Phthalates and Phthalate Alternatives Analysis Using Gas Chromatography Mass Spectrometry With Demonstration using Silicone Passive Samplers and Real-World Samples

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Introduction

Phthalates are common plasticizers found in everyday items from single use plastics, PVC pipes, vinyl flooring, medical devices, toys, and electronics. Phthalates increase the plastics flexibility, durability, and temperature range (1). Since they are not chemically bound to polymers, they are therefore able to leach out. Over the years, more phthalates have been synthesized. From 2000 to 2010, global production increased from 3.5 to 6 mil tons/yr (2). Phthalates have been found in the air, ocean, dust, wastewater, cosmetics, and food (3, 5-15). Exposure to phthalates is associated with allergies, asthma, rhinoconjunctivitis, reduced birth weight, and endocrine disruptions (3, 15, 16, 17, 18). Due to increasing awareness of the environmental and health impacts of phthalates within the last thirty years, regulations have been implemented across the globe for particular phthalates. In the US, for example, 8 phthalates are regulated. This has caused a shift to alterative phthalates in the global market (3). We developed a selective ion monitoring (SIM) gas chromatography mass spectrometry (GCMS) method on an Agilent 8890 5977B GCMS for quantitation of 27 phthalates and 3 alternative phthalates for passive samplers and biological matrices. See Table 1 for full analyte list.

Method Validation and Optimization

Oven profile
- Started with from Takeuchi et al (2014)
- Broad peaks for high molecular weight (HMW) compounds
- Series of oven profile experiments
- Reduced hold on 3rd ramp to improve HMW compounds
- 4th ramp was added to improve ditridecyl phthalate
- 5 minute post-run added to reduce carry over
- Final oven profile achieved shape and resolution (15 scans/peak) for all compounds (Table 1)

Table 1. Full compound list with peak number, structure, physical and chemical properties, and limits of detection (LODs) and of quantitation (LOQs). Orange compounds are alternative plasticizers.

