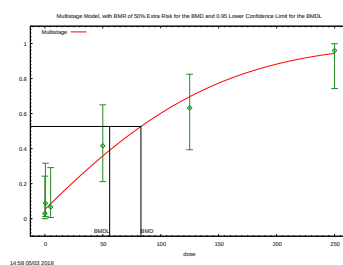
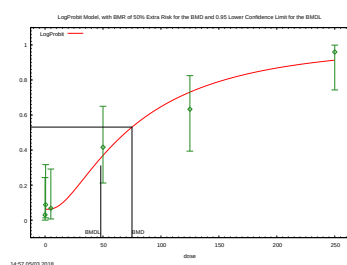
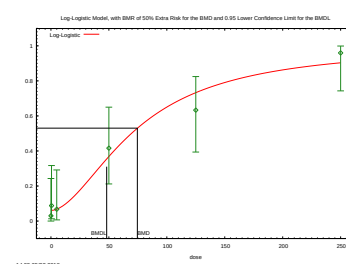
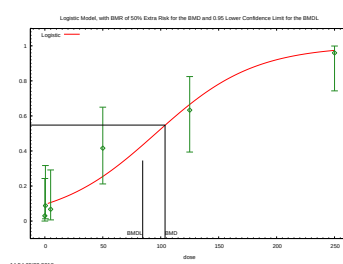
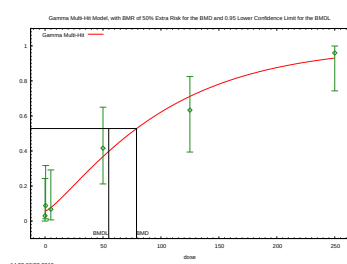
Model type	Risk type	BMFR	Restricted model	No of parameters	p-value (goodness of fit)	AIC	BMC50 (µg raw material/ml)	BMCL50 (µg raw material/ml)	BMC/ BMCL
Gamma	Extra	0.1	yes	2	0.8662	85.48	54.53	40.61	1.34
Logistic	Extra	0.1	no	2	0.0492	93.75	85.49	68.86	1.24
LogLogistic	Extra	0.1	yes	2	0.6408	88.13	43.90	25.36	1.73
LogProbit	Extra	0.1	no	2	0.671	87.97	42.16	24.97	1.69
Multistage	Extra	0.1	no	3	0.8662	85.48	54.53	40.61	1.34
Multistage Cancer	Extra	0.1	yes	2	0.8662	85.48	54.53	40.61	1.34
Probit	Extra	0.1	yes	2	0.0286	94.16	90.85	74.81	1.21
Weibull	Extra	0.1	yes	2	0.8662	85.48	54.53	40.61	1.34
Quantal Linear	Extra	0.1	na	2	0.8662	85.48	54.53	40.61	1.34



Tab. A6: Results from a BMD analysis of the data on ZET of sample #172 – gas oil (GO)

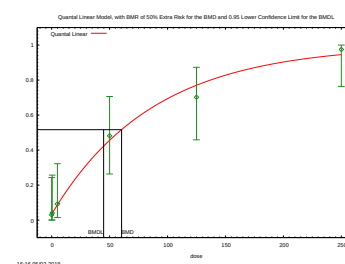
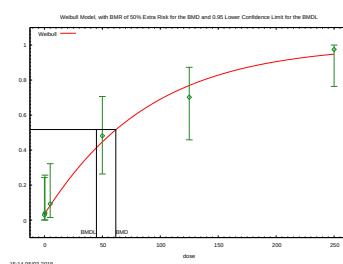
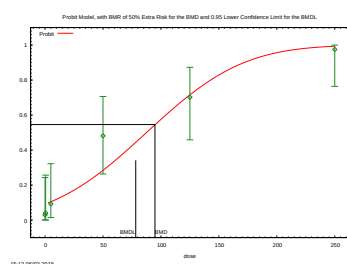
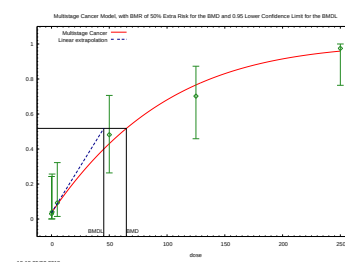
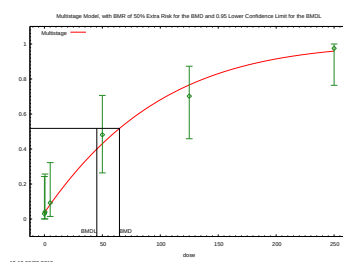
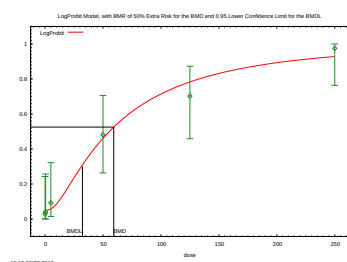
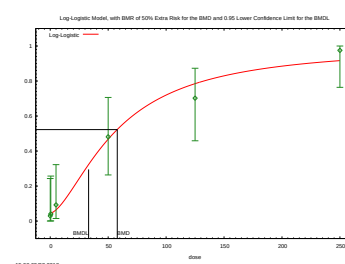
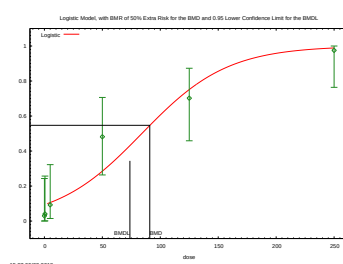
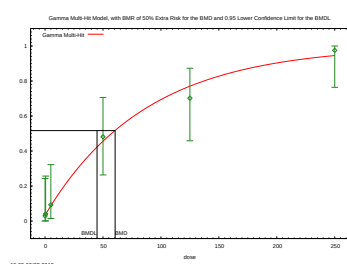
The table presents the benchmark concentration for 50% effect on differentiation (BMC50) and the 95% benchmark dose lower confidence limit (BMCL50) values for a BMR of 10% extra risk with characteristics of the model fit. The selected model (BMC/BMCL < 3, lowest AIC) is given in bold.

Model type	Risk type	BMFR	Restricted model	No of parameters	p-value (goodness of fit)	AIC	BMC50 (µg raw material/ml)	BMCL50 (µg raw material/ml)	BMC/ BMCL
Gamma	Extra	0.1	yes	2	0.6374	95.14	78.92	54.95	1.44
Logistic	Extra	0.1	no	2	0.3637	95.82	103.73	84.44	1.23
LogLogistic	Extra	0.1	yes	2	0.47	96.11	74.58	48.10	1.55
LogProbit	Extra	0.1	no	2	0.5147	95.83	75.02	48.08	1.56
Multistage	Extra	0.1	no	3	0.6973	94.84	82.92	55.93	1.48
Multistage Cancer	Extra	0.1	yes	2	0.6973	94.84	82.92	55.93	1.48
Probit	Extra	0.1	yes	2	0.3748	95.68	107.28	88.74	1.21
Weibull	Extra	0.1	yes	2	0.6547	95.05	80.57	55.24	1.46
Quantal Linear	Extra	0.1	na	2	0.7473	93.42	73.13	54.09	1.35



Tab. A7: Results from a BMD analysis of the data on ZET of sample #175 – vacuum tower overhead oil (VTO)
The table presents the benchmark concentration for 50% effect on differentiation (BMC50) and the 95% benchmark dose lower confidence limit (BMCL50) values for a BMR of 10% extra risk with characteristics of the model fit. The selected model (BMC/BMCL < 3, lowest AIC) is given in bold.

