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Supplemental Material

Estimated Transfer of Perfluoroalkyl Substances (PFAS) from Maternal Serum to Breast Milk in Women Highly Exposed from Contaminated Drinking Water: A Study in the Ronneby Mother–Child Cohort

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Table of Contents

Table S10. Estimated transfer efficiency (TE, 95% CI) by PFAS and lactation stage, calculated from a mixed-effects model with an interaction term between PFAS compound and lactation stage ($N_{participants}$ = 126 and $N_{observations}$ = 776). P-values are comparing transfer efficiency by lactation stage for each PFAS compound.

Table S11. Estimated transfer efficiency (TE, 95% CI) from serum into colostrum ($TE_{C:S}$) by PFAS and exposure level, calculated from a mixed-effects model with an interaction term between PFAS and exposure group ($N_{participants}$ = 85 and $N_{observations}$ = 340). P-values are for each PFAS compound and are relative to the background exposure group.

Table S12. Estimated transfer efficiency (TE, 95% CI) from serum into breastmilk ($TE_{B:S}$) by PFAS and exposure level, calculated from a mixed-effects model with an interaction term between PFAS and exposure group ($N_{participants}$ = 109 and $N_{observations}$ = 436). P-values are for each PFAS compound and are relative to the background exposure group.

Figure S1. Exposure category cutoffs by serum PFHxS concentrations, illustrated by area (Ronneby: n = 103; Karlshamn: n = 23).

Figure S2. Serum PFAS concentrations by area (Ronneby: n = 103; Karlshamn: n = 23).

Figure S3. Spearman correlations across PFAS and matrices ($N_{participants}$ = 126 and $N_{observations}$ = 2240).

Figure S4. Spearman correlations across PFAS and matrices, limited to participants in the intermediate and high exposure groups ($N_{participants}$ = 101 and $N_{observations}$ = 1827).

Figure S5. Spearman correlations across PFAS and matrices, limited to participants in the background exposure group ($N_{participants}$ = 25 and $N_{observations}$ = 239). Because of the small sample size, this correlation plot excludes PFAS measurements where the majority of measurements were less than the LOQ.

Supplemental Tables

Table S1: Between-run precision for four quality control (QC) samples and between-batch precision for 164 duplicate samples, expressed as the mean and coefficient of variation (CV, %).

PFAS	QC1	QC2	QC3	QC4	Duplicate
PFOA					
Mean	1.7	2.2	69.5	134.4	3.5
CV	14	10	12	11	14
PFNA					
Mean	1.4	1.4	2	131	0.4
CV	17	12	15	14	16
PFDA					
Mean	0.4	0.4	0.7	119	0.2
CV	24	18	15	12	30
PFHxS					
Mean	8.8	15.1	916	109	48.1
CV	8	8	7	8	7
PFOS					
Mean	12.7	19.1	1230	129	45
CV	11	8	8	8	11
PFUnDA					
Mean	0.5	0.5	0.6	114	0.2
CV	21	20	18	13	36
PFHpS					
Mean	0.5	0.8	74.6	0.3	1.4
CV	23	17	13	28	15

Table S2: Study population characteristics for women who did vs. did not provide provide at least one milk sample, limited to the 211 women with a serum delivery sample and displayed as either N (%) or Mean ± SD

Characteristic	At least one milk sample	No milk samples
N	126	85
Maternal Age at Delivery	30.71 ± 4.74	29.37 ± 4.69
Year of Delivery		
2015	6 (4.8)	7 (8.2)
2016	27 (21.4)	21 (24.7)
2017	32 (25.4)	15 (17.6)
2018	31 (24.6)	14 (16.5)
2019	27 (21.4)	24 (28.2)
2020	3 (2.4)	1 (1.2)
Missing	0 (0.0)	3 (3.5)
Parity = Multiparous	77 (61.6)	48 (60.8)
Smoking Status		
Never Smoker	80 (63.5)	37 (43.5)
Current Smoker	7 (5.6)	3 (3.5)
Past Smoker	32 (25.4)	27 (31.8)
Missing	7 (5.6)	18 (21.2)
Education Status		
Less than high school	3 (2.4)	5 (5.9)
High school	51 (40.5)	40 (47.1)
University (3 or more years)	63 (50.0)	21 (24.7)
Other	3 (2.4)	1 (1.2)
Missing	6 (4.8)	18 (21.2)
Location of Maternal Care		
Karlshamn	23 (18.3)	7 (8.2)
Ronneby	103 (81.7)	75 (88.2)
Missing	0 (0.0)	3 (3.5)

Table S3: Serum PFAS concentrations at delivery for women who did vs. did not provide provide at least one milk sample, limited to the 211 women with a serum delivery sample and displayed as median [IQR] or N (%)

