Supplementary Materials

Phthalate impurities in UV filters as an underrecognized source of exposure: evidence from European sunscreens and other personal care products

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SUPPLEMENTARY TEXT 1

Extraction and purification

The method was adapted from extraction methods reported by Guo and Kannan [1] and Koniecki et al. [2]. Approximately 100 mg of the sample was weighed using an analytical balance. Subsequently, 50 µL of a mixture of deuterated internal standards (D4-DEP, D4-BBzP, D4-DEHP, 4 ng/µL; Supelco and Cambridge Isotope Laboratories) was added. The sample was then homogenized with 2 mL of Milli-Q water using a shaker for 5 minutes. After homogenization, 4 mL of methyl tert-butyl ether (MTBE) was added, and the mixture was shaken for 10 minutes.

The sample was then centrifuged for 2 minutes at 1800 rpm, after which the organic layer was collected into a vial. The extraction procedure was repeated three times in total.

Column chromatography was used for purification. Activated pure silica gel was used as the solid phase (5 g per column), and hexane (10 mL) and DCM (20 mL) served as the mobile phases. The sample was added to the top of the column, and eluted using 10 mL of hexane, followed by 20 mL of DCM. Following purification, 1 mL of nonane was added, and the extract was concentrated to a final volume of 1 mL and transferred into a mini vial. The residue was rinsed twice with 200 µL of hexane. The final volume of the purified extract was approximately 1 mL.

Prior to instrumental analysis, 50 µL of p-terphenyl (4 ng/µL; Absolute Standards Inc.) was added as an internal syringe standard for calibration.

Instrumental Analysis

Selected phthalates were analyzed using an Agilent 7890A GC (Agilent Technologies, Inc., Santa Clara, CA, USA) equipped with a 30 m × 0.25 mm × 0.25 µm Rxi-5Sil-MS capillary column (Restek, Inc., France), coupled to a tandem mass spectrometer Agilent 7000B MS/MS (Agilent Technologies, Inc., Santa Clara, CA, USA). The temperature program of the GC oven started at 80 °C (3 min hold), then increased at a rate of 7 °C/min until 280 °C (no hold), and lastly at a rate of 20 °C/min until 320 °C (5 min hold). The inlet temperature was set at 280 °C. The injected sample volume was 1 µl in splitless mode. The carrier gas used was helium with a 1.5 mL/min flow rate. The transfer line and ion source temperatures were set at 280 °C. Electron impact ionization (EI) was used, and the mass spectrometer was operating in multiple reaction monitoring (MRM) mode, with nitrogen as the collision gas at a flow rate of 1.5 mL/min (Supplementary Table 3). Compound quantification was done using MassHunter Workstation 10.1 software (Agilent Technologies, Inc., Santa Clara, CA, USA) with an internal standard normalisation calibration curve approach, using p-Terphenyl area for calculation of compound relative responses, with calibration over a linear range of 10 ng/ml to 10 µg/ml.

QA/QC

Recoveries of D4-DEP, D4-BBzP and D4-DEHP to be 56.8±26.8%, 88.6±17.1%, and 96.1±20.4% respectively. Process blanks were run with the samples in each batch, and blank concentrations were used to determine method detection limits (MDLs) based on the average concentration of the blanks plus 3x standard deviation. If a compound concentration was not detected in the process blanks, the instrumental limit of quantification (LOQ) was used as the method detection limit. Instrumental limit of quantitation (LOQ) was calculated using lowest calibration point on signal-to-noise (S/N) ratio basis, as an amount of

compound, which gives S/N ratio of 10. Sample masses <MDL are reported as such.

Recoveries of internal standards were tracked for each sample, and in cases where sample recoveries of D4-DEHP were under 50% (attributed to variations in sunscreen matrix), the results were excluded from the analysis. This resulted in the exclusion of four samples in Round 1 and one sample in Round 2.