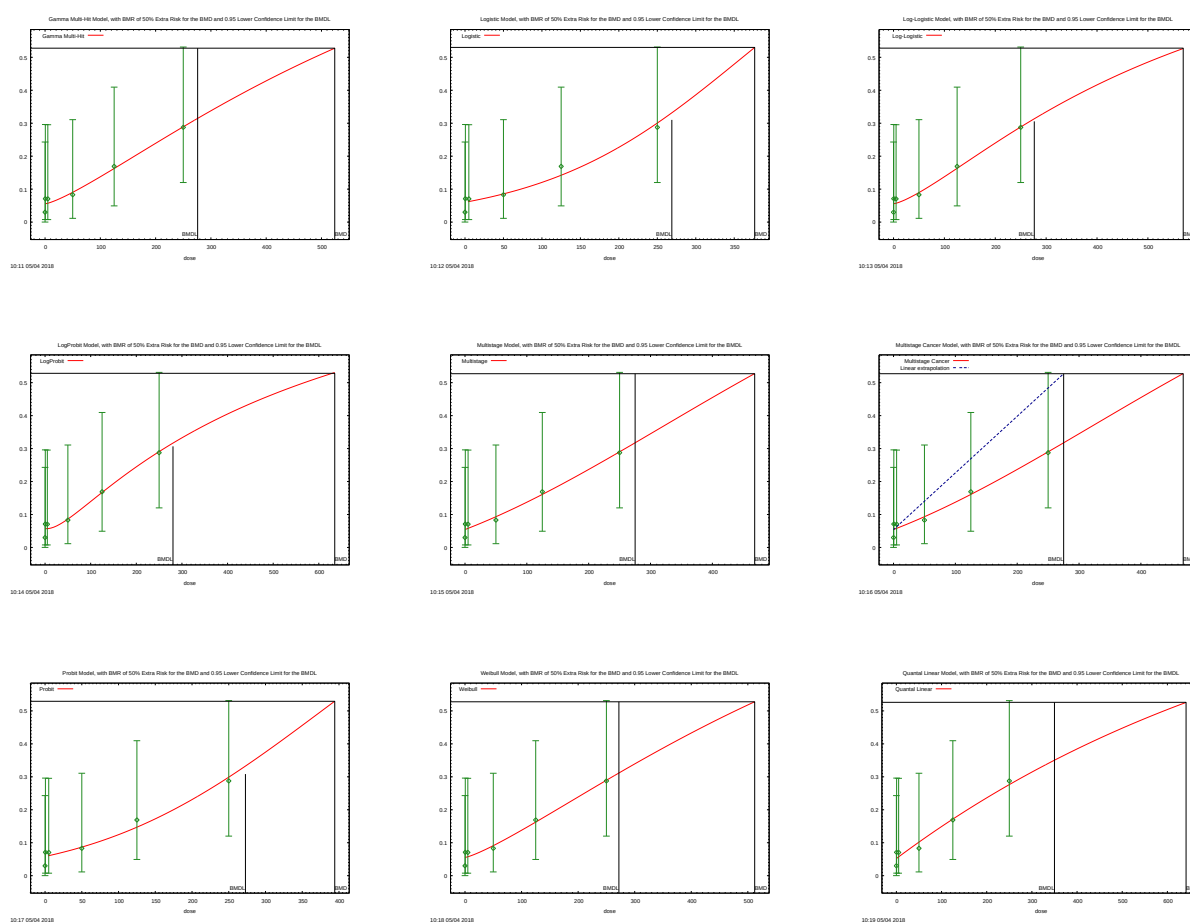
Model type	Risk type	BMFR	Restricted model	No of parameters	p-value (goodness of fit)	AIC	BMC50 (µg raw material/ml)	BMCL50 (µg raw material/ml)	BMC/ BMCL
Gamma	Extra	0.1	yes	2	0.8172	88.23	60.47	44.86	1.35
Logistic	Extra	0.1	no	2	0.1944	91.35	90.92	73.78	1.23
LogLogistic	Extra	0.1	yes	2	0.549	89.60	57.85	33.11	1.75
LogProbit	Extra	0.1	no	2	0.5422	89.50	59.26	32.27	1.84
Multistage	Extra	0.1	no	3	0.8399	88.08	64.77	45.23	1.43
Multistage Cancer	Extra	0.1	yes	2	0.8399	88.08	64.77	45.23	1.43
Probit	Extra	0.1	yes	2	0.1677	91.37	94.84	78.26	1.21
Weibull	Extra	0.1	yes	2	0.8154	88.22	61.62	44.89	1.37
Quantal Linear	Extra	0.1	na	2	0.9199	86.23	60.36	44.86	1.35



Tab. A8: Results from a BMD analysis of the data on ZET of sample #185 – residual aromatic extract (RAE)

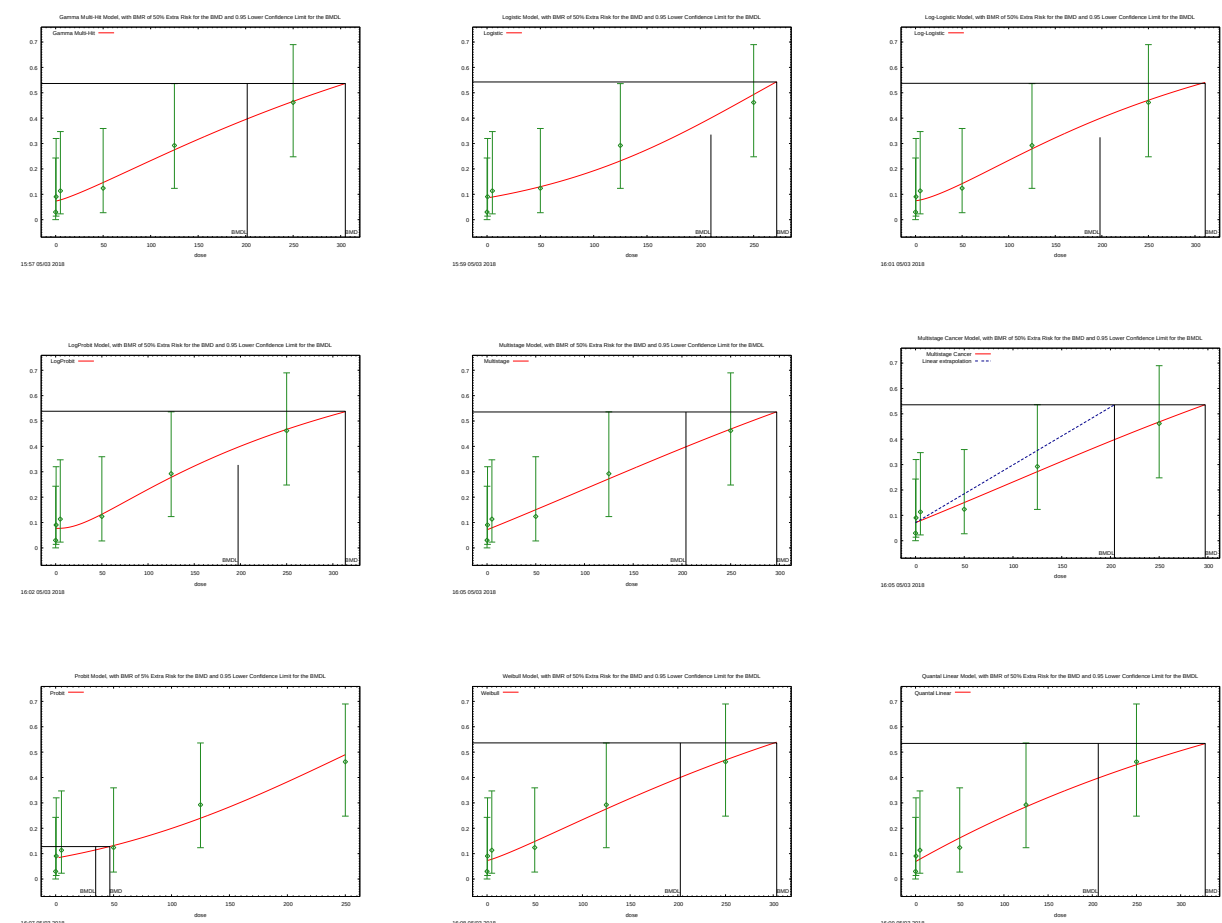
The table presents the benchmark concentration for 50% effect on differentiation (BMC50) and the 95% benchmark dose lower confidence limit (BMCL50) values for a BMR of 10% extra risk with characteristics of the model fit. The selected model (BMC/BMCL < 3, lowest AIC) is given in bold.

