Supplementary Material

Personal care product use and lifestyle affect phthalate and DINCH metabolite levels in teenagers and young adults

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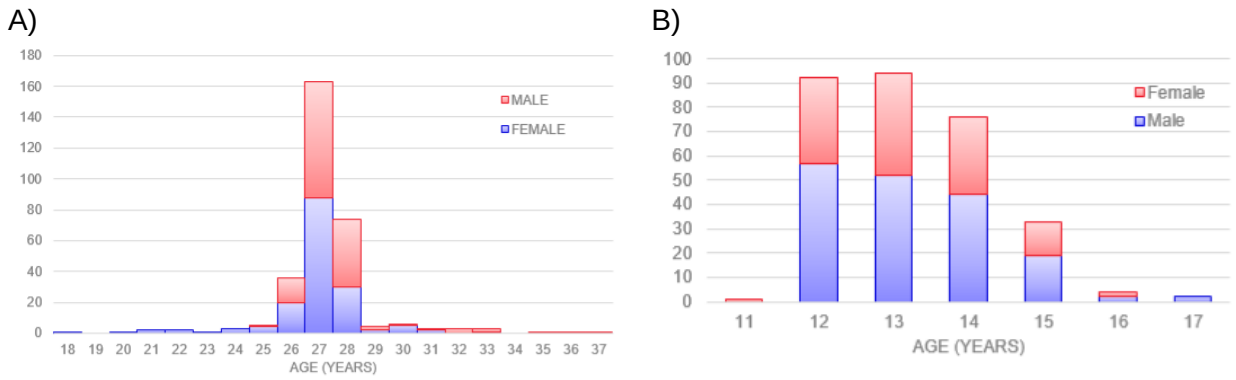


Figure S1 Distribution of age for participants in (A) YAC and (B) TAC.

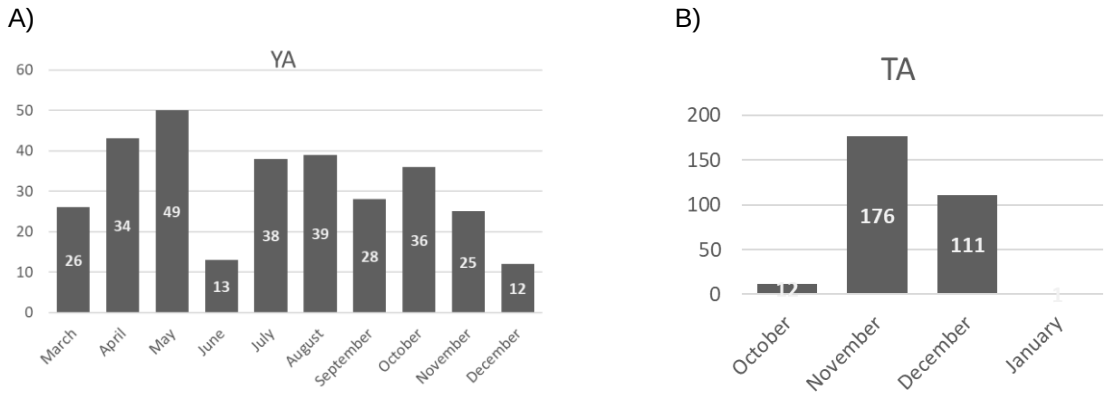


Figure S2 Distribution of sampling by (A) month for YAC and (B) seasons for TAC. The y-axis and the numbers on each column indicate the number of samples per month.

Table S1 Distribution of key cohort demographic, socioeconomic, and household variables

Cohort variables	Parameters	YAC	TAC
Gender distribution	Male	48%	58%
	Female	52%	42%
Monthly total gross income	Low	6%	25%
	Medium	33%	58%
	High	55%	17%
	Don't know/don't want to share	4%	-
	Missing	2%	-
Education - female (YAC)/mother (TAC)	Low	0.6%	1%
	Medium	20%	58%
	High	78%	36%
	Missing	1.4%	5%
Education - male (YAC)/father (TAC)	Low	1.4%	0.3%
	Medium	27%	60%
	High	71%	33%
	Missing	0.6%	6.7%
Smoking - passive	Yes	20%	13%
	No	78%	82%
	Missing	2%	5%
Smoking - active	Yes	13%	0%
	No	85%	95%
	Missing	2%	5%
Redecorations/renovations	Yes	59%	65%
	No	34%	28%
	Don't know	6%	-
	Missing	1%	7%

