

In Vitro Evaluation of the Carcinogenic Potential of Perfluorinated Chemicals

Supplementary Data

Tab. S1: PFOS – Preliminary cytotoxicity assay – Summary of results

Nominal concentration	Mean number of colonies/plate \pm SE	Absolute clonal efficiency \pm SE	Relative clonal efficiency (%)
UC	78.60 \pm 5.94	0.393 \pm 0.013	97.28
DMSO 0.1%	80.80 \pm 5.45	0.404 \pm 0.012	100
PFOS (μ g/mL)			
20	61.40 \pm 3.13 ^a	0.307 \pm 0.007 ^b	75.99
29	62.20 \pm 2.68 ^a	0.311 \pm 0.006 ^b	76.98
43	59.60 \pm 5.50 ^a	0.298 \pm 0.012 ^b	73.76
63	57.60 \pm 4.77 ^a	0.288 \pm 0.010 ^b	71.29
93	54.00 \pm 4.95 ^a	0.270 \pm 0.011 ^b	66.83
136	45.40 \pm 3.65 ^a	0.227 \pm 0.008 ^b	56.19
200	1.20 \pm 0.84 ^a	0.006 \pm 0.001 ^b	1.49
PFOS-K (μ g/mL)			
20	69.40 \pm 2.97 ^a	0.347 \pm 0.006 ^b	85.89
29	61.80 \pm 5.54 ^a	0.309 \pm 0.012 ^b	76.49
43	64.40 \pm 7.44 ^a	0.322 \pm 0.016 ^b	79.70
63	50.60 \pm 5.90 ^a	0.253 \pm 0.013 ^b	62.62
93	40.20 \pm 2.17 ^a	0.201 \pm 0.004 ^b	49.75
136	47.80 \pm 3.27 ^a	0.239 \pm 0.007 ^b	59.16
200	00.00 \pm 0.00 ^a	00.00 \pm 0.00 ^b	0.00

UC, untreated cells; n = 5

^a p < 0.01, one-tailed Student's t-test compared to the vehicle (DMSO 0.1%)

^b p < 0.01, one-tailed z test compared to the vehicle (DMSO 0.1%)

Tab. S2: PFOA – Preliminary cytotoxicity assays – Summary of results

Nominal concentration	Mean number of colonies/plate \pm SE	Absolute clonal efficiency \pm SE	Relative clonal efficiency (%)
UC	87.40 \pm 6.07	0.437 \pm 0.013	103.80
DMSO 0.1%	84.20 \pm 10.6	0.421 \pm 0.023	100
PFOA (μ g/mL)			
20	83.80 \pm 8.07	0.419 \pm 0.018	99.52
29	79.80 \pm 2.28	0.399 \pm 0.005	94.77
43	70.40 \pm 7.13 ^a	0.352 \pm 0.015 ^b	83.61
63	62.60 \pm 6.11 ^c	0.313 \pm 0.013 ^b	74.35
93	38.20 \pm 1.92 ^c	0.191 \pm 0.004 ^b	45.37
136	9.20 \pm 1.30 ^c	0.046 \pm 0.002 ^b	10.93
200	0.20 \pm 0.45 ^c	0.001 \pm 0.001 ^b	0.24
PFOA-NH ₄ (μ g/mL)			
20	75.60 \pm 4.93	0.378 \pm 0.011 ^d	89.79
29	73.80 \pm 10.76	0.369 \pm 0.024 ^b	87.65
43	67.00 \pm 5.48 ^c	0.335 \pm 0.012 ^b	79.57
63	50.00 \pm 3.61 ^c	0.250 \pm 0.008 ^b	59.38
93	34.00 \pm 4.30 ^c	0.170 \pm 0.009 ^b	40.38
136	6.20 \pm 1.30 ^c	0.031 \pm 0.002 ^b	7.36
200	0.60 \pm 0.55 ^c	0.003 \pm 0.001 ^b	0.71

UC, untreated cells; n = 5

^a p < 0.05, one-tailed Student's t-test compared to the vehicle (DMSO 0.1%)

^b p < 0.01, one-tailed z test compared to the vehicle (DMSO 0.1%)

^c p < 0.01, one-tailed Student's t-test compared to the vehicle (DMSO 0.1%)

^d p < 0.05, one-tailed z test compared to the vehicle (DMSO 0.1%)

Tab. S3: PFOS – Preliminary cytotoxicity assays – Summary of results

Nominal concentration	Mean number of colonies/plate ± SE	Absolute clonal efficiency ± SE	Relative clonal efficiency (%)
UC	92.20 ± 7.40	0.461 ± 0.016	102.67
DMSO 0.1%	89.80 ± 10.3	0.449 ± 0.023	100.00
PFOS (µg/mL)			
0.002	92.60 ± 5.13	0.463 ± 0.011	103.12
0.02	88.20 ± 4.02	0.441 ± 0.009	98.22
0.2	87.80 ± 3.35	0.439 ± 0.007	97.77
2	90.20 ± 6.61	0.451 ± 0.014	100.4
20	80.20 ± 3.03 ^a	0.401 ± 0.006 ^b	89.31
PFOS-K (µg/mL)			
0.002	92.40 ± 6.66	0.462 ± 0.014	102.9
0.02	92.60 ± 6.39	0.463 ± 0.014	103.12
0.2	92.60 ± 6.11	0.463 ± 0.013	103.12
2	90.00 ± 7.78	0.450 ± 0.017	100.22
20	75.80 ± 3.70 ^a	0.379 ± 0.008 ^c	84.41