Model type	Risk type	BMFR	Restricted model	No of parameters	p-value (goodness of fit)	AIC	BMC50 (µg raw material/ml)	BMCL50 (µg raw material/ml)	BMC/ BMCL
Gamma	Extra	0.1	yes	2	0.9354	85.90	524.02	276	1.90
Logistic	Extra	0.1	no	2	0.9715	84.02	376.59	268.89	1.40
LogLogistic	Extra	0.1	yes	2	0.936	85.90	569.45	276.69	2.06
LogProbit	Extra	0.1	no	2	0.9371	85.90	634.92	280.39	2.26
Multistage	Extra	0.1	no	3	0.9347	85.90	468.46	275.11	1.70
Multistage Cancer	Extra	0.1	yes	2	0.9347	85.90	468.46	275.11	1.70
Probit	Extra	0.1	yes	2	0.9761	83.97	394.24	272.71	1.45
Weibull	Extra	0.1	yes	2	0.9352	85.90	512.12	272.06	1.88
Quantal Linear	Extra	0.1	na	2	0.9743	83.96	641.36	349.99	1.83



Tab. A9: Results from a BMD analysis of the data on ZET of sample #186 – residual aromatic extract (RAE)
The table presents the benchmark concentration for 50% effect on differentiation (BMC50) and the 95% benchmark dose lower confidence limit (BMCL50) values for a BMR of 10% extra risk with characteristics of the model fit. The selected model (BMC/BMCL < 3, lowest AIC) is given in bold.

Model type	Risk type	BMFR	Restricted model	No of parameters	p-value (goodness of fit)	AIC	BMC50 (µg raw material/ml)	BMCL50 (µg raw material/ml)	BMC/ BMCL
Gamma	Extra	0.1	yes	2	0.7762	105.67	304.92	201.64	1.51
Logistic	Extra	0.1	no	2	0.8371	104.11	271.61	209.94	1.29
LogLogistic	Extra	0.1	yes	2	0.78	105.67	310.96	198.03	1.57
LogProbit	Extra	0.1	no	2	0.7824	105.67	313.50	197.45	1.59
Multistage	Extra	0.1	no	3	0.7779	105.66	297.25	204.12	1.46
Multistage Cancer	Extra	0.1	yes	2	0.7779	105.66	297.25	204.12	1.46
Probit	Extra	0.1	yes	2	0.8598	103.96	273.63	208.82	1.31
Weibull	Extra	0.1	yes	2	0.7764	105.67	303.65	202.61	1.50
Quantal Linear	Extra	0.1	na	2	0.8829	103.72	327.78	206.77	1.59

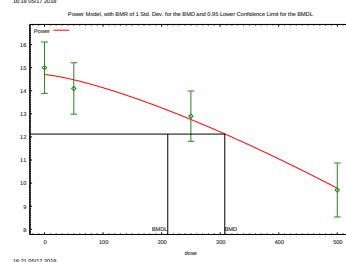
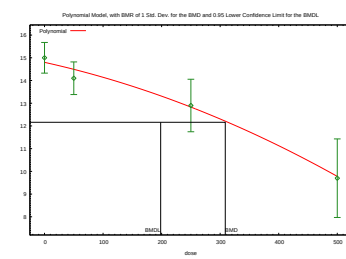
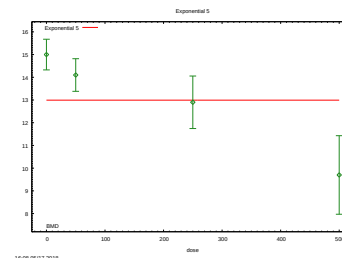
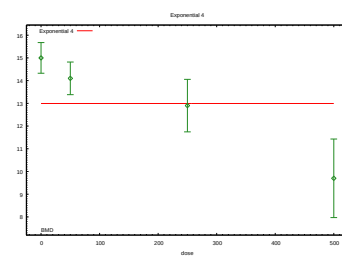
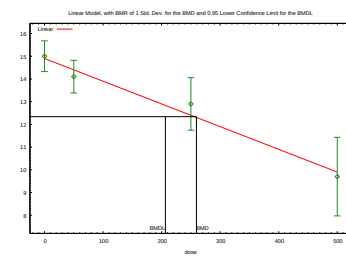
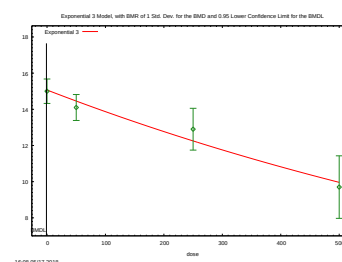
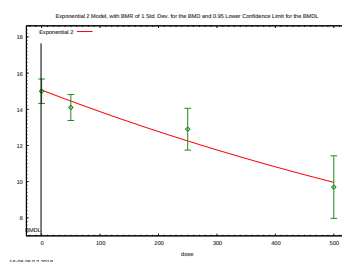
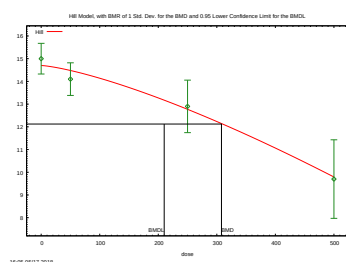


Appendix B: Results from dose-response modeling of the data on developmental toxicity occurring at higher incidences in petroleum substances exposed rats over controls
(ARCO 1993; Feuston et al. 1996; Hoberman et al. 1995; Mobil 1989)

Tab. B1: Results from a BMD analysis (continuous model) of the data on number of live fetuses/litter of female rats exposed to gas oil intermediate (64741-43-1) by dermal exposure on gestation days (GD) 0 to 19 (ARCO 1993)
The table presents the benchmark dose (BMD10) and the 95% benchmark dose lower confidence limit (BMDL10) values for a BMR of 1 standard deviation (SD) extra risk with characteristics of the model fit. The selected model (BMD/BMDL < 3, lowest AIC) is given in bold.

Model type	Risk type	BMR	Restricted model	No of parameters	p-value (goodness of fit)				AIC	BMD (mg/kg bw/day)	BMDL (mg/kg bw/day)	BMD/ BMDL
					Test 1	Test 2	Test 3	Test 4				
Hill	Extra	1.0 SD	yes	5	<.0001	<.0001	<.0001	NA	286.09	307.94	210.11	1.47
Hill	Extra	1.0 SD	no	6	<.0001	<.0001	<.0001	NA	263.00	160.91	*	-
Exponential	Extra	1.0 SD	na	-	-	-	-	-	-	*	*	-
Linear	Extra	1.0 SD	yes	3	<.0001	<.0001	<.0001	0.4959	282.57	259.47	206.45	1.26
Linear	Extra	1.0 SD	no	4	<.0001	<.0001	0.348	0.7289	261.05	174.04	131.14	1.33
Polynomial	Extra	1.0 SD	yes	4	<.0001	<.0001	<.0001	0.4022	283.87	308.72	198.39	1.56
Polynomial	Extra	1.0 SD	no	5	<.0001	<.0001	0.348	0.4423	263.01	163.23	92.00	1.77
Power	Extra	1.0 SD	yes	3	<.0001	<.0001	<.0001	0.3377	284.08	307.88	210.49	1.46
Power	Extra	1.0 SD	no	4	<.0001	<.0001	0.348	0.7289	261.05	174.04	131.14	1.33

* BMD computation failed.