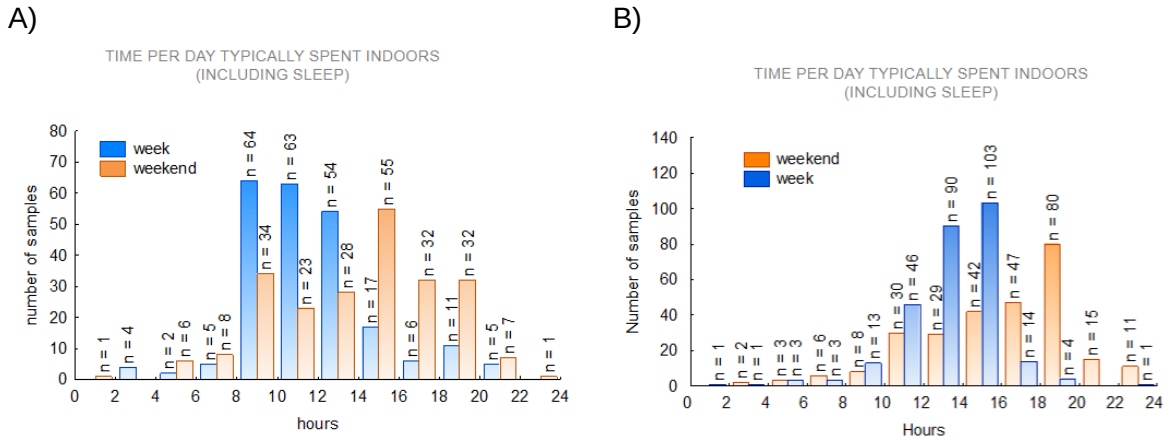
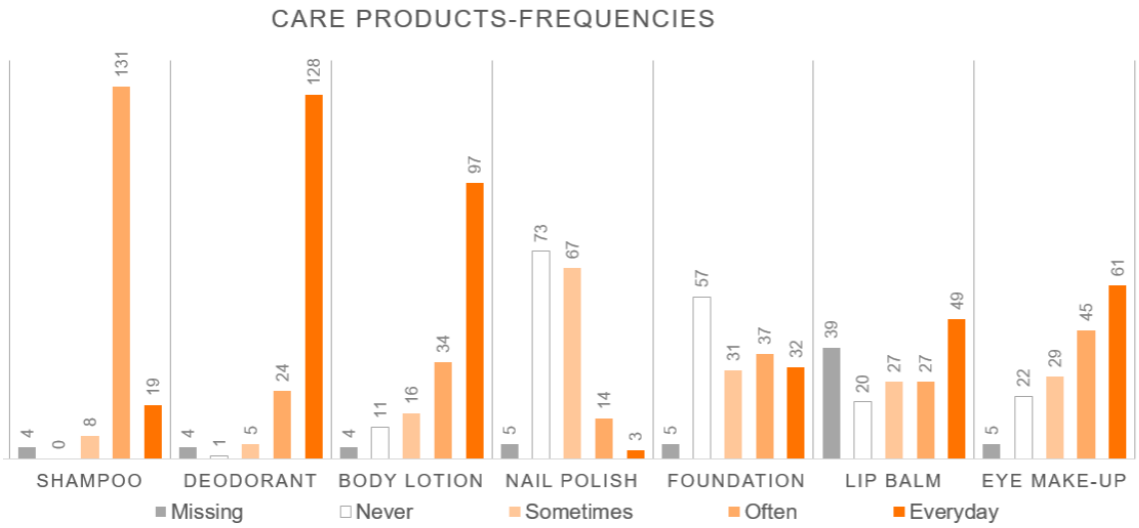


Figure S3 Time per day typically spent indoors during week days (blue bars) and weekends (orange bars), sleep included, for (A) YAC and (B) TAC.

A)



B)

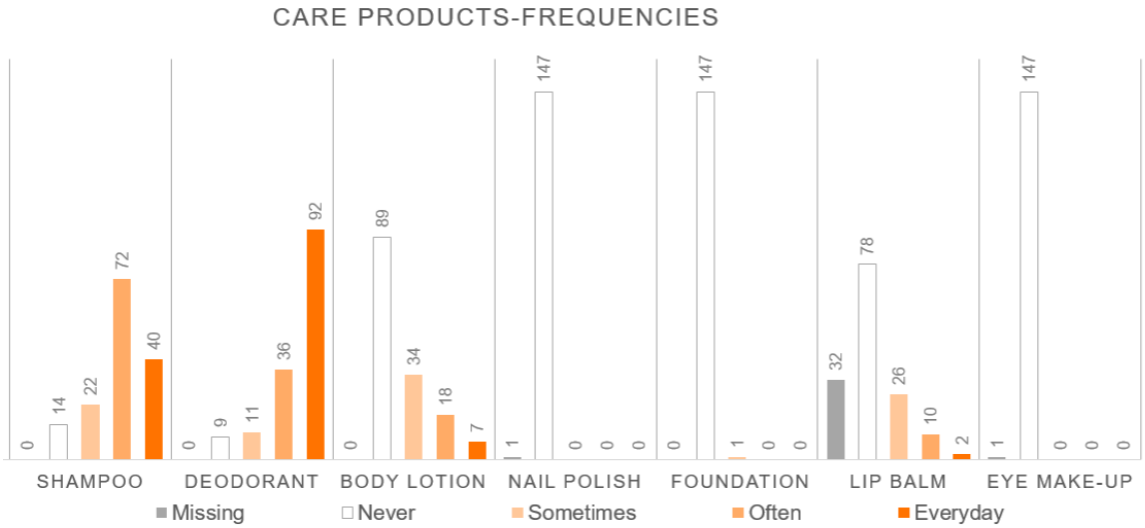
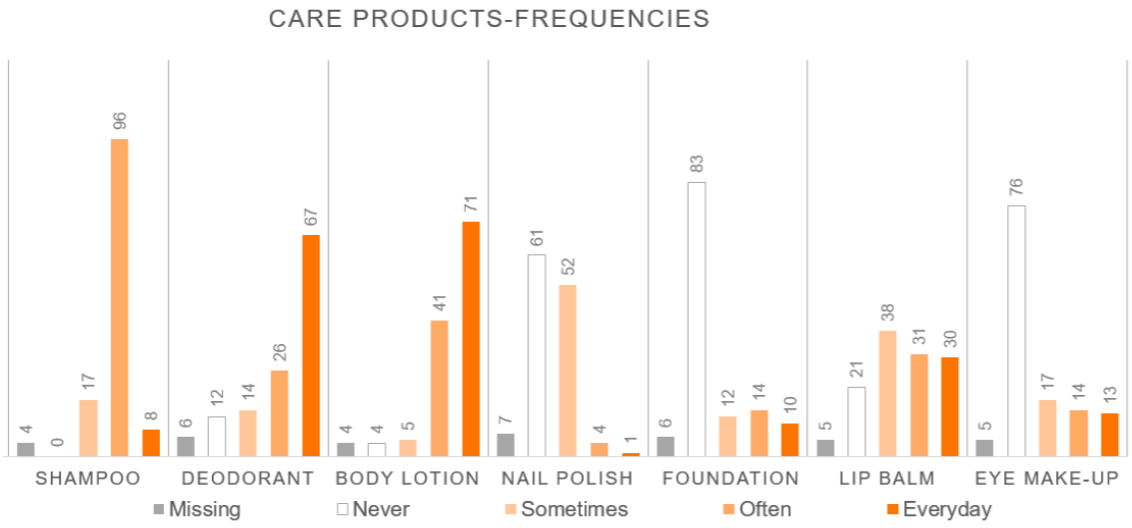


Figure S4 Frequency of PCP use for YAC by (A) females and (B) males. Categories are as follows: sometimes: once or less than one time per week, but more than one time per month; often: 2-6 times per week; everyday: seven times or more times per week.