SUPPLEMENTARY TEXT 2

Repeatability of analyses was compared between Rounds 1 and 2. A subset of samples from Round 1, covering a range of DnHxP concentrations, were selected to test method consistency. Three new 100 mg samples of each selected product were analyzed following the sample extraction and analytical procedures as in Round 1. In Round 2, analyses showed good consistency, with relative standard deviations of triplicate analyses under 20% (Table S5). The comparison between the single measurement concentrations in Round 1 and the triplicates in Round 2 also generally showed good repeatability, with the exception of two products (BB cream, SPF 30, and General sunscreen, SPF 50), which both had concentrations that were 50-60% lower in Round 2 compared with Round 1. This could be in part due to analytical variability, although triplicate consistency was good within Round 2 for both samples. Another possible likely contributor is option emulsion matrix inhomogeneity or phase separation within the product, or a possible contribution from degradation or reaction of DnHxP within the product between the first and second analytical rounds (approx. 4-month period). Although products were stored closed, at room temperature, and in an undisturbed location, sunscreen stability is unlikely to be tested for impurities and thus reaction of DnHxP with other formulation components could occur. A hypothesis was considered of whether the presence/absence of preservatives in the product formulation could impact the DnHxP stability. This did not offer a clear explanation, as one of the products with the concentration drop had the highest number of preservatives present, while the other had only one preservative (Table S1). As concentration information on the specific levels of preservatives is not available, it is difficult to test this hypothesis further. Therefore, apparent losses of DnHxP in some products between Rounds 1 and 2 are attributed to a combination of analytical variability, degradation within the product, inhomogeneity of the formulation and impurity distribution, and potential sorption to packaging.

Supplementary Table 1. List of products tested

Product description	UV blocker type	DHHB as ingredient	Vol. (mL)	Price/100 mL of prod. (€)	Number of preservatives*	Preservatives*	Notes
Anti-aging facial gel, SPF 40	chemical	no	30	130.90	3	Lactobacillus Ferment, BHT, Phenoxyethanol	
Sun tanning/spray oil, SPF 20	chemical	no	150	2.70	0	-	
Vegan sunscreen, SPF 20	chemical	no	150	2.83	3	Methylparaben, Hydroxyacetophenone, Benzyl Alcohol	
Kids sunscreen, SPF 50	mineral	no	30	7.90	0	-	
Baby and kids sunscreen spray, SPF 50+	chemical	yes	200	7.50	2	Hydroxyacetophenone, Ethylhexylglycerin	
Baby sunscreen, SPF 50+	chemical	yes	30	7.50	1	Hydroxyacetophenone	
Baby sunscreen, SPF 50+	chemical	yes	30	40.77	3	Phenoxyethanol, Sorbic Acid, Ethylhexylglycerin	
BB cream, SPF 30	chemical	yes	30	33.97	6	BHT, Phenoxyethanol, Methylparaben, Ethylhexylglycerin, Ethylparaben, Hydroxyacetophenone	
Children's sensitive sunscreen SPF 50+	chemical	yes	100	7.68	1	Hydroxyacetophenone	
Coconut lip balm A	chemical	yes	10	20.60	0	-	
Coconut lip balm B	chemical	yes	4.8	50.83	1	Pentaerythrityl Tetra-Di-T-Butyl Hydroxyhydrocinnamate	