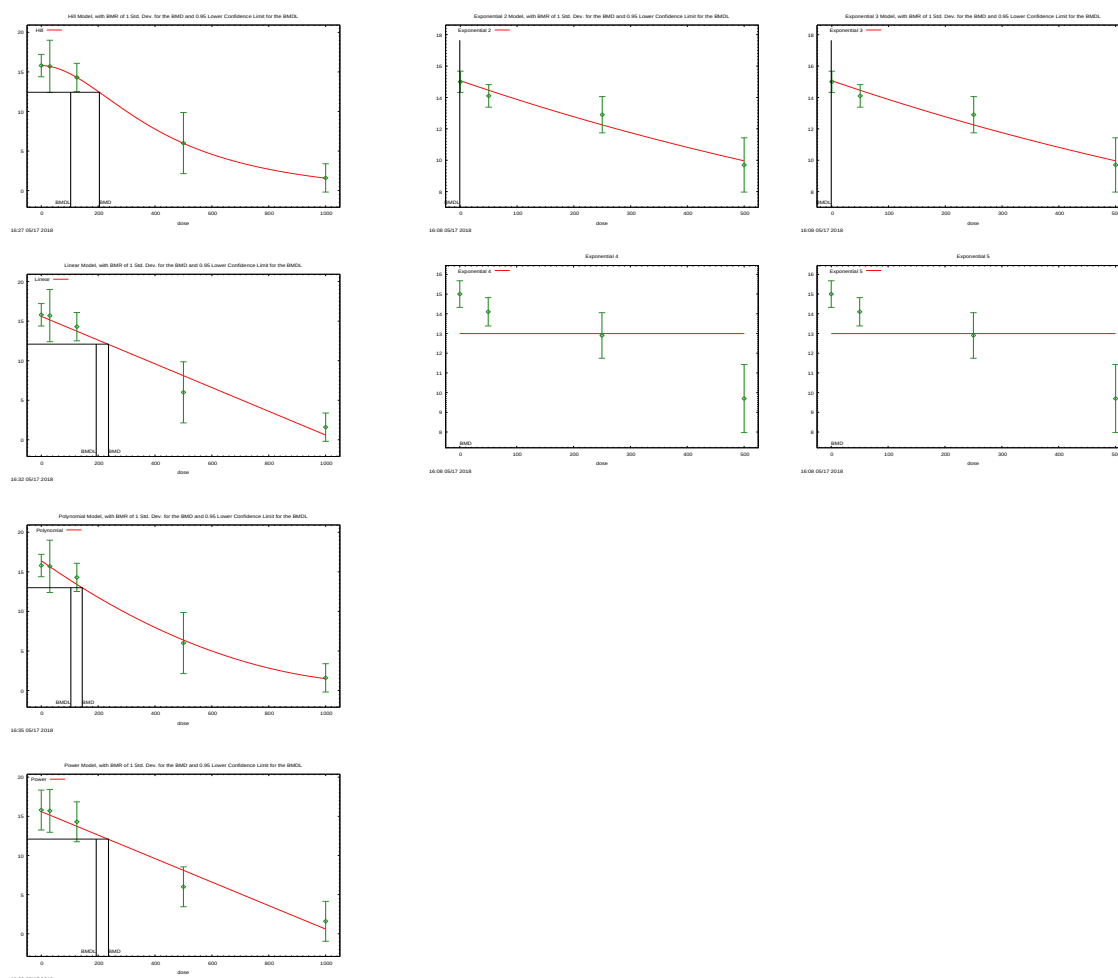


Tab. B2: Results from a BMD analysis (continuous model) of the data on number of live fetuses/litter of female rats exposed to vacuum tower overhead (64741-49-7) by dermal exposure on gestation days (GD) 0 to 19 (Mobil 1989)

The table presents the benchmark dose (BMD10) and the 95% benchmark dose lower confidence limit (BMDL10) values for a BMR of 1 standard deviation (SD) extra risk with characteristics of the model fit. The selected model (BMD/BMDL < 3, lowest AIC) is given in bold.

Model type	Risk type	BMR	Restricted model	No of parameters	p-value (goodness of fit)				AIC	BMD (mg/kg bw/day)	BMDL (mg/kg bw/day)	BMD/ BMDL
					Test 1	Test 2	Test 3	Test 4				
Hill	Extra	1.0 SD	yes	5	<.0001	0.006728	0.006728	0.996	178.21	204.40	103.38	1.98
Hill	Extra	1.0 SD	no	6	<.0001	0.006728	0.002678	0.993	180.20	201.87	102.76	1.96
Exponential	Extra	1.0 SD	na	-	-	-	-	-	-	*	*	-
Linear	Extra	1.0 SD	yes	3	<.0001	0.006728	0.006728	0.1689	179.25	236.42	193.20	1.22
Linear	Extra	1.0 SD	no	4	<.0001	0.006728	0.002678	0.2069	180.76	251.76	*	-
Polynomial	Extra	1.0 SD	yes	4	<.0001	0.006728	0.006728	0.5683	177.34	144.03	104.02	1.38
Polynomial	Extra	1.0 SD	no	5	<.0001	0.006728	0.002678	0.5748	179.30	148.89	92.78	1.60
Power	Extra	1.0 SD	yes	3	<.0001	0.006728	0.006728	0.1689	179.25	236.42	193.20	1.22
Power	Extra	1.0 SD	no	4	<.0001	0.006728	0.002678	0.2069	180.76	251.76	191.14	1.32

* BMD computation failed.

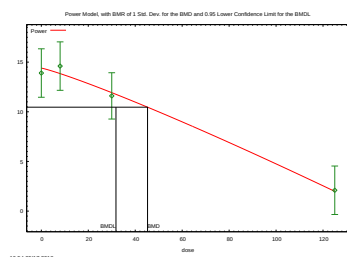
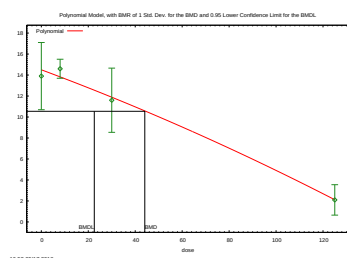
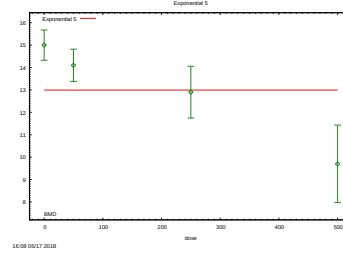
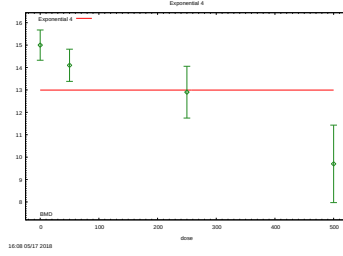
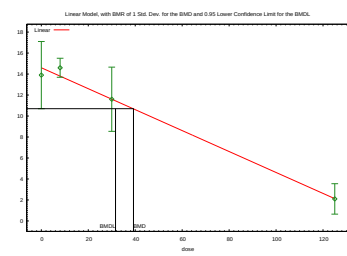
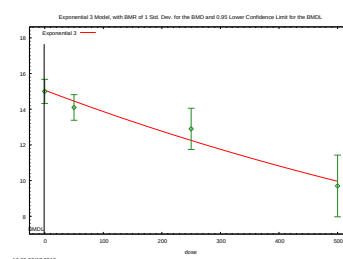
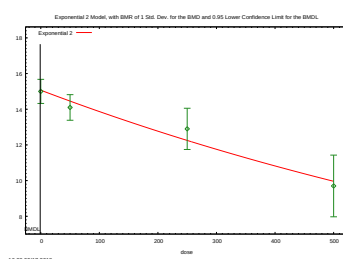
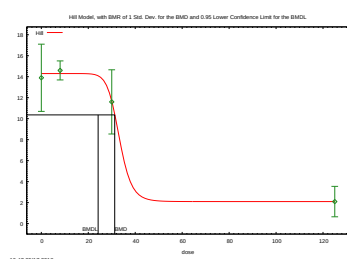


Tab. B3: Results from a BMD analysis (continuous model) of the data on number of live fetuses/litter of female rats exposed to distillate aromatic extract (64742-04-7) by dermal exposure on gestation days (GD) 0 to 19 (Feuston et al. 1996)

The table presents the benchmark dose (BMD10) and the 95% benchmark dose lower confidence limit (BMDL10) values for a BMR of 1 standard deviation (SD) extra risk with characteristics of the model fit. The selected model (BMD/BMDL < 3, lowest AIC) is given in bold.