Characteristic	At least one milk sample	No milk samples
N	126	85
PFOA, ng/ml	1.31 [0.84, 2.60]	2.15 [1.22, 3.25]
PFOA, N>LOQ	126 (100.0)	85 (100.0)
PFNA, ng/ml	0.38 [0.26, 0.52]	0.36 [0.26, 0.46]
PFNA, N>LOQ	125 (99.2)	85 (100.0)
PFDA, ng/ml	0.22 [0.15, 0.32]	0.21 [0.15, 0.29]
PFDA, N>LOQ	118 (93.7)	80 (94.1)
PFUnDA, ng/ml	0.20 [0.15, 0.32]	0.18 [0.12, 0.26]
PFUnDA, N>LOQ	114 (90.5)	73 (85.9)
PFHxS, ng/ml	6.02 [0.88, 26.12]	12.91 [4.20, 35.33]
PFHxS, N>LOQ	126 (100.0)	85 (100.0)
PFHpS, ng/ml	0.42 [0.10, 1.74]	0.86 [0.24, 2.07]
PFHpS, N>LOQ	96 (76.2)	71 (83.5)
PFOS, ng/ml	11.29 [3.81, 36.58]	22.84 [6.47, 50.57]
PFOS, N>LOQ	126 (100.0)	85 (100.0)

Table S4: PFAS concentrations (ng/ml) by location of maternal care and sample matrix

	Ronneby				Karlshamn			
PFAS	N	%>LOQ	Range	Median (IQR)	N	%>LOQ	Range	Median (IRQ)
Serum								
PFOA	103	100%	0.34-21.9	1.48 (1.02, 3.34)	23	100%	0.29-1.65	0.72 (0.475, 0.99)
PFNA	103	99%	<0.1-1.02	0.38 (0.26, 0.525)	23	100%	0.18-0.86	0.32 (0.285, 0.465)
PFDA	103	94%	<0.1-0.78	0.23 (0.15, 0.32)	23	91%	<0.1-0.5	0.21 (0.15, 0.255)
PFUnDA	103	91%	<0.1-0.58	0.2 (0.15, 0.3)	23	87%	<0.1-0.4	0.23 (0.17, 0.32)
PFHxS	103	100%	0.38-189	11.4 (2.55, 35.7)	23	100%	0.15-6.42	0.37 (0.28, 0.54)
PFHpS	103	91%	<0.1-11	0.69 (0.18, 2.04)	23	9%	<0.1-0.34	<0.1 (<0.1, <0.1)
PFOS	103	100%	1-310	18.3 (6.04, 43.6)	23	100%	0.92-12.1	2.33 (1.92, 2.64)
Colostrum								
PFOA	73	99%	<0.01-0.76	0.04 (0.03, 0.09)	12	92%	<0.01-0.03	0.02 (0.01, 0.02)
PFNA	73	51%	<0.01-0.06	0.01 (<0.01, 0.01)	12	8%	<0.01-0.01	<0.01 (<0.01, <0.01)
PFDA	73	3%	<0.01-0.01	<0.01 (<0.01, <0.01)	12	0%	-	-
PFUnDA	73	11%	<0.01-0.02	<0.01 (<0.01, <0.01)	12	0%	-	-
PFHxS	73	82%	<0.01-3.46	0.16 (0.03, 0.54)	12	0%	-	-
PFHpS	73	40%	<0.01-0.24	<0.01 (<0.01, 0.03)	12	0%	-	-
PFOS	73	99%	<0.01-2.66	0.14 (0.05, 0.36)	12	75%	<0.01-0.05	0.01 (0.00993, 0.02)
Breastmilk								
PFOA	90	97%	<0.01-0.24	0.04 (0.02, 0.07)	19	84%	<0.01-0.04	0.01 (0.01, 0.02)
PFNA	90	74%	<0.01-0.02	0.01 (<0.01, 0.01)	19	79%	<0.01-0.01	0.01 (0.01, 0.01)
PFDA	90	2%	<0.01-0.01	<0.01 (<0.01, <0.01)	19	0%	-	-
PFUnDA	90	3%	<0.01-0.01	<0.01 (<0.01, <0.01)	19	0%	-	-
PFHxS	90	83%	<0.01-1.76	0.125 (0.02, 0.348)	19	11%	<0.01-0.05	<0.01 (<0.01, <0.01)
PFHpS	90	41%	<0.01-0.14	<0.01 (<0.01, 0.02)	19	11%	<0.01-0.01	<0.01 (<0.01, <0.01)
PFOS	90	100%	0.01-2.1	0.185 (0.06, 0.442)	19	95%	<0.01-0.06	0.02 (0.015, 0.03)

Table S5: Serum PFHxS cutoffs for each exposure group

Exposure Category	PFHxS Cutoff (ng/ml)	N
Background	X <= 0.78	25
Intermediate	0.78 < X <= 36	76
High	X > 36	25

