Environ Health Perspect

DOI: 10.1289/EHP11811

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Supplemental Material

Exposure to Perfluoroalkyl Substances and Associations with Pubertal Onset and Serum Reproductive Hormones in a Longitudinal Study of Young Girls in Greater Cincinnati and the San Francisco Bay Area

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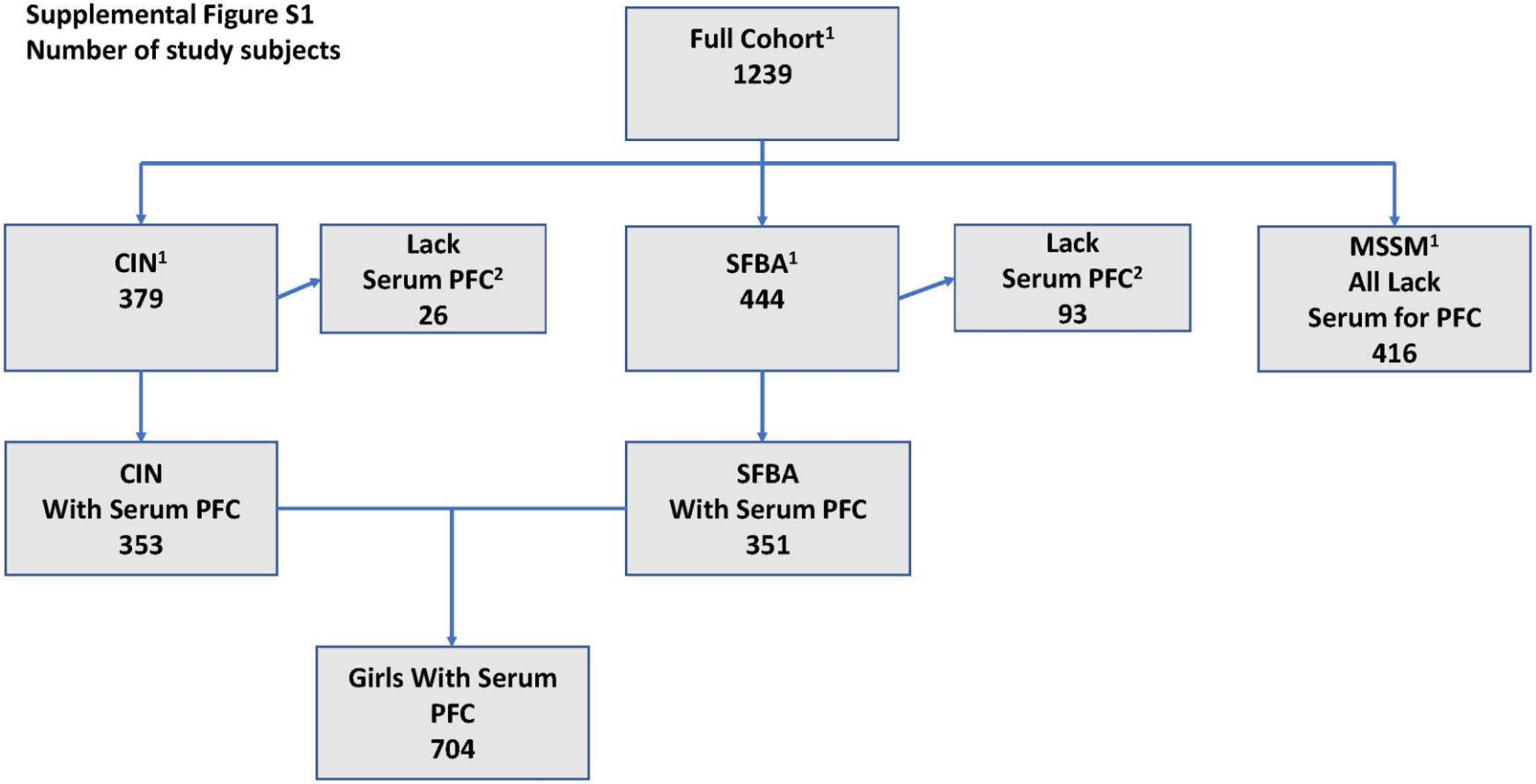
Supplemental Figure S1
Number of study subjects