Model type	Risk type	BMFR	Restricted model	No of parameters	p-value (goodness of fit)				AIC	BMD (mg/kg bw/day)	BMDL (mg/kg bw/day)	BMD/ BMDL
					Test 1	Test 2	Test 3	Test 4				
Hill	Extra	1.0 SD	yes	5	<.0001	<.0001	<.0001	NA	206.96	31.25	24.20	1.29
Hill	Extra	1.0 SD	no	6	<.0001	<.0001	<.0001	NA	203.85	31.53	29.90	1.05
Exponential	Extra	1.0 SD	na	-	-	-	-	-	-	*	*	-
Linear	Extra	1.0 SD	yes	3	<.0001	<.0001	<.0001	0.6176	203.71	39.26	31.61	1.24
Linear	Extra	1.0 SD	no	4	<.0001	<.0001	<.0001	0.8304	200.21	44.38	35.40	1.25
Polynomial	Extra	1.0 SD	yes	4	<.0001	<.0001	<.0001	0.3447	205.64	44.08	22.56	1.95
Polynomial	Extra	1.0 SD	no	5	<.0001	<.0001	<.0001	0.6377	202.06	52.43	26.37	1.99
Power	Extra	1.0 SD	yes	3	<.0001	<.0001	<.0001	0.3696	205.56	45.24	31.79	1.42
Power	Extra	1.0 SD	no	4	<.0001	<.0001	<.0001	0.6761	202.01	52.00	35.68	1.46

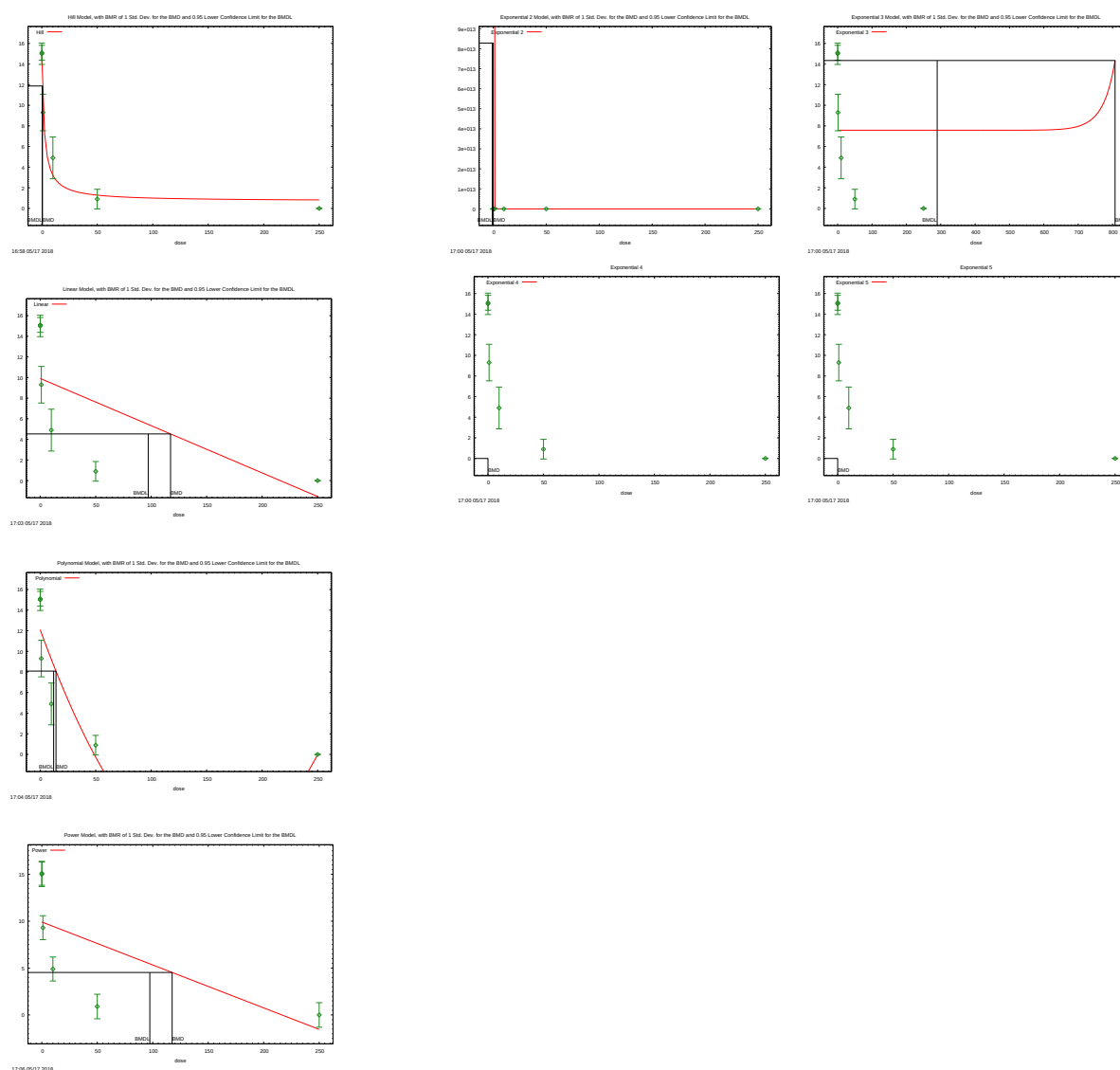
* BMD computation failed.



Tab. B4: Results from a BMD analysis (continuous model) of the data on number of live fetuses/litter of female rats exposed to heavy fuel oil (64741-62-4) by dermal exposure on gestation days (GD) 0 to 19 (Hoberman et al., 1995)
The table presents the benchmark dose (BMD10) and the 95% benchmark dose lower confidence limit (BMDL10) values for a BMR of 1 standard deviation (SD) extra risk with characteristics of the model fit. The selected model (BMD/BMDL < 3, lowest AIC) is given in bold.

Model type	Risk type	BMFR	Restricted model	No of parameters	p-value (goodness of fit)				AIC	BMD (mg/kg bw/day)	BMDL (mg/kg bw/day)	BMD/ BMDL
					Test 1	Test 2	Test 3	Test 4				
Hill	Extra	1.0 SD	yes	5	<.0001	<.0001	<.0001	0.004001	468.33	0.59	0.37	1.58
Hill	Extra	1.0 SD	no	6	<.0001	<.0001	<.0001	<.0001	692.05	4356.26	*	-
Exponential	Extra	1.0 SD	na	-	-	-	-	-	-	*	*	-
Linear	Extra	1.0 SD	yes	3	<.0001	<.0001	<.0001	<.0001	621.35	117.49	97.37	
Linear	Extra	1.0 SD	no	4	<.0001	<.0001	<.0001	<.0001	-470.67	189.32	*	-
Polynomial	Extra	1.0 SD	yes	4	<.0001	<.0001	<.0001	<.0001	541.32	14.22	12.11	1.17
Polynomial	Extra	1.0 SD	no	5	<.0001	<.0001	<.0001	<.0001	195.69	-9999.00	*	-
Power	Extra	1.0 SD	yes	3	<.0001	<.0001	<.0001	<.0001	621.35	117.49	97.37	1.21
Power	Extra	1.0 SD	no	4	<.0001	<.0001	<.0001	<.0001	729.67	74.75	52.12	1.43

* BMD computation failed.



Appendix C: Results from the ZET of samples tested in the present study: DMSO-extracts of 9 PS and 2 GTL products

0.25% (v/v) DMSO was used as solvent control for the ZET. The ZET results are presented as mean fraction of total general morphology scoring (GMS) system at 96 hpf. Results represent at least four independent experiments ($n \geq 4$) and are presented as mean $\pm$ standard error mean (SEM).