A)



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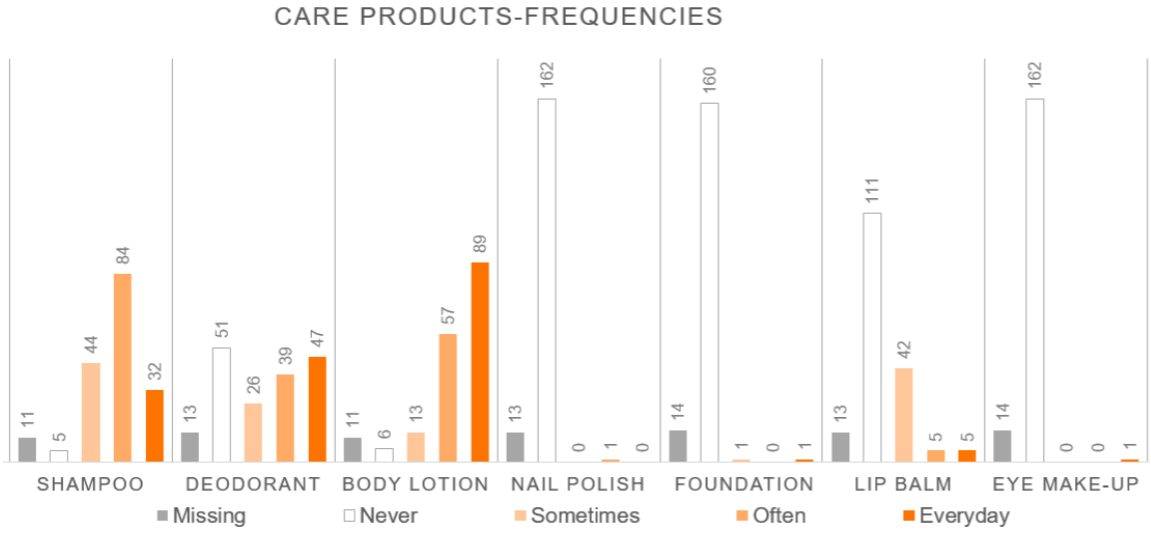


Figure S5 Frequency of PCP use for TAC by (A) females and (B) males. Categories are as follows: sometimes: once or less than one time per week, but more than one time per month; often: 2-6 times per week; everyday: seven times or more times per week.

Table S2 MS parameters to determine phthalate metabolites in negative mode – precursor ions, product ion [Da] with its collision energy (CE) [V], confirmation ion [Da] with its CE [V] and retention time (Rt) [min]

Analyte	Precursor Ion	Product Ion	$CE_{product\ ion}$	Confirmation Ion	$CE_{confirmation\ ion}$	Rt
MEP	193.1	121.1	-9	77	-13	1.3
MiBP	221.1	134	-13	77	-17	2.2
MBzP	255.1	183	-13	77	-17	2.4
MnBP	221.1	177.2	-9	76.9	-21	2.3
MCHP	247.1	97	-13	77.1	-17	2.9
MEHP	277.1	134	-13	127	-17	4.7
5OH-MEHP	293.1	1445	-9	121	-17	2.6
5oxo-MEHP	291.1	143.1	-9	120.9	-17	2.2
5cx-MEPP	307.1	159	-9	112.9	-29	2.4
MnOP	277.1	127.1	-13	77	-17	4.9
OH-MiNP	307.1	121.1	-21	77	-33	3.1
cx-MiNP	321.1	173.1	-13	120.9	-25	2.2
Oxo-MiNP	305.1	157.1	-9	121	-21	2.6
OH-MiDP	321.2	173.1	-13	120.9	-17	3.4
Oxo-MiDP	319.1	171.2	-13	121.2	-21	3.3
OH-MINCH	313.2	153	-17	109.1	-29	4.9
cx-MINCH	327.2	173.2	-21	153.1	-21	4.5
MEP-$13C_4$	197.2	123.1	-9	78.9	-17	1.3
MiBP-$13C_4$	225.1	137.1	-13	79.1	-21	2.2
MBzP-$13C_4$	259.2	107	-13	76.9	-21	2.4
MnBP-$13C_4$	225.1	151.1	-9	78.9	-21	2.3
MCHP-$13C_4$	251.2	97	-13	79.1	-25	2.9
MEHP-d_4	281.2	138.1	-17	127.1	-17	4.7
5OH-MEHP-$13C_4$	297.1	145.1	-13	124	-17	2.6
5oxo-MEHP-$13C_4$	295.1	143.1	-13	124.1	-17	2.2
5cx-MEPP-$13C_4$	311.1	159.2	-9	113	-37	2.4
MnOP-d_4	281.1	127.1	-13	125.1	-17	4.9
OH-MiNP-$13C_4$	311.2	124	-17	79	-29	3.1
cx-MiNP-$13C_4$	325.1	173.1	-13	124.1	-29	2.2
Oxo-MiNP-$13C_4$	309.1	123.9	-17	78.9	-29	2.6
OH-MiDP-$13C_4$	325.2	173.2	-13	123.8	-21	3.4
Oxo-MiDP-$13C_4$	323.2	171.1	-13	123.9	-17	3.3
OH-MINCH-d_4	217.2	153	-13	109.1	-29	4.9
cx-MINCH-d_2	329.2	175.3	-17	153.2	-25	4.5

Table S3 Average (minimum and maximum) values of the blank samples from Young adults cohort (YAC) and Teenage cohort (TAC) with LOD and LOQ values [µg/L]