Supplemental Table S1. Inclusion Criteria

Analysis of longitudinal association of PFOA with pubertal milestones N=704

Inclusion criteria	
	SFBA – enrolled in clinics of the Kaiser Premanente system at San Francisco Bay area since birth and at time of study recruitment
	GC – enrolled in selected public and parochial schools in Greater Cincinnati area or had a mother or grandmother in the Breast Cancer Registry of Greater Cincinnati
	Six to eight years old (calendar year, for example from 6.0 to 8.0) at time of study enrollment

Analysis of circulating sex hormones around the time of thelarche N=304

Inclusion criteria	
	Participant in the main study
	Serum samples for hormone analyses (Cincinnati site only)
	At least one of four hormone measurements from at least one of four time points (6 months prior to thelarche, thelarche, 6 months after thelarche, 12 months after thelarche)

Supplemental Table S2. Characteristics of the girls in the puberty cohort not in this analysis and in the analysis

	Both Sites Combined Not Included(n=119)	Both Sites Combined Included (n=704)	p-value[c]
Median BMI% at baseline[a]	65.73	66.39	0.9120
Mean[b] Age of Thelarche (months)	107.50±17.12	111.58 ± 14.57	0.0536
Mean[b] Age of Pubarche (months)	111.07±20.22	118.63 ± 17.19	0.0012
Mean[b] Age of Menarche (months)	144.45±20.31	147.99 ± 14.48	0.3747
Race/Ethnicity, n (%)			0.1140
White	56 (47.06%)	364 (51.70%)	
Asian	1 1(9.24%)	46 (6.53%)	
Hispanic	25 (21.01%)	98 (13.92%)	
Black	27 (22.69%)	196 (27.84%)	

[a]BMI% from CDC 2000 Female BMI-for-age table; height and weight at closest visit prior to pubertal event
[b]Mean ±standard deviation
[c]Test of significance of difference in values for "Both Sites Combined Not Included " for those without PFAS measurements who were not included in the analysis (N=119) to those in the analysis, Both Sites Combined Included (N=704, Table 1); Fisher exact test for categorical variables and Kruskal-Wallis for continuous variables.
BMI=body mass index

Supplemental Table S3: Pearson correlation coefficients[a] of PFCs biomarkers levels with each other