UC, untreated cells; n = 5

^a p < 0.05, one-tailed Student's t-test compared to the vehicle (DMSO 0.1%)

^b p < 0.05, one-tailed z test compared to the vehicle (DMSO 0.1%)

^c p < 0.01, one-tailed z test compared to the vehicle (DMSO 0.1%)

Tab. S4: PFOA – Preliminary cytotoxicity assays – Summary of results

Nominal concentration	Mean number of colonies/plate ± SE	Absolute clonal efficiency ± SE	Relative clonal efficiency (%)
UC	92.20 ± 7.40	0.461 ± 0.016	102.67
DMSO 0.1%	89.80 ± 10.3	0.449 ± 0.023	100.00
PFOA (µg/mL)			
0.002	96.40 ± 3.91	0.482 ± 0.008	107.35
0.02	91.80 ± 5.26	0.459 ± 0.011	102.23
0.2	91.40 ± 5.13	0.457 ± 0.011	101.78
2	93.40 ± 3.21	0.467 ± 0.007	104.01
20	87.00 ± 3.24 ^a	0.435 ± 0.007 ^b	96.88
PFOA-NH ₄ (µg/mL)			
0.002	87.60 ± 5.13	0.438 ± 0.011	97.55
0.02	82.80 ± 3.56	0.414 ± 0.008	92.20
0.2	84.60 ± 3.85	0.423 ± 0.008	94.21
2	83.60 ± 6.77	0.418 ± 0.015	93.10
20	82.20 ± 7.12 ^a	0.411 ± 0.015 ^c	91.54

UC, untreated cells; n = 5

^a p < 0.05, one-tailed Student's t-test compared to the vehicle (DMSO 0.1%)

^b p < 0.05, one-tailed z test compared to the vehicle (DMSO 0.1%)

^c p < 0.01, one-tailed z test compared to the vehicle (DMSO 0.1%)

Tab. S5: PFOS – GLP Study - Cytotoxicity assay – Summary of results

Nominal concentration	Mean number of colonies/plate ± SE	Absolute clonal efficiency ± SE	Relative clonal efficiency (%)
UC	85.80 ± 1.66	0.429 ± 0.008	97.95
DMSO 0.1%	87.60 ± 0.93	0.438 ± 0.005	100
PFOS (µg/mL)			
10	88.60 ± 1.57	0.443 ± 0.008	101.14
25	86.20 ± 1.07	0.431 ± 0.005	98.40
50	78.00 ± 1.14 ^a	0.390 ± 0.006 ^b	89.04
75	73.40 ± 1.54 ^a	0.367 ± 0.008 ^c	83.79
100	68.20 ± 1.77 ^a	0.341 ± 0.009 ^c	77.85
MCA (µg/mL)			
4	20.80 ± 1.66 ^a	0.104 ± 0.008 ^c	23.74

UC, untreated cells; n = 5

^a p < 0.01, one-tailed Student's t-test compared to the vehicle (DMSO 0.1%)

^b p < 0.05, one-tailed z test compared to the vehicle (DMSO 0.1%)

^c p < 0.01, one-tailed z test compared to the vehicle (DMSO 0.1%)

Tab. S6: PFOA – Cytotoxicity assay – Summary of results

Nominal concentration	Mean number of colonies/plate ± SE	Absolute clonal efficiency ± SE	Relative clonal efficiency (%)
NT	87.80 ± 6.46	0.439 ± 0.014	93.80
DMSO 0.1%	93.60 ± 7.20	0.468 ± 0.016	100.00
PFOA (µg/mL)			
5	83.40 ± 5.46 ^a	0.417 ± 0.012 ^b	89.10
10	78.00 ± 4.18 ^c	0.390 ± 0.009 ^d	83.33
25	73.80 ± 5.81 ^c	0.369 ± 0.013 ^d	78.85
50	44.80 ± 5.40 ^c	0.224 ± 0.012 ^d	47.86
75	38.00 ± 3.16 ^c	0.190 ± 0.007 ^d	40.60
MCA (µg/mL)			
4	15.80 ± 2.17 ^c	0.079 ± 0.004 ^d	16.88

UC, untreated cells; n = 5

^a p < 0.05, one-tailed Student's t-test compared to the vehicle (DMSO 0.1%)

^b p < 0.5, one-tailed z test compared to the vehicle (DMSO 0.1%)

^c p < 0.01, one-tailed Student's t-test compared to the vehicle (DMSO 0.1%)

^d p < 0.01, one-tailed z test compared to the vehicle (DMSO 0.1%)

Tab. S7: PFOS GPL Study - Test and reference items nominal and measured concentrations in the stock and working solutions

	Stock solutions concentration			Working solutions concentration				
	Nominal (mg/mL)	Measured (mg/mL)	Difference (%)	Time 0			Time 72	
				Nominal (µg/mL)	Measured (µg/mL)	Difference (%)	Measured (µg/mL)	Difference (%)
None	-----	-----	-----	0	< 0.001	-----	0.059	-----
DMSO	0	0.00002	n.d.	0	< 0.001	-----	< 0.001	-----
PFOS	10	13.7	37	10	12.6	26	10.2	2
	25	33.2	33	25	32.7	31	31.5	26
	50	48.9	-2	50	66.8	34	57.0	14
	75	74.8	0	75	72.6	-3	77.9	4
	100	89.5	-11	100	94.3	-6	101.5	2
None	-----	-----	-----	0	n.d.	-----	n.d.	-----
DMSO	0	n.d.	-----	0	n.d.	-----	n.d.	-----
MCA	4	3.98	-1	4	3.99	0	4.20	5

n.d., not detectable