Table S6: Serum PFAS concentrations (ng/ml) by exposure group

	Exposure Group		
Statistic	Background	Intermediate	High
PFOA			
N (%>LOQ)	25 (100)	76 (100)	25 (100)
Median (IQR)	0.7 (0.5, 0.98)	1.29 (0.908, 1.84)	5.06 (4.1, 6.01)
Range	0.29-2.22	0.34-4.49	1.44-21.9
Mean (SD)	0.786 (0.442)	1.55 (0.941)	5.79 (4.02)
PFNA			
N (%>LOQ)	25 (100)	76 (99)	25 (100)
Median (IQR)	0.31 (0.27, 0.44)	0.38 (0.25, 0.47)	0.49 (0.31, 0.65)
Range	0.13-0.58	<0.1-0.86	0.24-1.02
Mean (SD)	0.346 (0.123)	0.384 (0.173)	0.501 (0.21)
PFHxS			
N (%>LOQ)	25 (100)	76 (100)	25 (100)
Median (IQR)	0.37 (0.28, 0.51)	6.02 (2.19, 14.9)	56.1 (49.9, 68.5)
Range	0.15-0.65	0.78-36	37-189
Mean (SD)	0.392 (0.144)	9.92 (9.66)	64 (30.9)
PFOS			
N (%>LOQ)	25 (100)	76 (100)	25 (100)
Median (IQR)	2.49 (1.89, 3.05)	11.3 (5.63, 21.5)	69.3 (47.9, 96)
Range	0.92-4.51	1.89-79.3	30-310
Mean (SD)	2.43 (0.957)	17 (16)	80.6 (56.5)

Table S7: Colostrum PFAS concentrations (ng/ml) by exposure group

	Exposure Group		
Statistic	Background	Intermediate	High
PFOA			
N (%>LOQ)	13 (92)	56 (98)	16 (100)
Median (IQR)	0.02 (0.01, 0.03)	0.03 (0.02, 0.06)	0.145 (0.1, 0.202)
Range	<0.01-0.08	<0.01-0.14	0.04-0.76
Mean (SD)	0.0244 (0.0191)	0.0432 (0.0328)	0.185 (0.168)
PFNA			
N (%>LOQ)	13 (23)	56 (39)	16 (81)
Median (IQR)	<0.01 (<0.01, <0.01)	<0.01 (<0.01, 0.01)	0.01 (0.01, 0.01)
Range	<0.01-0.03	<0.01-0.06	<0.01-0.02
Mean (SD)	0.00931 (0.00642)	0.0095 (0.00741)	0.0115 (0.00435)
PFHxS			
N (%>LOQ)	13 (0)	56 (79)	16 (100)
Median (IQR)	<0.01 (<0.01, <0.01)	0.1 (0.02, 0.212)	1.09 (0.687, 1.81)
Range	<0.01-<0.01	<0.01-0.78	0.3-3.46
Mean (SD)	0.00658 (0.00155)	0.166 (0.19)	1.29 (0.827)
PFOS			
N (%>LOQ)	13 (85)	56 (96)	16 (100)
Median (IQR)	0.02 (0.01, 0.02)	0.115 (0.04, 0.195)	0.85 (0.428, 1.05)
Range	<0.01-0.08	<0.01-0.64	0.29-2.66
Mean (SD)	0.0231 (0.0217)	0.156 (0.163)	0.907 (0.617)

Table S8: Breastmilk PFAS concentrations (ng/ml) by exposure group

	Exposure Group		
Statistic	Background	Intermediate	High
PFOA			
N (%>LOQ)	21 (90)	68 (94)	20 (100)
Median (IQR)	0.02 (0.01, 0.02)	0.03 (0.02, 0.0425)	0.115 (0.08, 0.15)
Range	<0.01-0.05	<0.01-0.1	0.04-0.24
Mean (SD)	0.0189 (0.0111)	0.0352 (0.0222)	0.125 (0.0577)
PFNA			
N (%>LOQ)	21 (76)	68 (74)	20 (80)
Median (IQR)	0.01 (0.01, 0.01)	0.01 (<0.01, 0.01)	0.01 (0.01, 0.01)
Range	<0.01-0.01	<0.01-0.02	<0.01-0.02
Mean (SD)	0.00975 (0.00062)	0.0102 (0.00221)	0.0102 (0.00243)
PFHxS			
N (%>LOQ)	21 (0)	68 (84)	20 (100)
Median (IQR)	<0.01 (<0.01, <0.01)	0.07 (0.01, 0.2)	0.83 (0.698, 1)
Range	<0.01-<0.01	<0.01-0.57	0.34-1.76
Mean (SD)	0.00707 (0)	0.122 (0.133)	0.892 (0.361)
PFOS			
N (%>LOQ)	21 (95)	68 (100)	20 (100)
Median (IQR)	0.03 (0.01, 0.04)	0.135 (0.0575, 0.25)	0.865 (0.51, 1.16)
Range	<0.01-0.04	0.01-0.71	0.39-2.1
Mean (SD)	0.0252 (0.0122)	0.176 (0.162)	0.888 (0.441)

Table S9: The average relative contribution of each PFAS to the sum of PFAS (PFOA, PFNA, PFDA, PFUnDA, PFHxS, PFHpS and PFOS), by matrix and exposure category.