			Both Sites Combined			Greater Cincinnati			SF Bay Area		
Analyte 1	Analyte 2		N	Correlation Coefficient	p-value	N	Correlation Coefficient	p-value	N	Correlation Coefficient	p-value
PFOA	PFOS		704	0.578	<.0001	353	0.616	<.0001	351	0.600	<.0001
PFOA	PFOSA		704	0.309	<.0001	353	0.377	<.0001	351	0.173	0.001
PFOA	Et-PFOSA-AcOH		704	0.287	<.0001	353	0.377	<.0001	351	0.245	<.0001
PFOA	Me-PFOSA-AcOH		704	0.335	<.0001	353	0.333	<.0001	351	0.356	<.0001
PFOA	PFNA		704	0.310	<.0001	353	0.338	<.0001	351	0.537	<.0001
PFOA	PFHxS		704	0.407	<.0001	353	0.305	<.0001	351	0.494	<.0001
PFOA	PFDeA[b]		641	0.428	<.0001	293	0.447	<.0001	348	0.479	<.0001
PFOS	PFOSA		704	0.279	<.0001	353	0.310	<.0001	351	0.239	<.0001
PFOS	Et-PFOSA-AcOH		704	0.229	<.0001	353	0.271	<.0001	351	0.182	0.001
PFOS	Me-PFOSA-AcOH		704	0.548	<.0001	353	0.593	<.0001	351	0.492	<.0001
PFOS	PFNA		704	0.370	<.0001	353	0.423	<.0001	351	0.345	<.0001
PFOS	PFHxS		704	0.688	<.0001	353	0.735	<.0001	351	0.692	<.0001
PFOS	PFDeA[b]		641	0.352	<.0001	293	0.389	<.0001	348	0.318	<.0001
PFOSA	Et-PFOSA-AcOH		704	0.281	<.0001	353	0.428	<.0001	351	0.135	0.012
PFOSA	Me-PFOSA-AcOH		704	0.356	<.0001	353	0.349	<.0001	351	0.359	<.0001
PFOSA	PFNA		704	0.088	0.019	353	0.096	0.072	351	0.120	0.024
PFOSA	PFHxS		704	0.099	0.008	353	0.079	0.139	351	0.085	0.113
PFOSA	PFDeA[b]		641	0.047	0.233	293	0.139	0.017	348	0.016	0.765
Et-PFOSA-AcOH	Me-PFOSA-AcOH		704	0.113	0.003	353	0.139	0.009	351	0.091	0.088
Et-PFOSA-AcOH	PFNA		704	0.063	0.093	353	-0.003	0.958	351	0.101	0.060
Et-PFOSA-AcOH	PFHxS		704	0.092	0.015	353	0.072	0.178	351	0.145	0.006
Et-PFOSA-AcOH	PFDeA[b]		641	0.076	0.054	293	0.066	0.260	348	0.065	0.227
Me-PFOSA-AcOH	PFNA		704	0.299	<.0001	353	0.314	<.0001	351	0.328	<.0001
Me-PFOSA-AcOH	PFHxS		704	0.257	<.0001	353	0.287	<.0001	351	0.213	<.0001
Me-PFOSA-AcOH	PFDeA[b]		641	0.231	<.0001	293	0.301	<.0001	348	0.180	0.001
PFNA	PFHxS		704	0.128	0.001	353	0.251	<.0001	351	0.141	0.008
PFNA	PFDeA[b]		641	0.588	<.0001	293	0.602	<.0001	348	0.577	<.0001
PFHxS	PFDeA[b]		641	0.156	<.0001	293	0.251	<.0001	348	0.128	0.017

Notes:

a <LOD values substituted with LOD/sqrt(2); correlations calculated using log-transformed values

b PFDeA was not measured in the samples sent to CDC as part of the pilot study (N=55 for both sites) nor in the additional 30 samples from Cincinnati sent after the pilot study

Supplemental Table S4. Cox regression models of effects of PFOA serum concentration on time to pubertal event stratified by median BMI% for the girls in the study population.

	Thelarche					Pubarche					Menarche			
Sub cohort of girls with a BMI% < the median N=352	BMI% < 65.73%					BMI% < 67.57%					BMI% < 67.28%			
	n	Hazard	95% CI	p-value		n	Hazard	95% CI	p-value		n	Hazard	95% CI	p-value
PFOA (ng/mL)														
Serum PFOA ≤4.9	78	1.00	reference	-		80	1.00	reference	-		72	1.00	reference	-
4.9<Serum PFOA ≤6.4	80	0.99	0.71,1.37	0.94		78	0.85	0.61,1.18	0.33		83	0.98	0.7,1.38	0.90
6.4<Serum PFOA ≤8.55	87	0.85	0.61,1.17	0.31		88	**0.64**	**0.46,0.9**	**0.01**		89	0.91	0.65,1.30	0.61
8.55<Serum PFOA	107	0.92	0.66,1.28	0.63		104	**0.66**	**0.47,0.93**	**0.02**		105	1.00	0.70,1.43	1.00
BMIz closest to pubertal milestone	352	**1.43**	**1.22,1.68**	**<0.01**		350	**1.46**	**1.24,1.72**	**<0.01**		349	**1.45**	**1.20,1.74**	**<0.01**
Race														
White	207	1.00	reference	-		198	1.00	reference	-		211	1.00	reference	-
Asian	31	1.01	0.68,1.51	0.95		33	**0.57**	**0.38,0.87**	**0.01**		34	1.23	0.82,1.84	0.31
Hispanic	38	1.10	0.74,1.62	0.65		41	0.96	0.65,1.42	0.84		36	**1.70**	**1.13,2.56**	**0.01**
Black	**76**	**1.59**	**1.19,2.13**	**<0.01**		**78**	**1.53**	**1.14,2.06**	**0.01**		68	1.36	0.99,1.88	0.06
Maternal Age of Menarche														
Before Age 12											61	1.26	0.92,1.74	0.16
Between Age 12 and 14											199	1.00	reference	-
After Age 14											89	**0.72**	**0.54,0.96**	**0.03**
Site														
Cincinnati	177	1.00	reference	-		172	1.00	reference	-		171	1.00	reference	-
San Francisco Bay Area	175	**0.69**	**0.54,0.89**	**<0.01**		178	1.03	0.79,1.34	0.85		178	1.05	0.79,1.38	0.75
Sub cohort of girls with a BMI% ≥ the median N=351	BMI% ≥ 65.73%					BMI% ≥ 67.57%					BMI% ≥ 67.28%			
PFOA (ng/mL)														
Serum PFOA ≤4.9	104	1.00	reference	-		101	1.00	reference	-		109	1.00	reference	-
4.9<Serum PFOA ≤6.4	98	1.20	0.89,1.61	0.23		100	1.15	0.85,1.56	0.38		94	1.00	0.73,1.36	0.98
6.4<Serum PFOA ≤8.55	87	0.94	0.70,1.27	0.69		84	0.95	0.70,1.28	0.71		82	0.95	0.69,1.30	0.73