Eco kids sunscreen, SPF 50	chemical	yes	150	5.16	1	Hydroxyacetophenone
Face cream with sun protection, SPF 50 +	chemical	yes	40	50.73	0	-
Gel sunscreen, SPF 50	chemical	yes	50	13.12	1	Pentaerythrityl Tetra-Di-T-Butyl Hydroxyhydrocinnamate
General sunscreen, SPF 50	chemical	yes	150	3.79	1	Phenoxyethanol
General sunscreen, SPF 50+	chemical	yes	200	7.14	1	Ethylhexylglycerin
General sunscreen, SPF 50+	chemical	yes	50	21.18	1	Phenoxyethanol
Hydrating face cream	chemical	yes	50	26.90	1	Hydroxyacetophenone
Illuminating face cream, SPF 50	chemical	yes	50	13.44	3	BHT, Hydroxyacetophenone, Phenoxyethanol
Kids sunscreen milk, SPF 50+	chemical	yes	150	9.33	2	Phenoxyethanol, Hydroxyacetophenone
Kids sunscreen, SPF 50	chemical	yes	50	16.30	2	Hydroxyacetophenone, Phenoxyethanol
Kids sunscreen, SPF 50+	chemical	yes	150	7.61	4	BHT, Hydroxyacetophenone, Methylparaben, Phenoxyethanol
Oil-free gel sunscreen, SPF 50	chemical	yes	50	44.76	3	Phenoxyethanol, Ethylhexylglycerin, Sodium Benzoate
Shine control daycream, SPF 50	chemical	yes	50	26.08	4	Hydroxyacetophenone, Ethylhexylglycerin, Phenoxyethanol, Benzyl Alcohol
Sport sunscreen,	chemical	yes	50	16.22	2	Benzyl Alcohol, Chlorphenesin

SPF 50						
Sun protection and bronzing cream, SPF 30	chemical	yes	200	9.78	3	Benzyl Alcohol, Ethyl Benzoate, Methyl Benzoate
Sun protection and bronzing cream, SPF 50	chemical	yes	50	18.88	2	Phenoxyethanol, Ethylhexylglycerin
Sunbathing oil, SPF 20	chemical	yes	200	2.87	2	Pentaerythrityl Tetra-Di-T-Butyl Hydroxyhydrocinnamate, Benzoic Acid
Sunscreen spray, sensitive skin, SPF 50+	chemical	yes	200	7.50	1	Ethylhexylglycerin
Sunscreen stick, SPF 50+	chemical	yes	24	40.75	0	-
Tinted face cream, SPF 40	chemical	yes	30	13.60	1	Phenoxyethanol

*Preservatives were classified based on the “what it does” classification in the INCIDecoder database.

Supplementary Table 2. Target phthalates included in GC-MS method

Abbreviation	Common name	CAS RN	InChIKey	Avg mass	Mol. Formula
DMP	Dimethyl phthalate	131-11-3	NIQCNGHVCWTJSM-UHFFFAOYSA-N	194.186	C10H10O4
DEP	Diethyl phthalate	84-66-2	FLKPEMZONWLCSK-UHFFFAOYSA-N	222.24	C12H14O4
DBP	Di-n-butyl phthalate	84-74-2	DOIRQSBPFJWKBE-UHFFFAOYSA-N	278.348	C16H22O4
DIBP	Di-isobutyl phthalate	84-69-5	MGWAVDBGNNKXQV-UHFFFAOYSA-N	278.348	C16H22O4
DMEP	Di(2-methoxyethyl) phthalate	117-82-8	HSUIVCLOAAJSRE-UHFFFAOYSA-N	282.292	C14H18O6
DPP	Dipentyl phthalate	131-18-0	IPKKHRVROFYTEK-UHFFFAOYSA-N	306.402	C18H26O4
DEEP	1,2-Benzenedi- carboxylic acid, bis(2-ethoxyethyl) ester	605-54-9	RMKYMNRQXYPJHL-UHFFFAOYSA-N	310.346	C16H22O6
BBzP	Benzyl butyl phthalate	85-68-7	IRIAEXORFWYRCZ-UHFFFAOYSA-N	312.365	C19H20O4
DCHP	Dicyclohexyl phthalate	84-61-7	VOWAEIGWURALJQ-UHFFFAOYSA-N	330.424	C20H26O4
DMPP	Bis(4-methyl-2-pentyl) phthalate	84-63-9	UAFXUVUVOTXFND-UHFFFAOYSA-N	334.456	C20H30O4
DnHxP	Di-n-hexyl phthalate	84-75-3	KCXZNSGUUQJJTR-UHFFFAOYSA-N	334.456	C20H30O4
DBEP	Bis(2-butoxyethyl) phthalate	117-83-9	CMCJNODIWQEOAI-UHFFFAOYSA-N	366.454	C20H30O6
DEHP	Di(2-ethylhexyl) phthalate	117-81-7	BJQHLKABXJIVAM-UHFFFAOYSA-N	390.564	C24H38O4
DNOP	Di-n-octyl phthalate	117-84-0	MQIUGAXCHLFZKX-UHFFFAOYSA-N	390.564	C24H38O4
DNP	Di-nonyl phthalate	84-76-4	DROMNWUQASBTFM-UHFFFAOYSA-N	418.618	C26H42O4