Concentration (μg raw material/ml)	#034-HFO		#097-DAE		#098-DAE		#099-DAE		#171-GO		#172-GO		#175-VTO	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
0 (DMSO)	0.97	0.01	0.97	0.01	0.97	0.01	0.97	0.01	0.97	0.01	0.97	0.01	0.97	0.01
0.05	0.951	0.013												
0.5	0.939	0.018	0.916	0.033	0.957	0.017	0.968	0.012	0.968	0.012	0.912	0.023	0.959	0.010
1.5	0.354	0.089												
5	0.042	0.029	0.813	0.031	0.651	0.049	0.899	0.015	0.899	0.015	0.932	0.018	0.907	0.024
15	0.000	0.000												
50			0.543	0.054	0.415	0.035	0.434	0.044	0.434	0.044	0.584	0.031	0.519	0.048
125			0.418	0.061	0.282	0.066	0.271	0.024	0.271	0.024	0.367	0.053	0.298	0.047
250			0.205	0.025	0.048	0.022	0.030	0.013	0.030	0.013	0.040	0.020	0.025	0.016

Concentration (μg raw material/ml)	#185-RAE		#186-DAE		#091-GTLb		#092-GTLg	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
0 (DMSO)	0.97	0.01	0.97	0.01	0.97	0.01	0.97	0.01
0.5	0.929	0.029	0.910	0.032	0.94	0.01	0.97	0.03
5	0.929	0.017	0.886	0.050				
50	0.917	0.020	0.876	0.051				
125	0.831	0.044	0.707	0.066	0.960	0.024	0.979	0.020
250	0.710	0.060	0.538	0.069	0.960	0.021	0.955	0.017

Appendix D: Aromatic ring class (ARC) profiles of petroleum substances and GTL products tested in the present study

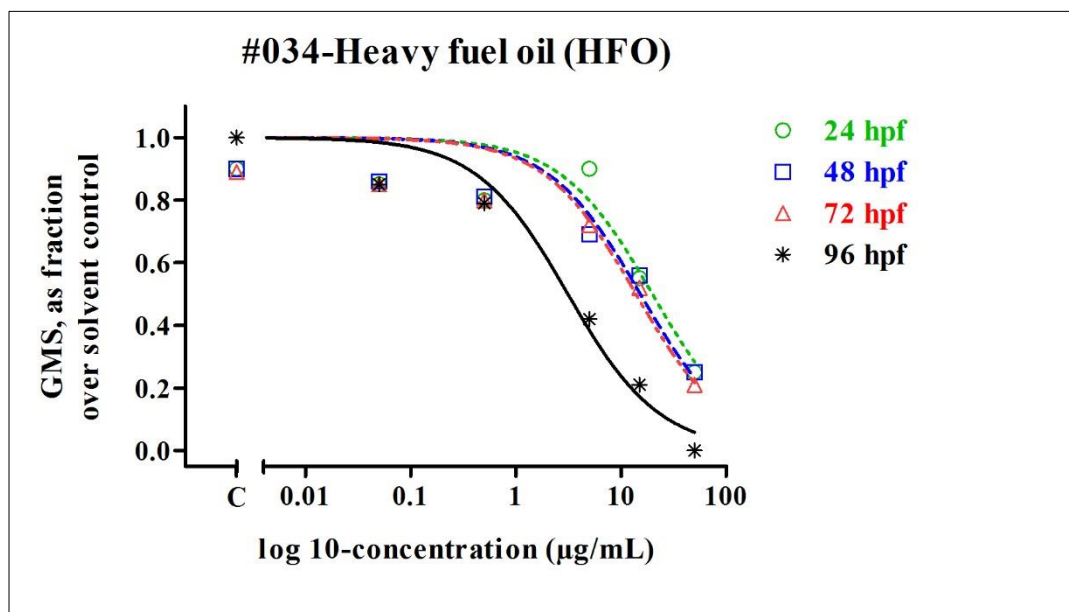
Compound	ARC profile ^a							Total wt. %
	1-ring wt. %	2-ring wt. %	3-ring wt. %	4-ring wt. %	5-ring wt. %	6-ring wt. %	≥ 7-ring wt. %	
#034/HFO	0	1	24	40	21	11	3	48
#097/DAE	0	2	1	6	22	36	33	9
#098/DAE	0	0	18	49	29	4	0	9.7
#099/DAE	0	0	22	41	25	11	1	12
#171/GO	0	12	76	12	0	0	0	5.5
#172/GO	0	20	77	2	0	0	0	4.2
#175/VTO	1	56	43	0	0	0	0	6.7
#185/RAE	0	4	4	4	16	30	42	1.5
#186/RAE	0	0	1	6	18	38	36	3.3
#091/GTLb	0	0	0	0	0	0	0	0
#092/GTLg	0	0	0	0	0	0	0	0

^a The weight percent of the DMSO-soluble 1- to ≥7 aromatic-ring compounds present in each petroleum substance, from the starting material of 4 gram samples, as determined by Method II chemical characterization procedure (see *Section 2.2*).

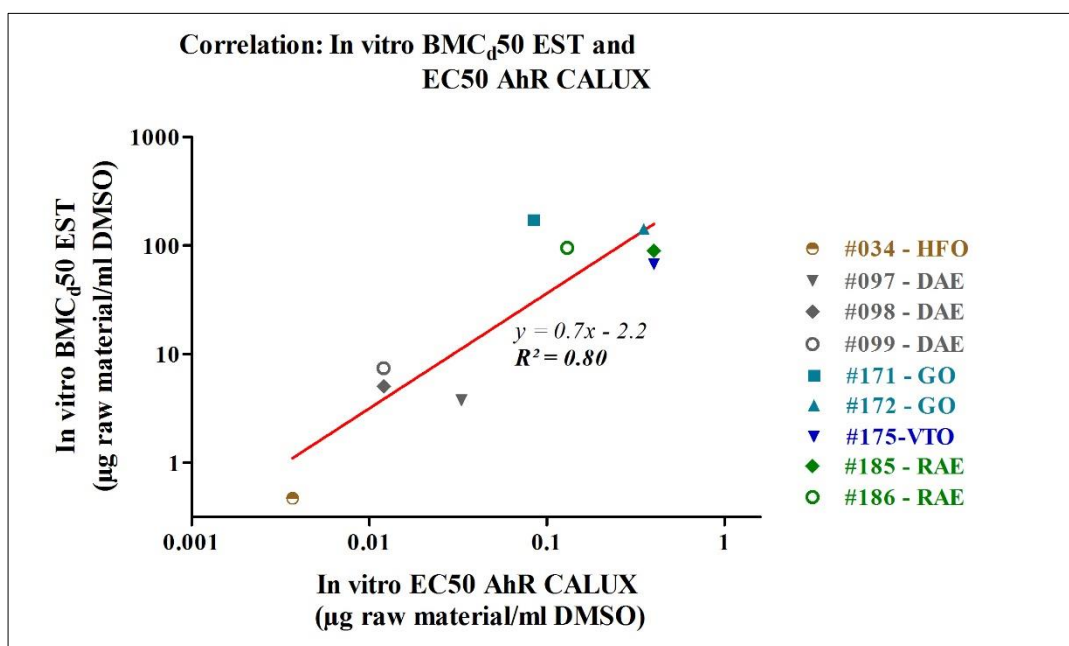
HFO, heavy fuel oil; DAE, distillate aromatic extract; GO, gas oil; VTO, vacuum tower overhead; RAE, residual aromatic extract; GTL-b, gas-to-liquid base oil; GTL-g, gas-to-liquid gas oil, wt%, weight percent

Appendix E:

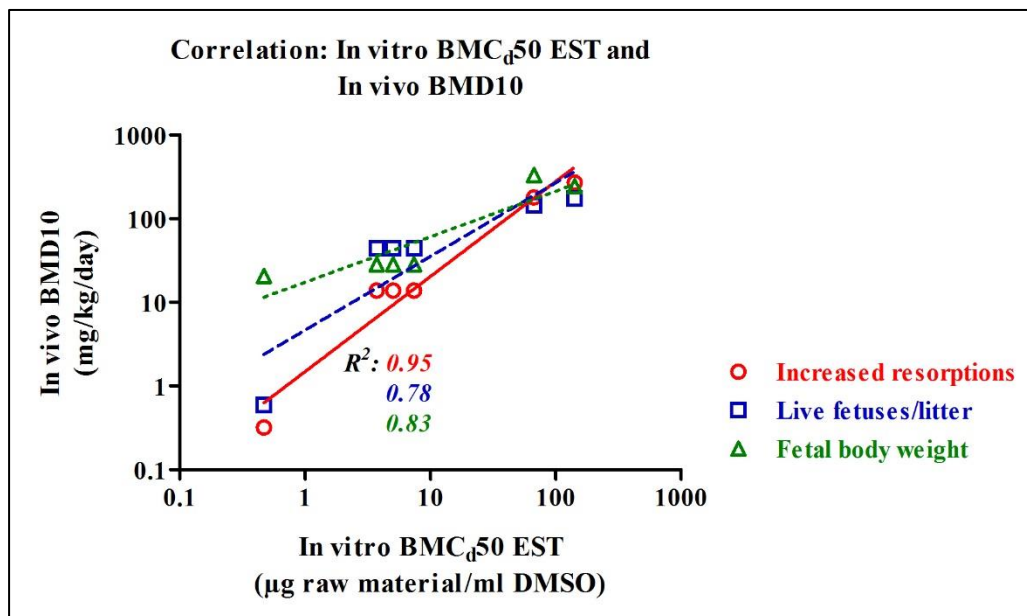
- Concentration-dependent effects of DMSO-extract of sample #034-HFO in the ZET at 24, 48, 72, and 96 hpf, based on the general morphology scoring (GMS) system.