8.55<Serum PFOA	62	0.95	0.67,1.34	0.76		65	0.93	0.65,1.32	0.68		64	0.80	0.54,1.19	0.27
BMIz closest to pubertal milestone	351	1.15	0.96,1.38	0.13		350	1.06	0.88,1.28	0.54		349	1.24	1.01,1.52	0.04
Race														
White	157	1.00	reference	-		165	1.00	reference	-		153	1.00	reference	-
Asian	15	1.42	0.79,2.55	0.24		13	0.71	0.38,1.33	0.29		12	1.88	0.98,3.64	0.06
Hispanic	60	0.95	0.68,1.34	0.79		56	0.93	0.65,1.33	0.70		60	0.97	0.68,1.39	0.87
Black	**119**	**1.85**	**1.42,2.41**	**<0.01**		**116**	**2.49**	**1.89,3.28**	**<0.01**		**124**	**1.43**	**1.07,1.92**	**0.02**
Maternal Age of Menarche														
Before Age 12											**92**	**1.42**	**1.07,1.89**	**0.01**
Between Age 12 and 14											200	1.00	reference	-
After Age 14											57	0.90	0.64,1.27	0.55
Site														
Cincinnati	175	1.00	reference	-		180	1.00	reference	-		180	1.00	reference	-
San Francisco Bay Area	176	**0.34**	**0.26,0.43**	**<0.01**		170	1.06	0.83,1.36	0.66		169	0.93	0.71,1.21	0.58

Note: For models in which the proportional hazards assumption failed, a time dependent covariate was included. BMIz from CDC 2000 tables.
CI=confidence interval, BMI=body mass index, PFOA= perfluorooctanoic acid.

Supplemental Table S5. Logistic regression model of the effect of PFOA on the odds of entering pubarche prior to thelarche for the girls in the study.

	N	Odds Ratio	95% CI	p-value
PFOA log transformed (ng/mL)	700	**0.60**	**0.37,0.95**	**0.03**
BMIz closest to first pubertal event	700	0.90	0.75,1.07	0.23
Race				
White	363	1.00	reference	-
Asian	46	**0.16**	**0.05,0.55**	**<0.01**
Hispanic	97	0.73	0.40,1.32	0.70
Black	194	**1.64**	**1.06,2.56**	**<0.01**
Site				
Cincinnati	352	1.00	reference	-
San Francisco Bay Area	348	**2.30**	**1.52,3.47**	**<0.01**

Note: Pubarche 'prior to thelarche' is defined as either pubarche before or at the same age as thelarche. BMI% from CDC 2000 tables. CI= Confidence Interval, BMIz=body mass index. PFOA= perfluorooctanoic acid.

Supplemental Table S6. Regression model of effects of PFOA serum concentrations on hormones stratified by median BMI% for the Cincinnati girls (n=304).