Supplementary Table 3. Retention times (RT), MRM ion pairs and collision energies (CE) for phthalates analysis

Analyte	**RT** (min)	**MRM 1** **m/z**	**CE** eV	**MRM 2** **m/z**	**CE** eV
DMP	11.29	162.6 -> 77.0	20	162.6 -> 135.0	10
D4-DEP (IS)	13.61	152.7 -> 125.0	10	152.7 -> 97.1	15
DEP	13.63	148.7 -> 65.0	25	148.7 -> 93.1	15
DIBP	17.83	148.7 -> 65.0	25	148.7 -> 93.1	15
DBP	19.17	148.7 -> 65.0	25	148.7 -> 93.1	15
DMEP	19.67	148.7 -> 65.0	25	148.7 -> 93.1	15
DMPP	20.65	148.7 -> 65.0	25	148.7 -> 93.1	15
DEEP	21.19	148.7 -> 65.0	25	148.7 -> 93.1	15
DPP	21.70	148.7 -> 65.0	25	148.7 -> 93.1	15
DnHxP	24.07	148.7 -> 65.0	25	148.7 -> 93.1	15
D4-BBzP (IS)	24.12	152.7 -> 125.0	10	152.7 -> 97.1	15
BBzP	24.15	148.7 -> 65.0	25	148.7 -> 93.1	15
DBEP	25.53	148.7 -> 65.0	25	148.7 -> 93.1	15
DCHP	26.06	148.7 -> 65.0	25	148.7 -> 93.1	15
D4-DEHP	26.26	152.7 -> 125.0	10	152.7 -> 97.1	15
DEHP	26.30	148.7 -> 65.0	25	148.7 -> 93.1	15
DNOP	28.35	148.7 -> 65.0	25	148.7 -> 93.1	15
DNP	30.23	148.7 -> 65.0	25	148.7 -> 93.1	15

Supplementary Table 4. Instrument limits of quantification (LOQ), average of procedural blanks, and calculated method detection limits (MDLs)

	LOQ (ng/sample)	Blank avg. (ng/sample)	MDL (ng/sample)
DMP	0.218	<LOQ	0.218
DEP	0.345	1.95	2.39
DBP	0.173	23.2	37.3
DIBP	0.153	15.9	26.1
DMEP	10.0	<LOQ	10.0
DPP	0.182	<LOQ	0.182
DEEP	4.2	<LOQ	4.20
BBzP	0.763	<LOQ	0.763
DCHP	0.235	<LOQ	0.235
DMPP	0.556	<LOQ	0.556
DnHxP	0.195	<LOQ	0.195
DBEP	3.33	<LOQ	3.33
DEHP	0.223	63.1	95.6
DNOP	0.272	<LOQ	0.272
DNP	0.288	<LOQ	0.288

Supplementary Table 5. Results from initial quantitative analysis of all products (Round 1). Phthalates with detection in at least 1 product are listed. "<MDL" indicates samples were below the blank-determined method detection limit, while "LOQ" indicates that samples were below instrumental quantification limits. NR indicates that the results were below analytical performance criteria and results are not reported.