Phthalate metabolite	Average (min-max) of YAC blanks	Average (min-max) of TAC blanks	LOD	LOQ
MEP	0.06 (0.00 – 0.35)	0.02 (0.00 – 0.32)	0.20	0.60
MiBP	0.29 (0.00 – 0.80)	0.15 (0.00 – 0.81)	0.07	0.20
MBzP	0.02 (0.00 – 0.15)	0.02 (0.00 – 0.14)	0.10	0.30
MnBP	0.27 (0.00 – 0.86)	0.06 (0.00 – 0.90)	0.18	0.40
MCHP	0.01 (0.00 – 0.06)	0.03 (0.00 – 0.08)	0.20	0.60
MEHP	0.07 (0.00 – 0.95)	0.03 (0.00 – 0.18)	0.18	0.50
5OH-MEHP	0.03 (0.00 – 0.16)	0.10 (0.00 – 0.38)	0.07	0.20
5oxo-MEHP	0.02 (0.00 – 0.17)	0.02 (0.00 – 0.16)	0.07	0.20
5cx-MEPP	0.04 (0.00 – 0.21)	0.08 (0.01 – 0.27)	0.07	0.20
MnOP	0.01 (0.00 – 0.06)	0.01 (0.00 – 0.07)	0.20	0.50
OH-MiNP	0.03 (0.00 – 0.24)	0.09 (0.00 – 0.79)	0.30	0.70
cx-MiNP	0.02 (0.00 – 0.13)	0.04 (0.01 – 0.20)	0.20	0.40
oxo-MiNP	0.02 (0.00 – 0.16)	0.05 (0.00 – 0.15)	0.20	0.50
OH-MiDP	0.02 (0.00 – 0.13)	0.03 (0.00 – 0.07)	0.20	0.60
oxo-MiDP	0.02 (0.00 – 0.16)	0.05 (0.02 – 0.09)	0.10	0.40
OH-MINCH	0.02 (0.00 – 0.10)	0.04 (0.00 – 0.14)	0.30	1.00
cx-MINCH	0.04 (0.00 – 0.56)	0.03 (0.00 – 0.11)	0.25	1.00

Table S4 Basic statistical characteristics for young adult cohort – detection frequency, median, minimal and maximal value and 5th and 95th percentiles, SG corrected data [µg/L]

Phthalate metabolite	Detection frequency	Median	Min	Max	5^{th} percentile	95^{th} percentile
MEP *	297/300	17.6	0.02	860	2.51	192
MiBP *	299/300	52.6	0.07	446	10.5	270
MBzP *	227/300	0.69	0.10	25.3	0.10	6.14
MnBP *	289/300	60.7	0.18	665	1.65	249
MCHP	0/300	0.20	0.20	0.20	0.20	0.20
MEHP	281/300	2.29	0.18	40.1	0.18	10.8
5OH-MEHP *	299/300	7.85	0.07	150	1.76	30.6
5oxo-MEHP	298/300	3.65	0.07	113	0.82	14.2
5cx-MEPP *	299/300	5.04	0.07	108	1.17	18.7
MnOP	1/300	0.20	0.20	2.95	0.20	0.20
OH-MiNP *	295/300	7.92	0.30	1649	1.16	131
cx-MiNP *	149/300	0.20	0.20	105	0.20	3.65
oxo-MiNP	276/300	1.99	0.20	111	0.20	14.3
OH-MiDP *	95/300	0.20	0.20	102	0.20	3.30
oxo-MiDP	125/300	0.10	0.10	60.4	0.10	1.99
OH-MINCH *	184/300	1.34	0.30	952	0.30	45.9
cx-MINCH	119/300	0.25	0.25	277	0.25	17.5

** indicates a metabolite was selected as priority biomarker for further analysis*

Table S5 Basic statistical characteristics for teenage cohort – detection frequency, median, minimal and maximal value and 5th and 95th percentiles, SG corrected data [µg/L]

Phthalate metabolite	Detection frequency	Median	Min	Max	5th percentile	95th percentile
MEP *	300/300	38.2	3.04	704	12.3	194
MiBP *	292/300	28.8	0.03	486	7.86	127
MBzP *	284/300	1.74	0.07	112	0.30	16.2
MnBP *	273/300	45.5	0.08	303	0.15	148
MCHP	1/300	0.14	0.08	1.07	0.09	0.31
MEHP	281/300	2.14	0.11	63.6	0.35	10.5
5OH-MEHP *	298/300	13.9	0.03	678	4.81	49.5
5oxo-MEHP	300/300	5.56	1.33	74.5	2.24	22.1
5cx-MEPP *	300/300	7.52	1.74	128	2.94	34.6
MnOP	10/300	0.14	0.08	6.63	0.10	0.42
OH-MiNP *	295/300	11.5	0.16	169	4.52	44.8
cx-MiNP *	300/300	5.34	0.96	503	2.13	26.6
oxo-MiNP	292/300	2.75	0.16	228	0.85	14.8
OH-MiDP *	157/300	0.55	0.09	13.5	0.11	3.25
oxo-MiDP	165/300	0.39	0.05	14.2	0.05	2.07
OH-MINCH *	225/300	1.71	0.15	493	0.19	19.3
cx-MINCH	153/300	0.76	0.10	204	0.13	12.7

** indicates a metabolite was selected as priority biomarker for further analysis*

Table S6 Number of samples (N) and medians for categories of the exposure determinants used for non-parametric testing of the phthalate metabolites. S1 corresponds to cold season (October-April) and S2 to warm season (May-September).