Peak #	Compounds	CAS	Structure	Mol Wt (g/mol)	Log Koa	Log Kow	Henry's Law	LOD (ppb)	LOQ (ppb)
1	Dimethyl Phthalate	131-11-3		194.2	6.69	1.6	1.97E-07	200	1000
2	Diethyl Phthalate	84-66-2		222.24	7.44	2.65	6.10E-07	150	750
3	Diisopropyl Phthalate	605-45-8		250.29	8.03	3.48	6.14E-07	50	250
4	Diallyl Phthalate	131-17-9		246.26	8.16	3.36	1.17E-07	50	250
5	Di-n-propyl Phthalate	131-16-8		250.3	8.18	3.63	4.03E-07	50	250
6	Diisobutyl Phthalate	84-69-5		278.35	8.76	4.46	6.43E-07	150	750
7	Di-n-butyl Phthalate	84-74-2		278.35	8.63	4.5	1.22E-06	200	1000
8	Bis(2-methoxyethyl) Phthalate	117-82-8		282.29	9.77	1.11	2.81E-13	50	250
9	Diisopentyl Phthalate	605-50-5		306.4	9.5	5.45	1.29E-06	50	250
10	Bis(2-ethoxyethyl) Phthalate	605-54-9		310.34	10.5	2.1	5.11E-13	50	250
11	Diamyl Phthalate	131-18-0		306.4	9.67	5.59	8.89E-07	50	250
12	Bis(4-methylpentyl) Phthalate	146-50-9		334.46	10.2	6.43	2.56E-06	50	250
13	Di-n-hexyl Phthalate	84-75-3		334.4	10.4	6.57	2.57E-05	150	750
14	Butyl Benzyl Phthalate	85-68-7		312.4	10.6	4.83	1.26E-06	150	750
15	Di(2-ethylhextl) Adipate	103-23-1		370.58	11.2	8.12	2.15E-05	150	750
16	Bis(2-butoxyethyl) Phthalate	117-83-7		366.4	12	4.06	2.03E-12	50	250
17	Bis(2-ethylhexyl) Phthalate	117-81-7		390.57	11.7	8.39	2.70E-07	200	1000
17	Diheptyl Phthalate	3648-21-3		362.5	11.1	7.56	3.54E-06	200	1000
18	Dicyclohexyl Phthalate	84-61-7		330.4	11.6	6.2	1.00E-07	200	1000
19	Diphenyl Phthalate	84-62-8		318.32	10	4.1	3.06E-08	50	250
20	Bis(2-ethylhexyl) Isophthalate	137-89-3		390.56	11.7	8.39	2.44E-07	50	250
21	Di-n-octyl Phthalate	117-84-0		390.6	11.9	8.54	2.57E-06	150	750
22	Bis(2-ethylhexyl) Terephthalate	744-45-6		318.3	10	4.1	3.06E-08	150	750
22	Diphenyl Isophthalate	6422-86-2		390.56	11.7	8.39	1.02E-05	50	250
23	Dibenzyl Phthalate	523-31-9		346.4	12.3	5.08	1.02E-11	50	250
24	Bis(2-propylheptyl) Phthalate	53306-54-0		446.66	13.2	10.4	1.55E-07	50	250
25	Di-n-nonyl Phthalate	84-76-4		418.6	####	9.52	1.41E-05	250	1250
27	Didecyl Phthalate	84-77-5		446.66	13.3	9.05	2.81E-06	250	1250
28	Diundecyl Phthalate	3648-20-2		474.7	14.1	11.5	5.60E-05	50	250
33	Tris(2-ethylhexyl) Trimellitate	3319-31-1		546.78	16.2	11.6	4.45E-07	200	1000
34	Ditridecyl Phthalate	119-06-2		530.8	15.5	13.5	2.23E-04	50	250
2	Diethyl Phthalate-d4	93952-12-6		225.26	--	--	--	--	--
7	Di-n-butyl Phthalate-d4	93952-11-5		282.37	--	--	--	--	--
11	Diamyl Phthalate-d4	358730-89-9		310.43	--	--	--	--	--
25	Di-n-nonyl Phthalate-d4	1202865-43-7		422.6	--	--	--	--	--
26	Diisodecyl Phthalate-d4	1346604-79-2		450.69	--	--	--	--	--
27	Di-n-decyl Phthalate-d4	1276197-18-2		450.66	--	--	--	--	--

Table 2. Full GC/MS settings for method

Description	Conditions
Instrument	Agilent GC/MSD 8890/5977, EI mode 70eV
Column	J&W Sicentific DB 5MS 30m x 250µm x 0.25µm, Helium 99.9% at 1.157 mL/min
Temperature	Injection 290 °C MSD 300 °C MS Source 300 °C MS quadripole 180 °C Column 40 °C (2 min hold) to 100 °C at 10 °C/min, to 200 °C at 25 °C/min, to 280 °C at 25 °C/min (3 min hold), to 335 °C at 20 °C/min (7 min hold). Than a 5 min post run at 340 °C. Total run time of 31.75 min
Inlet	Draw Speed 300 µL/min, Eject Speed 6000 µL/min, Pulsed Splitless mode 25psi until 0.5 min, Setpum Purge 3 mL/min, Purge to Split Vent 20 mL/min at 0.45 min
Scan Mass Range	50-600
Injection Volume	1 µL

Calibration curve
- Multi-level calibration curve
  - 250 to 10000 ng/mL & 2000 to 25000 ng/mL
- Average r^2 of 0.995
  - Only 5 quadratic fits needed.