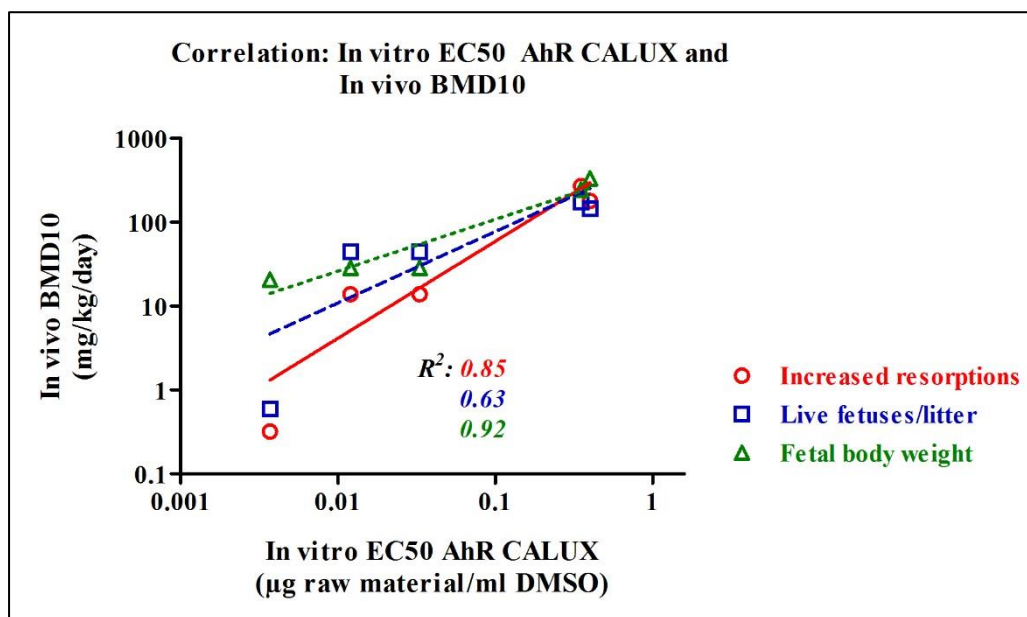
- Correlation between *in vitro* potencies in the EST and AhR-mediated activity of the DMSO-extracts of 9 PS (Kamelia et al., 2018).



- Correlation between *in vitro* potencies of the DMSO-extracts of 9 PS in the EST and potencies observed *in vivo* of the same substances, based on three different developmental endpoints: increased resorptions, number of live fetuses/litter, and fetal body weight (sum and average of the three correlations: 0.85).



- Correlation between AhR-mediated activity of the DMSO-extracts of 9 PS and potencies observed *in vivo* of the same substances, based on three different developmental endpoints: increased resorptions, number of live fetuses/litter, and fetal body weight (sum and average of the three correlations: 0.80).



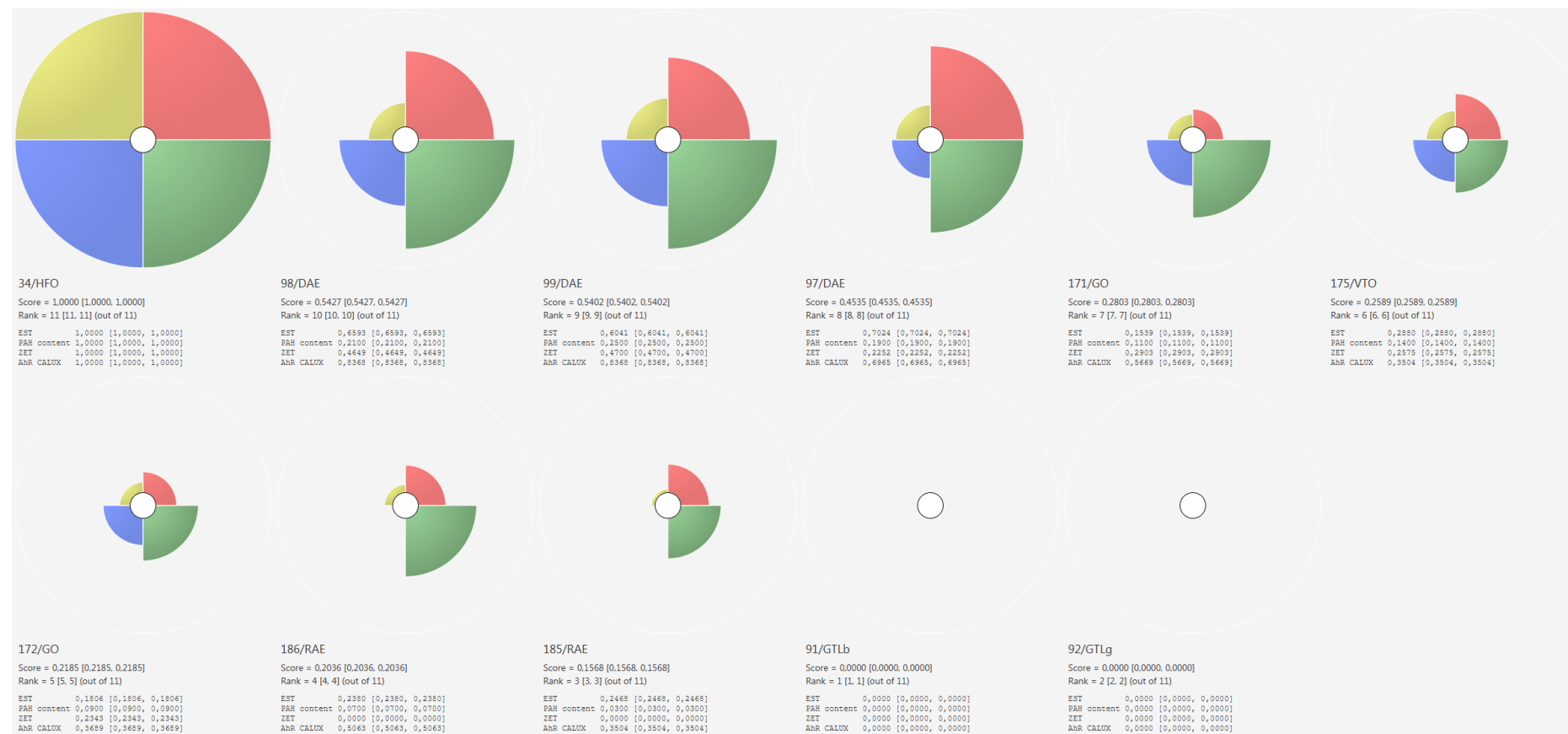
Appendix F: Correlation between *in vitro* developmental toxicity potency in the ZET (BMC50s) and specific PAH content (2- to 7-ring PAHs) present in each PS sample