Age	*TAC*		*YAC*								*TAC*	*YAC*
Season	*S1*		*S1*		*S2*		*ALL*		*S1*	*S2*	*S1*	
gender	*M*	*F*	*M*	*F*	*M*	*F*	*M*	*F*	*ALL*		*ALL*	
N	176	124	60	73	85	82	145	155	433	167	300	300
MEP	37.69	39.57	14.79	22.95	16.98	26.61	15.92	25.46	19.66	22.65	38.14	19.88
MiBP	28.68	29.06	64.75	61.60	57.86	64.14	58.10	62.09	62.14	60.50	28.81	61.14
MBzP	1.74	1.76	0.84	0.66	0.98	0.96	0.94	0.86	0.74	0.98	1.74	0.87
MnBP	45.47	44.73	68.89	74.59	72.26	77.27	69.60	75.21	72.06	73.61	45.47	73.16
MEHP	2.34	1.91	2.85	2.50	2.82	2.83	2.83	2.68	2.65	2.83	2.14	2.75
5OH-MEHP	14.96	13.15	7.88	8.95	9.71	10.46	8.99	9.93	8.52	10.18	13.87	9.27
5oxo-MEHP	5.79	5.25	3.67	4.30	4.49	5.26	4.11	4.86	3.95	4.84	5.56	4.48
5cx-MEPP	7.74	7.17	5.25	6.29	5.22	7.20	5.23	6.57	5.84	6.06	7.52	6.01
OH-MiNP	11.43	11.61	6.03	5.58	11.17	12.96	8.74	9.21	5.95	11.58	11.45	9.08
cx-MiNP	5.32	5.39	0.38	0.29	0.46	0.53	0.43	0.43	0.31	0.52	5.34	0.43
MiNP	0.24	0.25	0.42	0.39	0.76	0.39	0.54	0.39	0.41	0.42	0.25	0.42
oxo-MiNP	2.78	2.74	2.29	2.33	2.44	2.64	2.34	2.52	2.33	2.51	2.75	2.36
OH-MiDP	0.54	0.59	0.27	0.24	0.23	0.25	0.24	0.25	0.25	0.23	0.55	0.24
oxo-MiDP	0.39	0.38	0.24	0.14	0.13	0.15	0.18	0.14	0.18	0.14	0.39	0.15
MCPP	0.30	0.27	0.72	1.20	1.19	0.46	0.91	0.94	0.85	1.07	0.29	0.91
OH-MINCH	1.83	1.62	1.30	2.01	1.36	0.94	1.34	1.47	1.68	1.21	1.71	1.41
cx-MINCH	0.85	0.50	0.68	1.05	0.27	0.28	0.36	0.35	0.71	0.28	0.76	0.35

Table S7 Results of nonparametric Kruskal-Wallis ANOVA (for frequencies of PCPs use) and Mann-Whitney U test (for two categories of PCPs use, YES/NO). Number of participants using personal care products (PCPs) were stratified by age (cohort), gender and season (1- cold season – October-April, 2-warm season – May-September), and phthalate metabolites with a statistically significant difference ($p < 0.001$) in PCP use are shown. Tests were performed only for PCP categories with sample size greater than 20 and for metabolites (MEP, MiBP, MBzP, MnBP, 5OH-MEHP, 5cx-MEPP, OH-MiNP) with more than 75% sample above the LOD for each cohort. Asterisks indicate categories of PCPs use between which a statistically significant difference was found. TAC was only sampled in the cold season.

	age (cohort)	***gender***	***season***	***N, according to frequency of PCP use***				*Significant differences in phthalate metabolites*
PCPs				***0+1x per week***	***4x per week***	***7x per week***	***total***	Kruskal-Wallis ANOVA
Shampoo	TA	M	1	49	84	32	165	
	YA	M	2	21	37	27	85	
Deodorant	TA	M	1	77*	39	47*	163	MEP
	TA	F	1	26*	26	66*	118	MEP
				N, according to PCP use or not				**Whitney U test**
				NO	**YES**		**total**	
Lotion	YA	M	1	31	29		60	
	YA	M	2	56	29		85	
Nail polish	TA	F	1	61	56		117	
	YA	F	1	37*	33*		70	5cx-MEPP, OH-MiNP
	YA	F	2	34	46		80	
Foundation	TA	F	1	82	36		118	
	YA	F	1	30	40		70	
	YA	F	2	23	57		80	
Lip balm	TA	M	1	111	52		163	
	TA	F	1	20	99		119	
Eye make-up	TA	F	1	75	44		119	

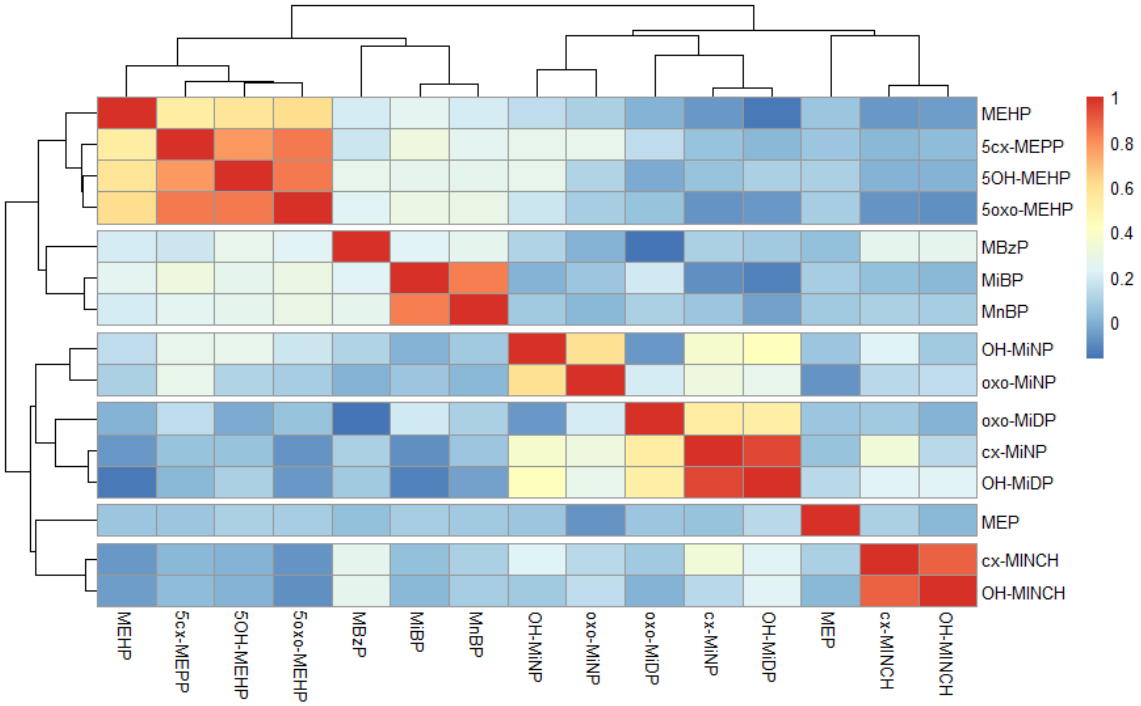


Figure S6 Correlation structure of phthalate metabolites for combined YAC and TAC cohorts.