LODs and LOQs
- Ran 15 repetitions over 3 days.
- From interday repetitions, limits of detection (LODs) calculated by standard deviation * t-value (99% confidence interval).
  - Average LOD is 83 ng/mL, from 1.6-231 ng/mL
- Limits of quantitation (LOQs) =LOD *5
  - Average LOQ is 535 ng/µL, from 250-1250 ng/mL
- Average percent recoveries for the targets: 111%
- LODs and LOQs values are shown in Table 1

Storage Stability
- Three aliquots of the same full curve mix were taken four times at 0, 12, 50, 133 days.
- Percent recovery (Figure 2)
  - Average at 0 days: 84%
  - Average at 12 days: 87%
  - Average at 50 days: 94%
  - Average at 133 days: 86%
- All compounds are stable for at least 133 days

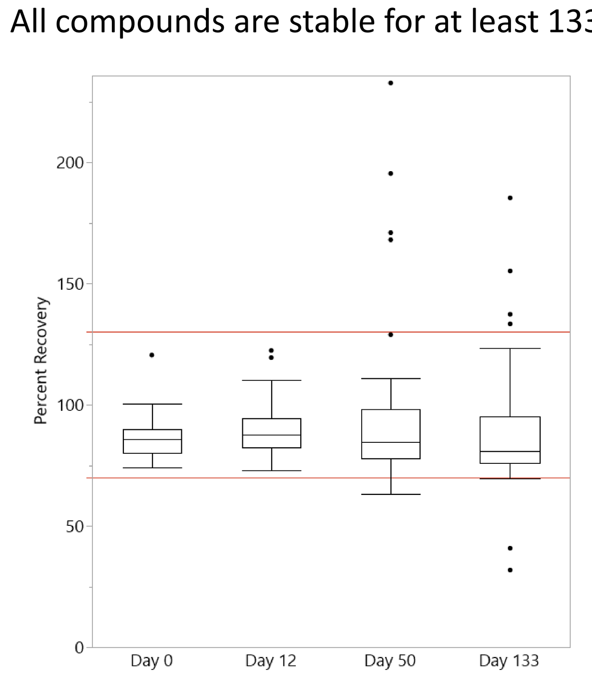


Figure 2. Storage stability. Red lines are at 70% and 130% FSES DQO's

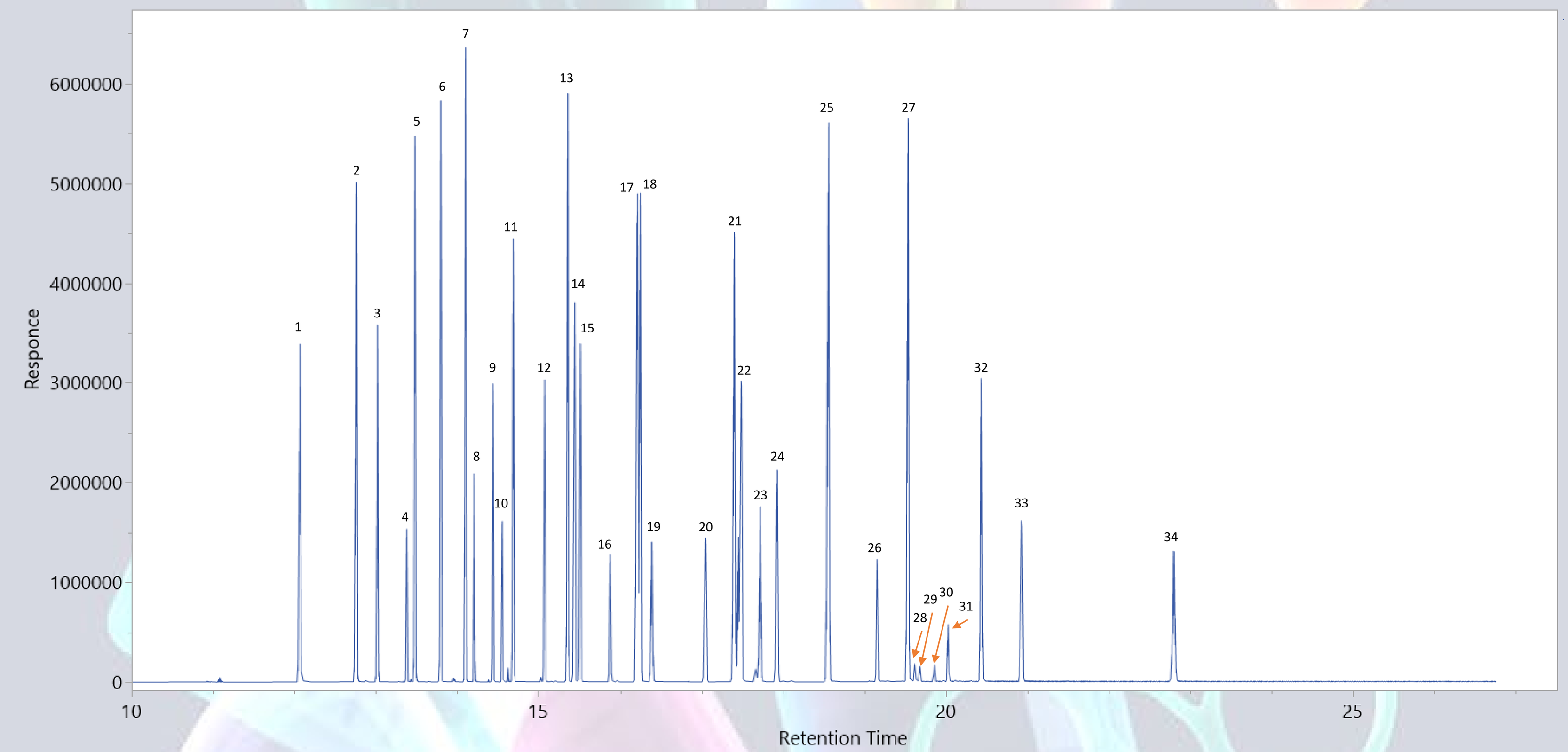
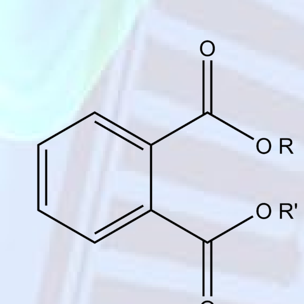


Figure 1. Full chromatogram. Target peaks are numbered. See Table 1 for targets names and structures

- Phthalates are in personal care products, medical devices, building materials, food packaging and more
- Over half all wristbands analyzed have phthalates detected
- Targeted analysis for over 30 phthalates and phthalate alternatives with a 30-minute run time
- Comparable with other published work for food stuffs and passive sampler technology