Percent of Total PFAS (%)			
PFAS	Background	Intermediate	High
Serum ($N_{samples}$)	25	76	25
PFOA	18.0	8.0	3.70
PFNA	8.0	2.5	0.36
PFOS	54.0	55.0	50.00
PFHxS	9.4	29.0	43.00
Other PFAS	3.7	1.7	0.94
Colostrum ($N_{samples}$)	13	56	16
PFOA	29.0	16.0	7.40
PFNA	12.0	5.1	0.65
PFOS	25.0	36.0	36.00
PFHxS	10.0	32.0	51.00
Other PFAS	7.8	3.7	1.30
Breastmilk ($N_{samples}$)	21	68	20
PFOA	24.0	14.0	6.40
PFNA	13.0	5.3	0.59
PFOS	31.0	45.0	44.00
PFHxS	9.8	26.0	46.00
Other PFAS	7.2	3.2	1.00

Table S10: Estimated transfer efficiency (TE, 95% CI) by PFAS and lactation stage, calculated from a mixed-effects model with an interaction term between PFAS compound and lactation stage ($N_{participants}$ = 126 and $N_{observations}$ = 776). P-values are comparing transfer efficiency by lactation stage for each PFAS compound.

PFAS	$TE_{C:S}$	$TE_{B:S}$	p-value
PFOA	2.71 (2.44, 3.01)	2.21 (2.01, 2.43)	<0.01
PFNA	2.58 (2.32, 2.87)	2.69 (2.45, 2.95)	0.55
PFHxS	1.35 (1.22, 1.5)	1.21 (1.1, 1.32)	0.09
PFOS	0.81 (0.73, 0.9)	1 (0.91, 1.1)	<0.01

Table S11: Estimated transfer efficiency (TE, 95% CI) from serum into colostrum ($TE_{C:S}$) by PFAS and exposure level, calculated from a mixed-effects model with an interaction term between PFAS and exposure group ($N_{participants}$ = 85 and $N_{observations}$ = 340). P-values are for each PFAS compound and are relative to the background exposure group.

Exposure Category	$TE_{C:S}$	p-value
PFOA		
Background	2.75 (2.05, 3.68)	-
Intermediate	2.61 (2.27, 3.01)	0.75
High	3.08 (2.37, 4.01)	0.56
PFNA		
Background	2.4 (1.79, 3.21)	-
Intermediate	2.65 (2.3, 3.05)	0.54
High	2.54 (1.95, 3.3)	0.78
PFHxS		
Background	1.73 (1.3, 2.32)	-
Intermediate	1.2 (1.04, 1.38)	0.03
High	1.72 (1.32, 2.23)	0.96
PFOS		
Background	0.67 (0.5, 0.9)	-
Intermediate	0.78 (0.67, 0.89)	0.37
High	1.11 (0.86, 1.45)	0.01

Table S12: Estimated transfer efficiency (TE, 95% CI) from serum into breastmilk ($TE_{B:S}$) by PFAS and exposure level, calculated from a mixed-effects model with an interaction term between PFAS and exposure group ($N_{participants}$ = 109 and $N_{observations}$ = 436). P-values are for each PFAS compound and are relative to the background exposure group.

Exposure Category	$TE_{B:S}$	p-value
PFOA		
Background	2.42 (2.01, 2.91)	-
Intermediate	2.16 (1.95, 2.39)	0.3
High	2.17 (1.79, 2.62)	0.42
PFNA		
Background	2.99 (2.49, 3.6)	-
Intermediate	2.8 (2.52, 3.1)	0.54
High	2.06 (1.71, 2.5)	0.01
PFHxS		
Background	1.89 (1.57, 2.27)	-
Intermediate	1 (0.9, 1.11)	< 0.01
High	1.42 (1.17, 1.71)	0.03
PFOS		
Background	0.96 (0.79, 1.15)	-
Intermediate	0.98 (0.88, 1.08)	0.84
High	1.15 (0.95, 1.39)	0.18