Table S8a Regression analysis results (regression coefficients and standard error, SE), relationship between each exposure factor (gender, age, season of sample collection, time indoors, and sum of all PCP products used) and metabolite were adjusted for other factors to explain the main effects of each factor. Only metabolites with more than 75% of samples above the LOD for each cohort were used for regression analysis; no relationship found for MnBP; results significant at p<0.1 are shown in the table.

Main effect	**Age**		**Gender (male)**		**Season (S1)**		**Indoor**		**Total PCPs**	
	β; SE	p	β; SE	p	β; SE	p	β; SE	p	β; SE	p
5cx-MEPP	-0.2; 0.06	0.0001	-0.13; 0.042	0.0001	-0.024; 0.049	ns	0.01; 0.004	ns	0.011; 0.025	ns
5OH-MEHP	-0.294; 0.064	0.0001	0.037; 0.055	ns	-0.1; 0.038	0.045	0.054; 0.045	ns	-0.015; 0.064	ns
OH-MINP	-0.289; 0.063	0.0001	-0.014; 0.047	ns	-0.471; 0.074	0.0001	0.024; 0.044	ns	0.049; 0.063	ns
MEP	-0.345; 0.12	0.06	-0.15; 0.067	0.0001	-0.067; 0.07	ns	-0.007; 0.044	ns	0.059; 0.062	ns
MiBP	0.375; 0.061	0.0001	-0.053; 0.078	ns	0.005; 0.081	ns	0.07; 0.033	0.09	0.083; 0.061	ns
MBzP	-0.363; 0.063	0.0001	-0.006; 0.081	ns	-0.142; 0.09	0.09	0.007; 0.044	ns	-0.033; 0.063	ns

Table S8b Regression model (regression coefficients and standard error, SE) for each metabolite with all factors and their interactions to determine the maximum explained variability (coefficient of determination, R^2) in phthalate concentrations by the given factors. Effects and interactions listed are significant at p<0.05; only the results of the best model are shown here. Only metabolites with more than 75% of samples above the LOD for each cohort were used for regression analysis; no relationship found for MnBP.

Best model		**Exposure Factors**		
	R^2	β; SE	β; SE	β; SE
5cx-MEPP	0.11	**age**	**gender (male)**	**age x gender (male)**
		-0.21; 0.04	-0.13; .04	-0.31;0.012
5OH-MEHP	0.13	**age**	**season (S1)**	**indoor x age**
		-0.36; 0.068	-0.1; 0.038	0.1; 0.04
OH-MINP	0.14	**age**	**season (S1)**	**season(S1) x indoor**
		-0.29; 0.06	-0.49; 0.09	0.11; 0.05
MEP	0.25	**age**	**gender (male)**	**age x total PCPs**
		-0.35; 0.09	-0.15; 0.067	0.15; 0.09
MiBP	0.18	**age**	**age x total PCPs**	
		0.39; 0.06	0.1; 0.04	
MBzP	0.1	**age**		
		-0.363; 0.063		

*Table S9 Statistically significant differences between the phthalate metabolites and the exposure determinants; *more than 25 % values below the LOD; red values – statistically significant differences p< 0.05, black values – statistically significant differences p< 0.01. Cold season refers to months October-April; warm season to May-September. TAC was only sampled in the cold season.*

Age	*Teenagers (TAC)*	*Young Adults (YAC)*				*TAC x YAC*
Season	*cold season*	*cold season*	*warm season*	*ALL*	*cold season x warm season*	*Cold season*
gender	*MxF*	*MxF*	*MxF*	*MxF*	*ALL*	*ALL*
MEP*		**F**		**F**		**TAC**
MiBP*						**YAC**
MBzP*						**TAC**
MnBP*						**YAC**
5OH-MEHP*					**S2**	**TAC**
5oxo-MEHP			**F**	**F**		**TAC**
5cx-MEPP*			**F**	**F**		**TAC**
OH-MiNP*					**S2**	**TAC**
cx-MiNP*					**S2***	**TAC***
OH-MiDP*						
OH-MINCH*						

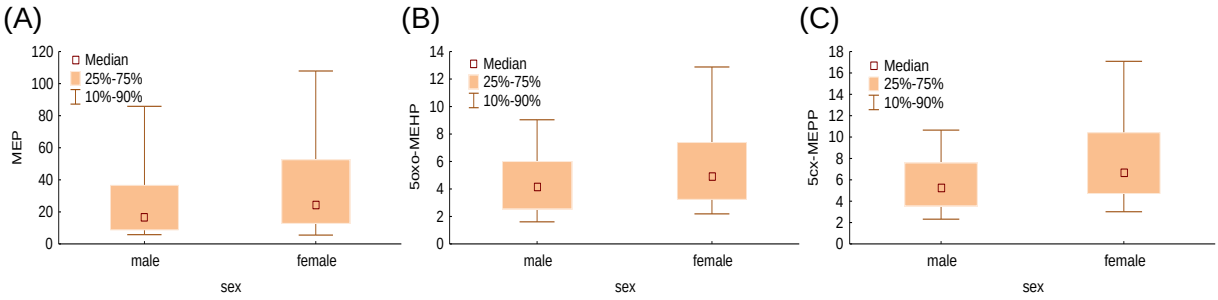


Figure S7 Gender differences (in µg/L) in YAC for MEP (A), 5oxo-MEHP (B) and 5cx-MEPP (C)