Representative phthalate structure

References:
1. Rahman, Mustafizur, and Christopher S Brazel. "The Plasticizer Market: An Assessment of Traditional Plasticizers and Research Trends to Meet New Challenges." *Progress in polymer science* 29, no. 12 (2004): 1223-48.
2. Liang, Yirui, Olivier Caillot, Jianshun Zhang, Jiping Zhu, and Ying Xu. "Large-Scale Chamber Investigation and Simulation of Phthalate Emissions from Vinyl Flooring." *Building and Environment* 89 (2015): 141-49.
3. Bui, Thuy T, Georgios Giovanoulis, Anna Palm Cousins, Jörgen Magnér, Ian T Cousins, and Cynthia A de Wit. "Human Exposure, Hazard and Risk of Alternative Plasticizers to Phthalate Esters." *Science of the Total Environment* 541 (2016): 451-67.
4.*Method 8061a: Phthalate Esters by Gas Chromatography with Electron Capture Detection (Gc/Ecd)*, by Agency, U.S. Environmental Protection. 1996.
5. Guo, Zhiyong, Sui Wang, Danyi Wei, Meili Wang, Huina Zhang, Panpan Gai, and Jing Duan. "Development and Application of a Method for Analysis of Phthalates in Ham Sausages by Solid-Phase Extraction and Gas Chromatography–Mass Spectrometry." *Meat science* 84, no. 3 (2010): 484-90.
6. Gimeno, P., et al. (2012). "Analytical method for the identification and assay of 12 phthalates in cosmetic products: Application of the ISO 12787 international standard "Cosmetics–Analytical methods–Validation criteria for analytical results using chromatographic techniques"." Journal of Chromatography A 1253: 144-153.
7.Feng, S. H., Brian J (2013). Sensitive and Reproducible Phthalate Analysis Using the Agilent 5977A Series GC/MSD, Agilent Technologies, Inc.: 8.
8.Raveane, L., et al. (2013). Phthalates determination in wine and spirits using GC-MS and LC-MS/MS. 3rd MS Food day, Fondazione Edmund Mach.
9. Gimeno, P., et al. (2014). "Identification and quantification of 14 phthalates and 5 non-phthalate plasticizers in PVC medical devices by GC–MS." Journal of Chromatography B 949: 99-108.
10.Ye, X., et al. (2014). "Analysis of 21 phthalate leachables in metered dose inhalers by gas chromatography tandem mass spectrometry." Analytical Methods 6(12): 4083-4089.
11.Orecchio, S., et al. (2015). "Determination of selected phthalates by gas chromatography–mass spectrometry in personal perfumes." Journal of Toxicology and Environmental Health, Part A 78(15): 1008-1018.
12.Dong, C.-D., et al. (2020). "Detecting phthalate esters in sludge particulates from wastewater treatment plants." Journal of Environmental Science and Health, Part A 55(10): 1233-1240.
13.Kartalović, B., et al. (2021). "Detection of microplastic residues-developing a method for phthalates in honey."
14.Sambolino, A., et al. (2022). "Determination of phthalic acid esters and di (2-ethylhexyl) adipate in fish and squid using the ammonium formate version of the QuEChERS method combined with gas chromatography mass spectrometry." Food Chemistry 380: 132174.
15. Giovanoulis, G., et al. (2018). "Multi-pathway human exposure assessment of phthalate esters and DINCH." Environment International 112: 115-126.
16.Jaakkola, J. J. and T. L. Knight (2008). "The role of exposure to phthalates from polyvinyl chloride products in the development of asthma and allergies: a systematic review and meta-analysis." Environmental health perspectives 116(7): 845-853.
17. Mariana, Melissa, et al. (2016) "The effects of phthalates in the cardiovascular and reproductive systems: A review." *Environment international* 94: 758-776
18. Engel, S. M. and M. S. Wolff (2013). "Causal Inference Considerations for Endocrine Disruptor Research in Children's Health." Annual Review of Public Health 34(1): 139-158.
19.Takeuchi, S., et al. (2014). "Detection of 34 plasticizers and 25 flame retardants in indoor air from houses in Sapporo, Japan." Science of the Total Environment 491: 28-33.

Method Validation and Optimization (Continued)

SPE
- Sample clean-up used several types of solid phase extraction (SPE).
- C18, florisil, and primary secondary amine (PSA) columns were tested with a 15 pg/µL matrix spike. (Figure 3)
- C18 was effective for smaller MW, however the HMW compounds were not recovered.
  - Florisil percent recovery 90%
  - PSA percent recovery 96%
- PSA had 91% compounds within data quality objectives (DQO's)
  - florisil (77%)
  - C18 (68%)