Supplemental Figures

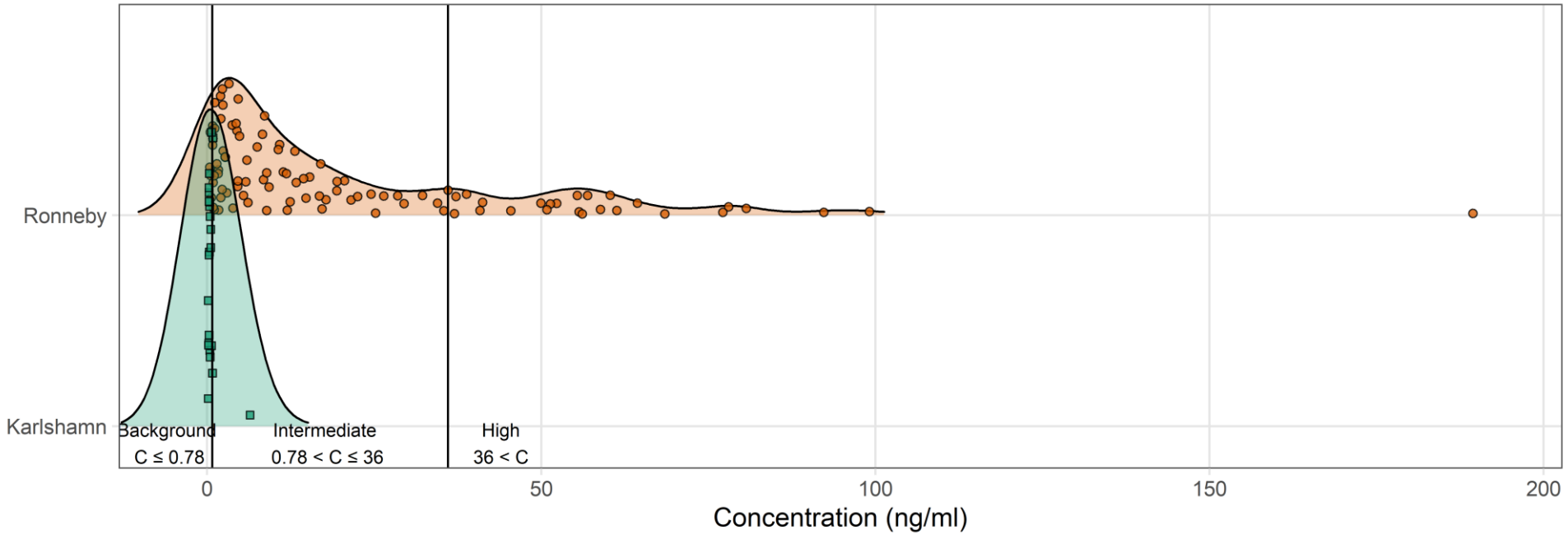

Figure S1: Exposure category cutoffs by serum PFHxS concentrations, illustrated by area (Ronneby: n = 103; Karlshamn: n = 23)