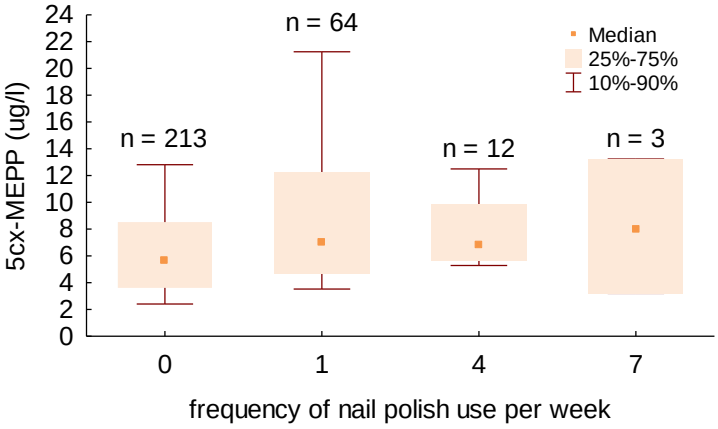


Figure S8 Differences of phthalate metabolites concentrations for different frequency (times per week) of nail polish usage, Young Adults cohort

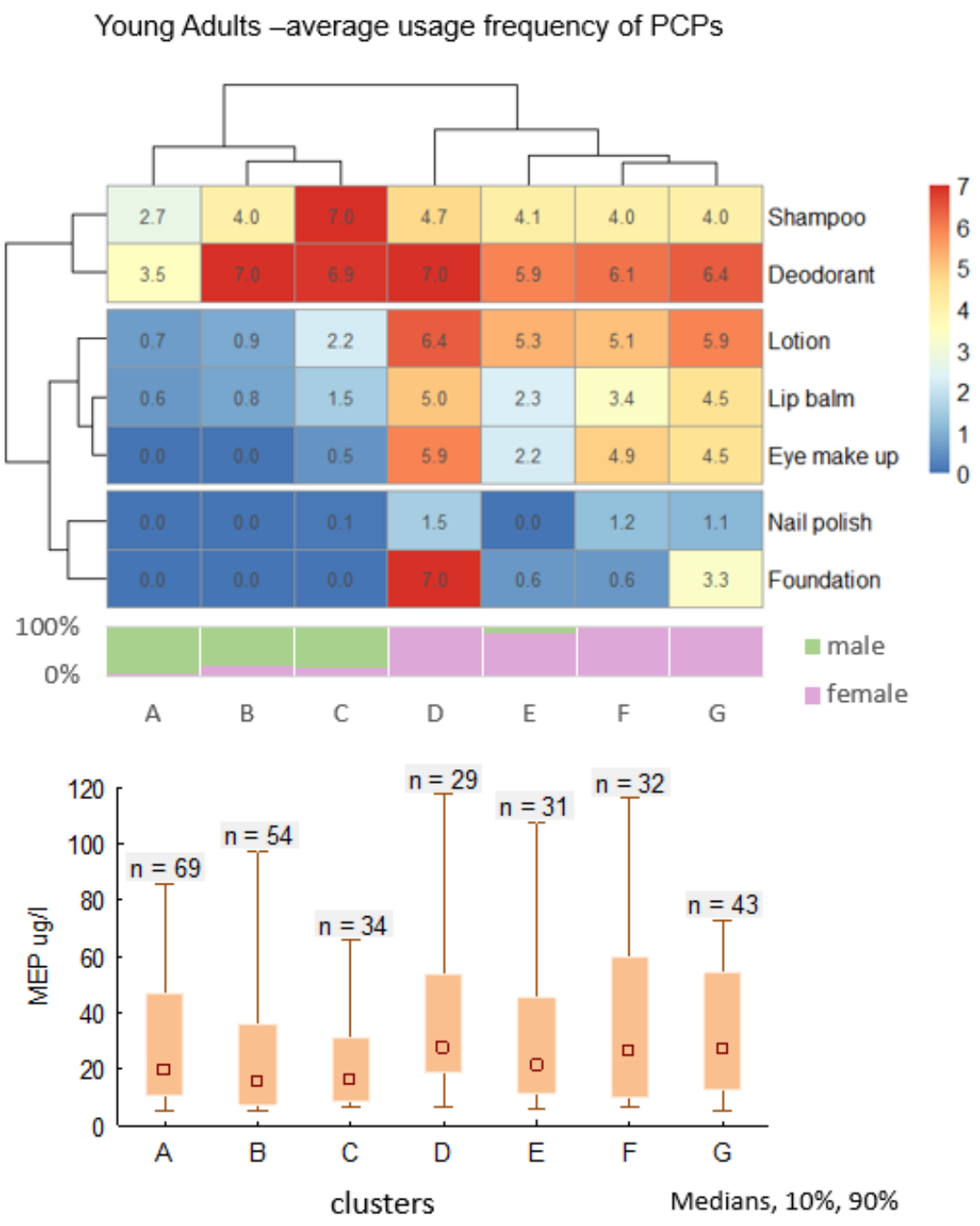
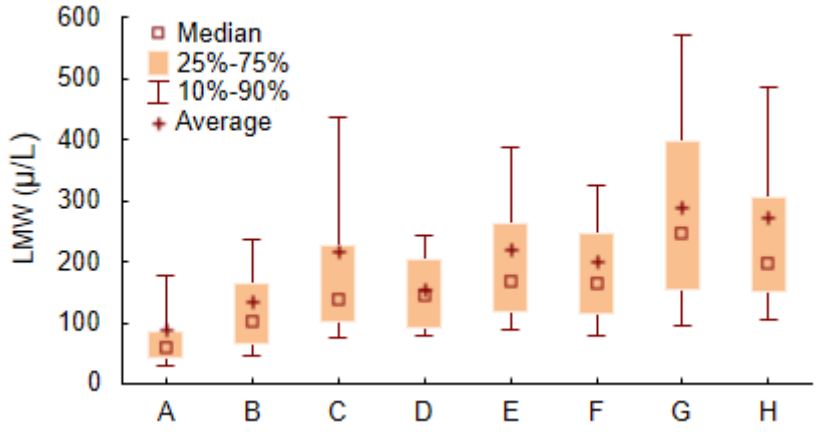
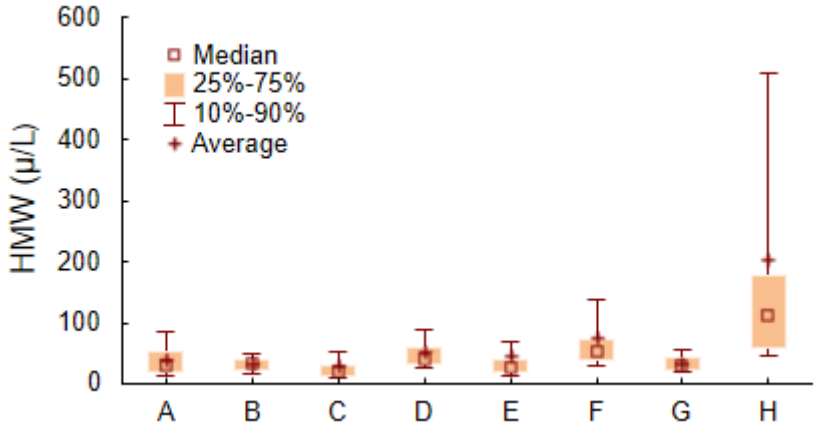