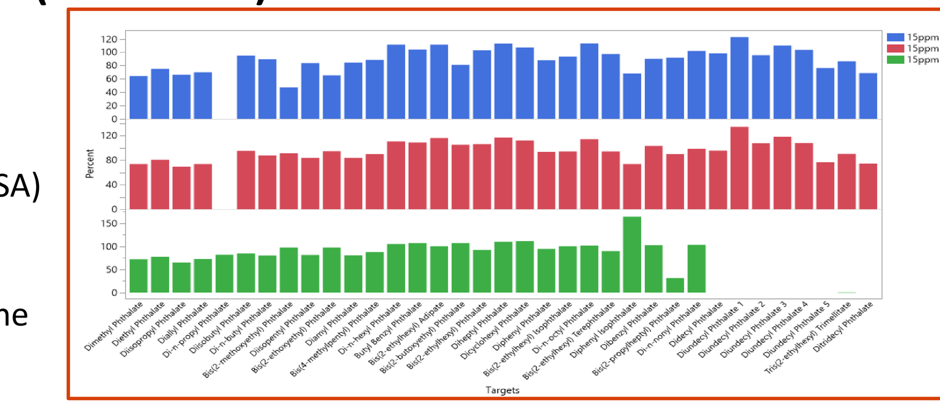


Figure 3: Comparison of the percent recoveries of C18, Florisil, and PSA SPE cartridges. Di-n-propyl phthalate was detected for both Florisil and PSA but 15 ng/µL is over its calibration range of 0.25 ng/µL -10 ng/µL. It is a quadratic fit, and the ratio of compound to ISTD was 5.3 which is over the vertex of 4.55 and therefore no concentration was given by MassHunter software.

Method Comparison

- Of the 11 phthalate method papers identified, the number of phthalates in the method ranged from 6 to 21 with run times of 16 minutes to 40 minutes. (Table 3)
- This method has at least 10 more phthalate compounds and kept the total run time comparable with most other published phthalate methods.
- This method had 9 unique compounds:
  - diamyl phthalate
  - bis (4-methylpentyl) phthalate
  - diheptyl phthalate
  - diphenyl isophthalate
  - dibenzyl phthalate
  - bis (2-propylheptyl) phthalate
  - didecyl phthalate
  - diundecyl phthalate
  - ditridecyl phthalate.

Table 3. Method comparison table comparing this method with 11 other phthalate method papers

Method Paper	This Method	EPA Method 8061A	Guo et al, 2010	Gimeno et al, 2012	Feng et al, 2013	Raveane et al, 2013	Gimeno et al, 2014	Ye et al, 2014	Orecchio et al, 2015	Dong et al, 2020	Kartalovic et al, 2021	Sambolino et al, 2022	Takeuchi, Kojima et al, 2014
Phthalates	28	7	6	12	6	17	9	21	15	10	6	12	19
Alternatives	3	-	-	-	-	-	5	-	-	-	-	1	15
Matrixes	PSD (Silicone and LDPE)	-	Ham Sausages	Cosmetics	-	Wine	Medical devices	Meter Dose	Perfumes	Wastewater	Honey	Fish and Squid	Indoor air
Run time	31 min	40 min	19 min	27 min	16 min	-	31 min	30 min	30 min	34 min	31 min	20 min	28 min
Percent Recovery (%)	89-130	-	87-108	80-120	-	70-120	78-127	86-108	89-102	84-108	88-112	70-117	-
Unique compounds	8	-	-	-	-	-	1	-	-	-	-	-	14
LOD (ng/mL)	0.05-0.25	0.042-0.64	0.00031-0.00061	0.10-0.01	0.016-0.002	0.06-10	0.25-0.07	0.0001-0.0042	>0.01	0.046-0.003	0.00028-0.00138	0.0005-0.005	-
LOD Calculation method	SD * t value(n-1,0.99)	SD * t value(n-1,0.99)	3σ	y intercept + (3*SD of y intercepts)	Etune and Atune	3σ	y intercept + (3*SD of y intercepts)	response=signal+3 or 10*SD	3σ	Not discussed	3σ	LCL	S/N=3

Real World Samples

Table 4. Data for environmental samples to evaluate the method. Table is separated out by sample type and further delineated by sample or matrix spike.