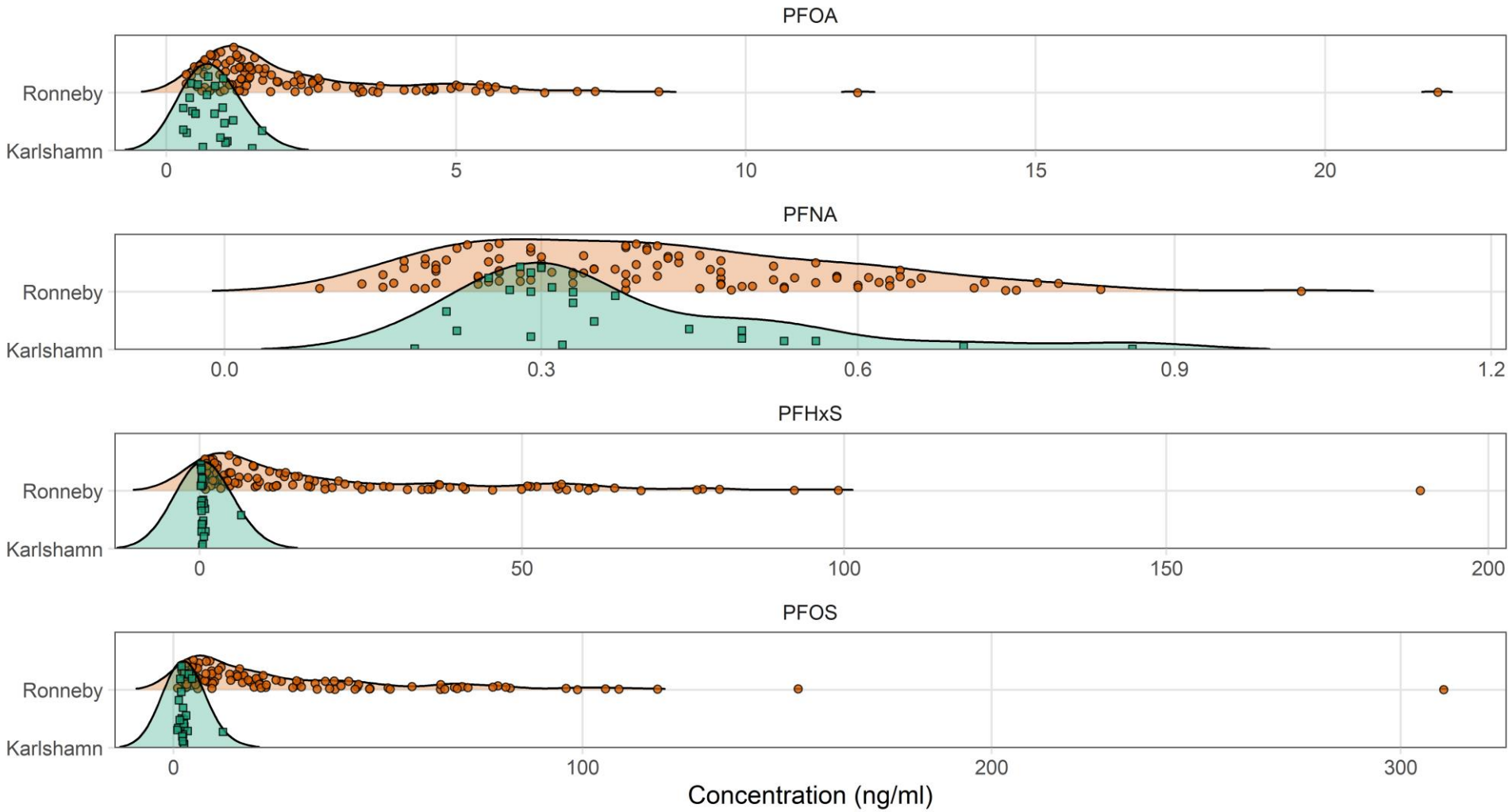

Figure S2: Serum PFAS concentrations by area (Ronneby: n = 103; Karlshamn: n = 23)