Product	DEP (ng/g)	DIBP (ng/g)	DBP (ng/g)	DnHxP (ng/g)
Kids sunscreen, SPF 50	<MDL	<MDL	81.9	<LOQ
Kids sunscreen, SPF 50+	<LOQ	2600	195	247
Baby sunscreen, SPF 50+	<MDL	<MDL	<MDL	<LOQ
General sunscreen, SPF 50+	<MDL	<MDL	<MDL	<LOQ
Sun tanning/spray oil, SPF 20	<MDL	<MDL	595	<LOQ
Baby sunscreen, SPF 50+*	NR	NR	NR	NR
General sunscreen, SPF 50	<MDL	<MDL	<MDL	2550
Vegan sunscreen, SPF 20*	NR	NR	NR	NR
Sport sunscreen, SPF 50	<MDL	<MDL	<MDL	2960
Kids sunscreen, SPF 50	<LOQ	<MDL	<MDL	<LOQ
Eco kids sunscreen, SPF 50	<MDL	<MDL	<MDL	474
Oil-free gel sunscreen, SPF 50	<MDL	<MDL	<MDL	1270
Gel sunscreen, SPF 50	<MDL	<MDL	<MDL	<LOQ
Hydrating face cream	<MDL	<MDL	<MDL	<LOQ
Sun protection and bronzing cream, SPF 30	<LOQ	<MDL	<MDL	<LOQ
Sun protection and bronzing cream, SPF 50	3900	<MDL	<MDL	<LOQ
Sunbathing oil, SPF 20*	NR	NR	NR	NR
Shine control daycream, SPF 50	<MDL	<MDL	<MDL	<LOQ
Illuminating face cream, SPF 50*	NR	NR	NR	NR
General sunscreen, SPF 50+	<MDL	<MDL	<MDL	804
Coconut lip balm A	<LOQ	<MDL	3,538	1980
Coconut lip balm B	<MDL	<MDL	75.2	1310
Sunscreen stick, SPF 50+	<MDL	<MDL	248	1020
Tinted face cream, SPF 40	<MDL	<MDL	<MDL	954
BB cream, SPF 30	<MDL	<MDL	<MDL	416
Anti-aging facial gel, SPF 40	<MDL	<MDL	111	<LOQ
Sunscreen spray, sensitive skin, SPF 50+	<LOQ	<MDL	<LOQ	<LOQ
Kids sunscreen milk, SPF 50+	<MDL	<MDL	<MDL	<LOQ
Children's sensitive sunscreen SPF 50+	<MDL	<MDL	<MDL	<LOQ
Face cream with sun protection, SPF 50 +	<MDL	<MDL	<MDL	3100
Baby and kids sunscreen spray, SPF 50+	<MDL	<MDL	<MDL	<LOQ

* 4 samples analyzed had low recoveries (D4-DEHP <50%) and therefore values are not reported (NR).

Supplementary Table 6. Round 2 results for all phthalates, products meeting QA/QC criteria. "<MDL" indicates samples were below the blank-determined method detection limit, while "LOQ" indicates that samples were below instrumental quantification limits.

Product	DMP	DIBP	DEP	DBP	DnHxP	DEHP	DNOP	DNP
Kids sunscreen, SPF 50+	<LOQ	<MDL	<MDL	<MDL	197	<MDL	<LOQ	<LOQ
General sunscreen, SPF 50+	<LOQ	<MDL	<MDL	<MDL	361	<MDL	<LOQ	<LOQ
Coconut lip balm A	<LOQ	510	13.2	4430	1490	2440	<LOQ	<LOQ
Sunscreen stick, SPF 50+	<LOQ	<MDL	<MDL	472	962	479	<LOQ	<LOQ
BB cream, SPF 30	5.34	<MDL	53.8	<MDL	158	<MDL	17.8	18.8
Face cream with sun protection, SPF 50 +	6.78	<MDL	10.7	<MDL	3070	<MDL	<LOQ	<LOQ

Supplementary Table 7. Synthesis of Round 1 and 2 results for products with detection; in case of Round 1 analysis only, Round 1 results are directly reported. In case of repeated analyses (indicated with an asterisk) concentrations are the average of all replicates, excluding values below detection. "<MDL" indicates samples were below the blank-determined method detection limit, while "LOQ" indicates that samples were below instrumental quantification limits.