BMC50 ZET (µg/ml)	Sample	ii-iii	ii-iv	ii-v	ii-vi	ii-vii	iii-iv	iii-v	iii-vi	iii-vii	iv-v	iv-vi	iv-vii	v-vi	v-vii	vi-vii
1.31695	#34	24.20	62.91	83.24	93.89	96.79	61.95	82.27	92.92	95.82	59.04	69.69	72.59	30.97	33.88	13.55
76.7146	#97	0.54	1.62	5.58	12.06	18.00	1.26	5.22	11.70	17.64	5.04	11.52	17.46	10.44	16.38	12.42
21.818	#98	3.60	13.40	19.20	20.00	20.00	13.40	19.20	20.00	20.00	15.60	16.40	16.40	6.60	6.60	0.80
21.2347	#99	5.28	15.12	21.12	23.76	24.00	15.12	21.12	23.76	24.00	15.84	18.48	18.72	8.64	8.88	2.88
54.5297	#171	9.94	11.30	11.30	11.30	11.30	9.94	9.94	9.94	9.94	1.36	1.36	1.36	0.00	0.00	0.00
73.1288	#172	8.16	8.33	8.33	8.33	8.33	6.64	6.64	6.64	6.64	0.17	0.17	0.17	0.00	0.00	0.00
64.7671	#175	13.33	13.33	13.33	13.33	13.33	5.79	5.79	5.79	5.79	0.00	0.00	0.00	0.00	0.00	0.00
641.356	#185	0.24	0.36	0.84	1.75	3.01	0.24	0.72	1.63	2.89	0.60	1.51	2.77	1.38	2.65	2.17
327.779	#186	0.07	0.46	1.65	4.16	6.54	0.46	1.65	4.16	6.54	1.59	4.10	6.48	3.70	6.08	4.89
	R square	0.81	0.93	0.96	0.94	0.85	0.94	0.96	0.87	0.62	0.43	0.31	0.85	0.69	0.08	-

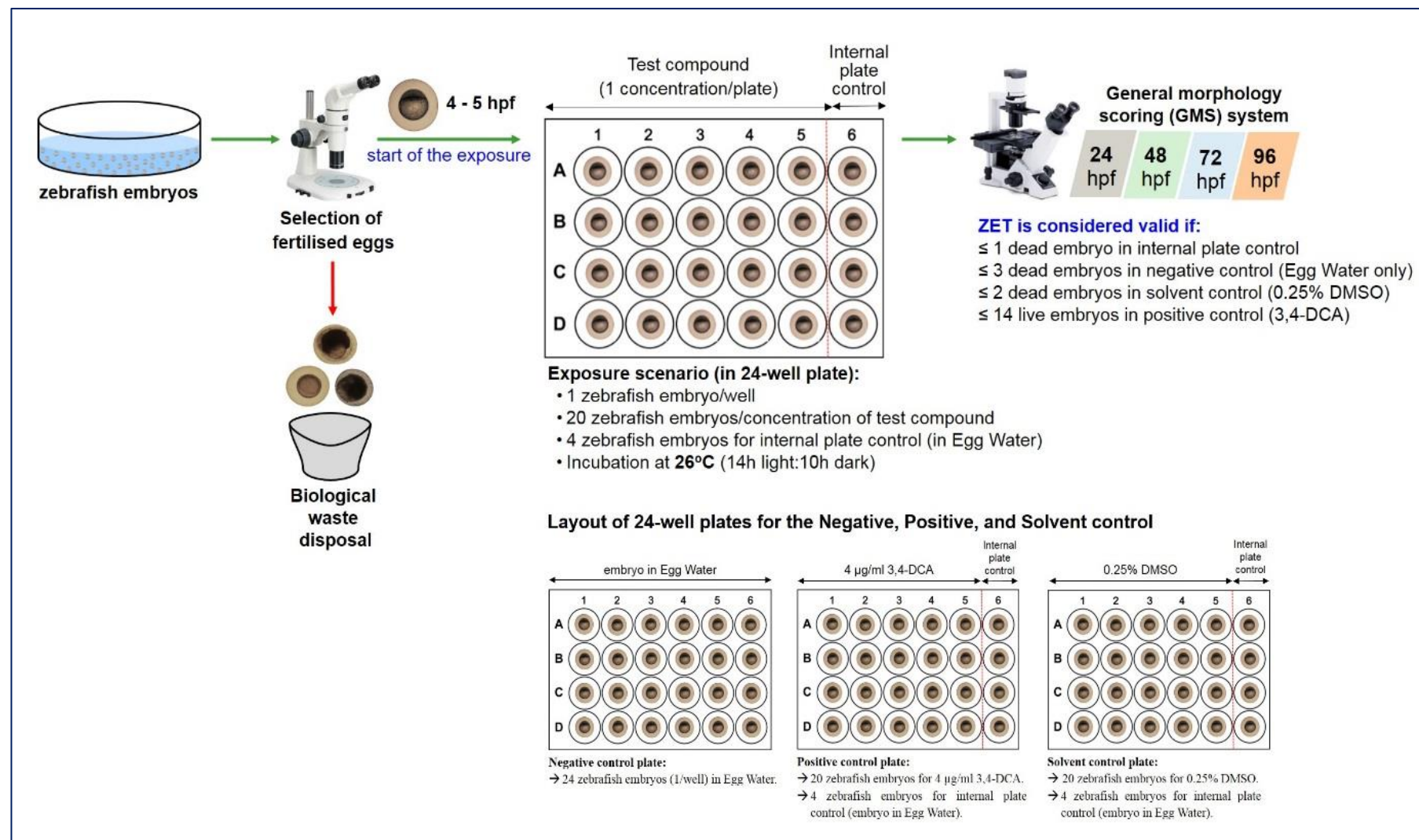
Appendix G: Bioactivity profiling using the ToxPi GUI

ToxPi score of each test compound is also listed below the pie-chart (ToxPi profile).

Data are taken from the ZET, EST, AhR CALUX assay, and PAH content of the respective test compound of the present study: DMSO-extracts of 9 PS and 2 GTL products.

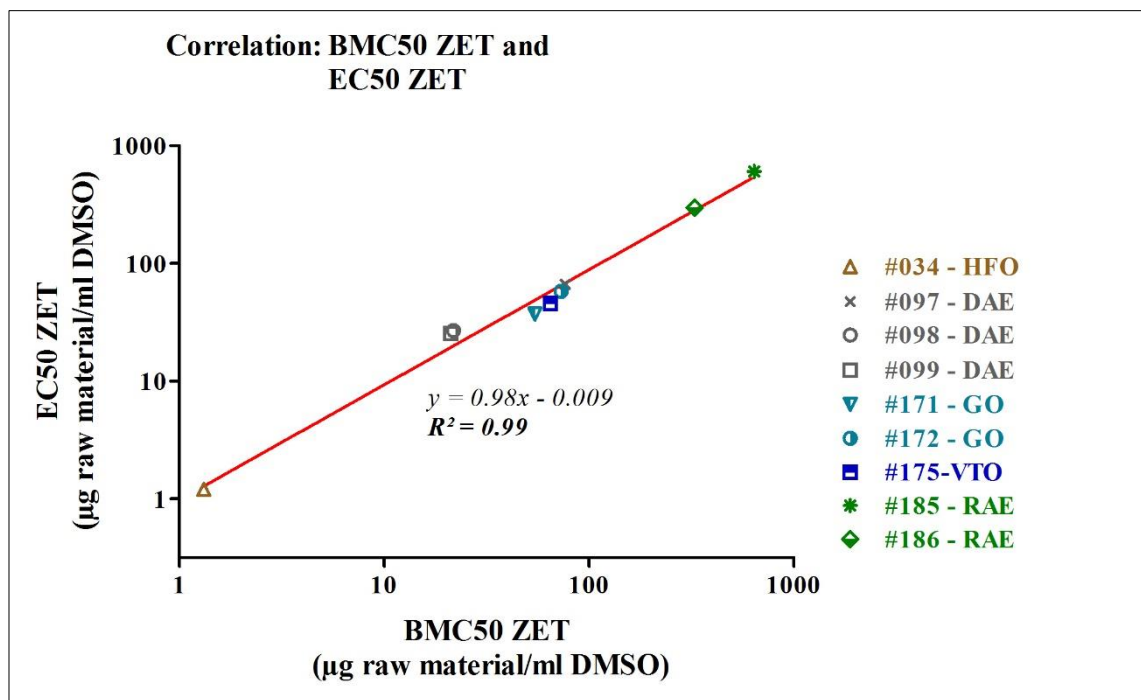


Appendix H: Schematic overview of the zebrafish embryotoxicity test (ZET) performed in the present study



Appendix I:

Correlation between the calculated BMCd50s, as determined using the BMD software from US-EPA, and EC50s, as determined using the GraphPad Prism software, in the ZET of the present study.



Comparison of results when using either (A-D) the BMC50s-ZET, as determined using BMD software from US-EPA, or (E-H) the EC50s-ZET, as determined using GraphPad Prism 5.0; for the correlation analysis of the present study.