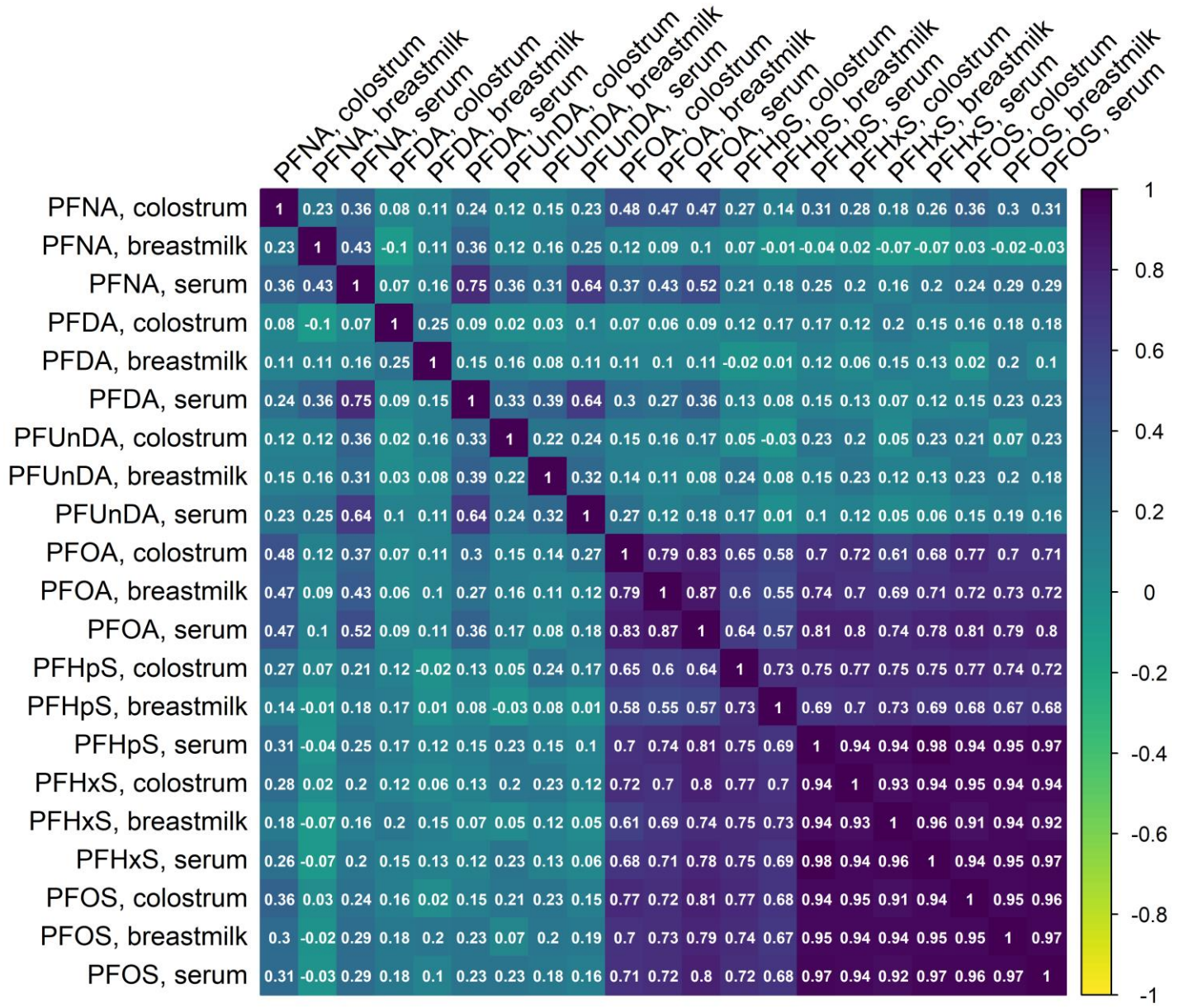

Figure S3: Spearman correlations across PFAS and matrices ($N_{participants}$ = 126 and $N_{observations}$ = 2240)

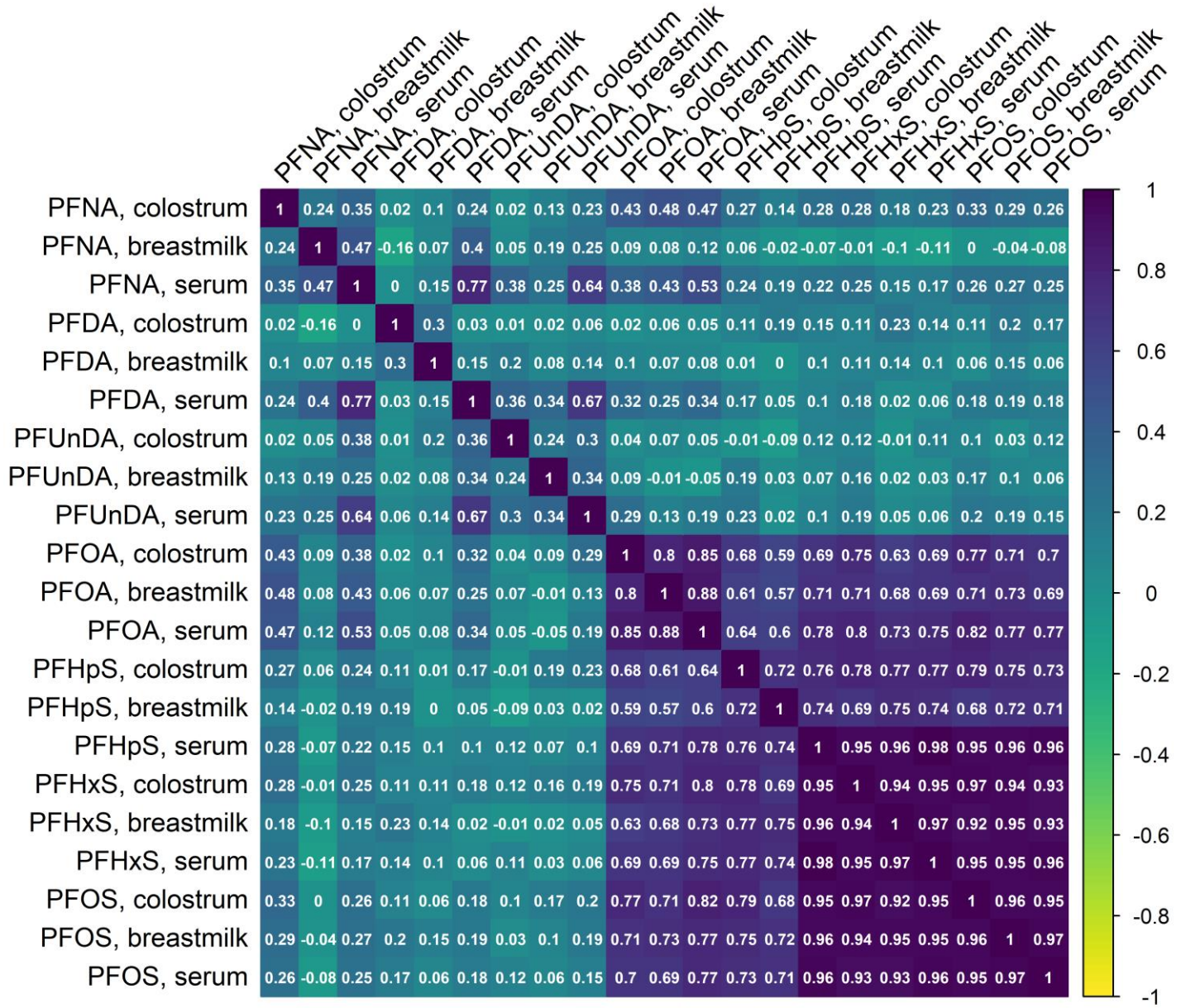

Figure S4: Spearman correlations across PFAS and matrices, limited to participants in the intermediate and high exposure groups ($N_{participants}$ = 101 and $N_{observations}$ = 1827)