Figure S9 Cluster analysis of average frequency of PCP usage for YAC. Numbers in the coloured table refer to average frequency of use per week of an individual product. TAC had relatively uniform distribution of PCP use, so cluster analysis specific to PCPs did not provide additional insight.

(A)



(B)



(C)

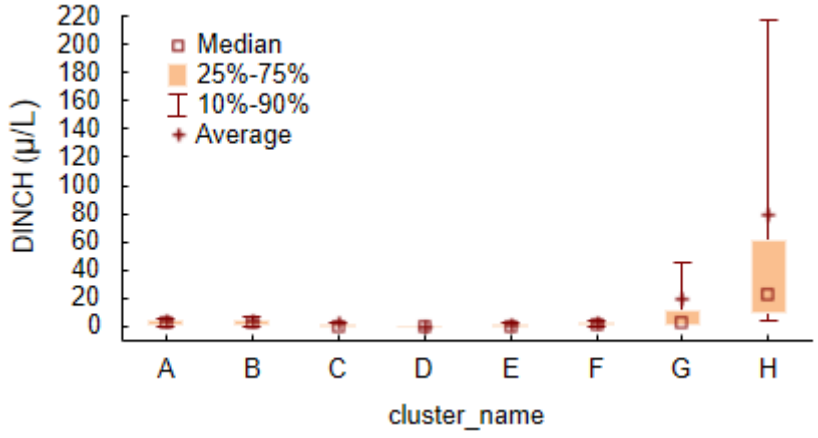


Figure S10 Contributions of (A) LMW, (B) HMW, and (C) DINCH metabolites to each cluster in the cluster analysis shown in Figure 3.

Table S10 Evaluation of statistical significance between phthalate metabolites in clusters in Figure 3, L is low molecular weight and H is high molecular weight phthalate metabolites, D is DINCH; where L, H or D is indicated a statistically significant difference p<0.05.

	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**
A	-		LD	LD	LD	LH	L	LHD
B		-	LD	HD	LD	LH	L	LHD
C	LD	D	-	HD		HD	LD	HD
D	LD	HD	HD	-	HD	D	LHD	LHD
E	LD	LD		HD	-	HD	D	HD
F	LH	LH	HD	D	HD	-	LHD	HD
G	L	L	LD	LHD	D	LHD	-	HD
H	LHD	LHD	HD	LHD	HD	HD	HD	-

Table S11 Median urinary concentrations of phthalate metabolites (µg/L) for teenagers and adults in different countries

Study name	Country	Sampling campaign	Population	MEP	MiBP	MnBP	MBzP	5cx-MEPP	5OH-MEHP	5oxo-MEHP	cx-MINP	OH-MINP	oxo-MINP	OH-MIDP	oxo-MIDP	cx-MINCH	OH-MINCH
This study*	Czech Republic	10.2019 – 01.2020	Teenagers (11-17 y; N=300)	38.14	28.81	45.47	1.74	7.52	13.87	5.56	5.34	11.45	2.75	0.55	0.39	0.76	1.71
			Young adults (18-37 y; N=300)	17.55	52.6	60.65	0.69	5.04	7.85	3.65	0.20	7.92	1.99	0.20	0.10	0.25	1.34
(Schwedler et al., 2020a); (Schwedler et al., 2020b)	Germany	01.2015-06.2017 GerES V study	Teenagers (14-17 y; N=660 approx.)	31.0	22.2	19.5	2.3	8.1	8.1	5.4	4.2	5.5	2.1	1.2	0.5	0.76	1.42
(Health Canada, 2019)	Canada	01.2016-12.2017 CHMS, cycle 5	Teenagers (12-19 y; N=533)	21	13	18	5.2	7.8	6.6	4.5	1.2	0.84	0.9	0.27	0.37		<LOD
			Young adults (20-39 y; N=370)	17	10	11	4.1	5.3	4.8	3	1	0.54	0.59	0.18	0.21		<LOD
(CDC, 2019)	USA	2015-2016 NHANES	Teenagers (12-19 y; N=405)	34.1	11.70	12.20	6.50	9.60	5.70	3.80	9.00		2.20			0.60	0.80
			Adults (>20 y; N=1690)	29.0	8.70	9.80	3.70	8.30	5.40	3.50	6.90		1.80			0.50	0.50

**Current study. Specific gravity adjusted concentrations*
White background: teenagers; grey background: adults

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