	All values in ng/g	DMP	DEP	DALP	DiBP	DBP	DMEP	BBP	DEHA	DBEP	DEHP	DnOP	DBzP	DuDP
Honey														
	Honey Plastic Package (LL) (n=4)	BDL	4.41	BDL	BDL	BDL	BDL	BDL	BDL	BDL	481	BDL	BDL	BDL
	Honey Plastic Bottle (LL) (n=5)	BDL	6.22	BDL	BDL	BDL	BDL	BDL	BDL	BDL	<RL	BDL	373	BDL
	Honey Glass (LL) (n=5)	BDL	89.3	BDL	BDL	BDL	BDL	BDL	127	960	<RL	BDL	473	BDL
	Honey Glass (n=2)	BDL	BDL	BDL	BDL	BDL	BDL	BDL	6.36	10.9	13.7	BDL	BDL	BDL
	Notardonato et al 2020 (n=47)	13	96		222	184.5					108	98		
Olive Oil														
	Olive Oil Plastic (n=3)	BDL	155.92	BDL	BDL	BDL	BDL	BDL	361	1556	805	BDL	BDL	BDL
	Olive Oil Glass (n=3)	BDL	BDL	494	BDL	BDL	2682	BDL	BDL	BDL	834	BDL	2181	BDL
	Cavaliere et al 2008 (n=8)					210		480			940			
Vegetable Oil														
	Vegetable Oil (n=3)	BDL	BDL	BDL	BDL	BDL	BDL	BDL	262	2872	<RL	BDL	1128	152
	Liu et al 2013 (n=5)					280					510			
Coconut Oil														
	Coconut Oil (n=3)	BDL	BDL	BDL	BDL	BDL	BDL	BDL	922	BDL	<RL	BDL	286	BDL
	Kiralan et al 2020 (n=2)					100					750			

	All Values in ng/g	DMP	DEP	DiBP	DnBP	DnHxP	BBP	DEHA	DEHP	DHtP	DEHiP	DnOP	DEHT	DPrHtP	DnNP	DDP	DuDP	TOTM	DtDP
Children																			
	Urban Children (n=3)	830	6083	1307	46.7	327	4713	194933	35967	3097	60000	BDL	240000	BDL	12100	570	6000	1287	10013
	Rural Children (n=3)	870	3607	2997	10793	BDL	4533	32000	102967	BDL	BDL	BDL	265667	BDL	26133	BDL	2937	1880	14220
	Hammel et al 2019 (n=77)	22.6	739	976	275		1059	1000	9010				8493						
Adults																			
	Urban Adult (n=5)	BDL	3668	1022	42.0	BDL	1660	8876	74860	BDL	BDL	904	48120	BDL	BDL	BDL	2628	542	8214
	Young et al 2019 (USA) (n=85)			1000	500				5000				23000						
	Young et al 2019 (UK) (n=42)			500	500				4000				16000						
	Young et al 2019 (China) (n=70)			5000	1000				28000				13000						
	Young et al 2019 (India) (n=54)		3000	1500	2000				36000				18000						
Ocupational																			
	Roofers (air) (n=4)	20.0	56.7	BDL	BDL	BDL	33.3	113	1190	BDL	BDL	BDL	210	BDL	66.7	BDL	16.7	197	403
	Roofers (WB) (n=3)	643	1523	957	BDL	127	287	2263	9220	440	BDL	BDL	1513	153	1060	110	2560	1863	3747
	Craig et al 2019 (air) (n=9)							5.00	251				81.9						
	Craig et al 2019 (WB) (n=9)							2.30	42.4				20.8						

- To evaluate the method, we analyzed real world samples such as honey, coconut oil, vegetable oil and olive oil. In addition, we measured phthalates in silicone wristbands from urban and rural children, pregnant people, and roofers.
- Roofers – detections for HMW compounds seen in building material.
  - Example: trimellitate, ditridecyl phthalate, didecyl phthalate, and diundecyl phthalate
- Wristbands worn for 48 hours by pregnant people had high hits for phthalates associated with personal care products.
  - High as 74860ng/g.
- Rural farm working children showed hits of 1000 – 200,000 ng/g.

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