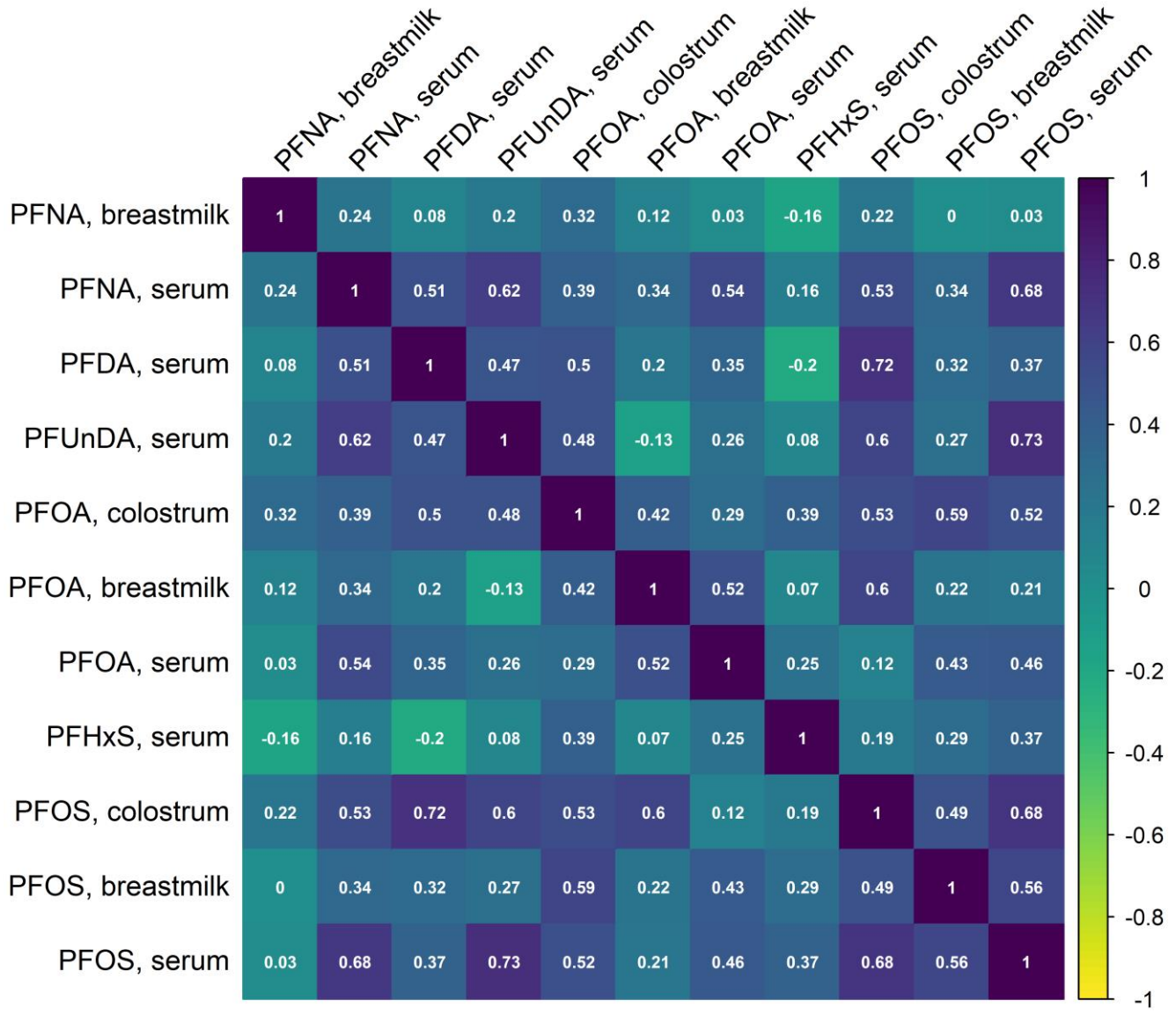

Figure S5: Spearman correlations across PFAS and matrices, limited to participants in the background exposure group ($N_{participants}$ = 25 and $N_{observations}$ = 239). Because of the small sample size, this correlation plot excludes PFAS measurements where the majority of measurements were less than the LOQ.