Product	DMP	DEP	DBP	DIBP	DnHxP	DEHP	DNOP	DNP
*Coconut lip balm A	<LOQ	13.2	4200	510	1610	2440	<LOQ	<LOQ
Sun protection and bronzing cream, SPF 50	<LOQ	3900	<MDL	<MDL	<LOQ	<MDL	<LOQ	<LOQ
*Face cream with sun protection, SPF 50 +	6.78	10.7	<MDL	<MDL	3080	<MDL	21.2	1.44
Sport sunscreen, SPF 50	<LOQ	<MDL	<MDL	<MDL	2960	<MDL	<LOQ	<LOQ
General sunscreen, SPF 50	<LOQ	<MDL	<MDL	<MDL	2550	<MDL	<LOQ	<LOQ
*Sunscreen stick, SPF 50+	<LOQ	<MDL	416	<MDL	976	479	<LOQ	<LOQ
Coconut lip balm B	<LOQ	<MDL	75.2	<MDL	1310	<MDL	<LOQ	<LOQ
Oil-free gel sunscreen, SPF 50	<LOQ	<MDL	<MDL	<MDL	1270	<MDL	<LOQ	<LOQ
Tinted face cream, SPF 40	<LOQ	<MDL	<MDL	<MDL	954	<MDL	<LOQ	<LOQ
Sun tanning/spray oil, SPF 20	<LOQ	<MDL	595	<MDL	<LOQ	<MDL	<LOQ	<LOQ
Eco kids sunscreen, SPF 50	<LOQ	<MDL	<MDL	<MDL	474	<MDL	<LOQ	<LOQ
*General sunscreen, SPF 50+	<LOQ	<MDL	<MDL	<MDL	472	<MDL	<LOQ	<LOQ
*Kids sunscreen, SPF 50+	<LOQ	<MDL	195	<MDL	210	<MDL	<LOQ	<LOQ
*BB cream, SPF 30	5.34	53.8			223	<MDL	17.8	18.8
Anti-aging facial gel, SPF 40	<LOQ	<MDL	111	<MDL	<LOQ	<MDL	<LOQ	<LOQ
Kids sunscreen, SPF 50	<LOQ	<MDL	81.9	<MDL	<LOQ	<MDL	<LOQ	<LOQ

Supplementary Table 8. Comparison of Round 1 and Round 2 concentrations for DnHxP for compounds with positive detection

Product	Round 1 (ng/g)	Round 2 Average ± Stand. Dev. (n=3) (ng/g)	Round 2 Relative Stand. Dev.	% decrease Round 1 vs. Round 2
Kids sunscreen, SPF 50+	247	197 ± 36.6	19%	20%
BB cream, SPF 30	416	158 ± 24.3	15%	62%
Eco kids sunscreen, SPF 50	474	NA	NA	NA
General sunscreen, SPF 50+	804	361 ± 26.7	7.4%	55%
Tinted face cream, SPF 40	954	NA	NA	NA
Sunscreen stick, SPF 50+	1020	962 ± 66.7	6.9%	5.5%
Oil-free gel sunscreen, SPF 50	1270	NA	NA	NA
Coconut lip balm B	1310	NA	NA	NA
Coconut lip balm A	1980	1490 ± 245	16%	25%
General sunscreen, SPF 50	2550	NA	NA	NA
Sport sunscreen, SPF 50	2960	NA*	NA	NA
Face cream with sun protection, SPF 50 +	3100	3070 ± 127	4.1%	0.73%

* Sport sunscreen, SPF 50 was analyzed in Round 2 but recoveries did not exceed the set threshold, therefore was excluded from interpretation.

REFERENCES

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