	6 months prior to thelarche			thelarche			6 months after thelarche			12 months after thelarche	
Sub cohort of girls with BMI% < median	BMI%< 62.12			BMI% < 68.35			BMI % < 65.40			BMI % < 64.75	
	estimate	p-value		estimate	p-value		estimate	p-value		estimate	p-value
Estradiol (pg/mL)	(n=100)			(n=105)			(n=114)			(n=59)	
PFOA (ng/mL)	-0.14	0.29		0.17	0.23		-0.17	0.27		0.18	0.52
BMIz	-0.16	0.18		-0.14	0.26		-0.21	0.17		0.15	0.55
race	0.07	0.74		0.06	0.78		0.24	0.34		0.51	0.25
Estrone (pg/mL)	(n=101)			(n=91)			(n=114)			(n=59)	
PFOA (ng/mL)	-0.09	0.28		-0.06	0.53		-0.12	0.20		-0.04	0.83
BMIz	-0.04	0.62		-0.10	0.23		0.03	0.71		0.17	0.28
race	0.05	0.69		-0.18	0.21		0.05	0.72		0.24	0.37
Testosterone (ng/dL)	(n=101)			(n=97)			(n=120)			(n=75)	
PFOA (ng/mL)	-0.07	0.21		-0.05	0.62		0.01	0.87		-0.02	0.86
BMIz	-0.01	0.92		0.08	0.31		0.02	0.77		0.08	0.39
race	-0.02	0.90		**-0.29**	**0.05**		-0.02	0.99		-0.12	0.53
DHEA-S (ug/dL)	(n=113)			(n=99)			(n=127)			(n=75)	
PFOA (ng/mL)	-0.21	0.07		0.01	0.94		0.03	0.73		-0.01	0.96
BMIz	**0.23**	**0.02**		0.09	0.39		**0.26**	**0.01**		**0.22**	**0.03**
race	-0.26	0.17		-0.27	0.19		-0.07	0.66		-0.27	0.22
Sub cohort of girls with BMI% ≥ median	BMI% ≥ 62.12			BMI% ≥ 68.35			BMI % ≥ 65.40			BMI % ≥ 64.75	
Estradiol (pg/mL)	(n=107)			(n=94)			(n=109)			(n=56)	
PFOA (ng/mL)	0.08	0.49		-0.01	0.92		0.07	0.71		-0.02	0.93
BMIz	-0.07	0.58		-0.01	0.94		**-0.37**	**0.03**		**-0.56**	**0.01**
race	0.28	0.09		**0.42**	**0.04**		**0.53**	**0.01**		0.17	0.56

Estrone (pg/mL)	(n=107)			(n=95)			(n=108)			(n=66)	
PFOA (ng/mL)	**-0.18**	**0.03**		-0.17	0.08		-0.21	0.16		0.00	0.99
BMIz	0.08	0.35		0.04	0.71		-0.03	0.81		-0.19	0.14
race	0.09	0.45		0.01	0.98		0.02	0.87		0.17	0.31
Testosterone (ng/dL)	(n=105)			(n=93)			(n=121)			(n=67)	
PFOA (ng/mL)	**-0.24**	**0.03**		-0.12	0.16		**-0.21**	**0.02**		-0.15	0.34
BMIz	0.05	0.60		0.06	0.58		-0.04	0.63		-0.05	0.72
race	-0.13	0.26		-0.12	0.31		-0.20	0.08		-0.06	0.72
DHEA-S (ug/dL)	(n=108)			(n=98)			(n=123)			(n=66)	
PFOA (ng/mL)	**-0.35**	**<0.01**		**-0.25**	**0.04**		-0.18	0.12		-0.12	0.51
BMIz	-0.00	0.98		**0.32**	**0.02**		-0.04	0.73		-0.07	0.65
race	0.11	0.51		-0.20	0.21		-0.03	0.85		0.19	0.35

Note: All models control for race. 6 months prior to thelarche defined as 9 months prior to < -3 months prior to thelarche. Thelarche defined as 3 months prior to <3 months after thelarche. 6 months after thelarche defined as 3 months after thelarche to <9 months after thelarche. 12 months after thelarche defined as 9 months after thelarche to <15 months after thelarche. BMI%=body mass index percentile based on 2000 Center for Disease Control tables. PFOA=perfluorooctanoic acid. BMI=body mass index.