

Supplementary Material

Assessing population exposure to phthalate plasticizers in thirteen Spanish cities through the analysis of wastewater

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Table S1. Details of the sampled wastewater treatment plants (WWTPs): name, population served, method used to estimate this population, locations/districts served, percentage of the main city population covered by the WWTP, sampling details and daily flow rates.

City ¹	Barcelona	Bilbao	Castellón	Guadalajara	Lleida	Madrid	Madrid	Móstoles	Palma de Mallorca	Palma de Mallorca	Reus	Santiago de Compostela	Tarragona	Toledo	Valencia	Valencia	Valencia
WWTP name (code)	Prat de Llobregat	Galindo	Castellón De La Plana	Guadalajara	Lleida	Madrid Centre	Madrid North	El Soto	Palma I	Palma II	Reus	Silvouta	Tarragona	Estiviel	Pinedo I (Valencia PI)	Pinedo II (Valencia PII)	Quart Benager (Valencia QB)
Population served by the WWTP	1163154	860237	171669	94755	143612	727176	227869	187281	406492	47961	115000	136500	142635	79793	527222	788242	162249
Method used to estimate the population served²	C (2017)	C (2016)	C (2015)	Average BOD Jan-April 2018	C (2017)	Average COD sampling period	Average BOD 2016 (with 60g BOD/d)	H x 3.5 (WWTP recommendation)	C (2017)	C (2017)	C (2017)	H x 2.5 (WWTP recommendation)	C (2017)	Average BOD April-May 2018	COD	COD	COD
Locations/districts served by the WWTP	Barcelona, Cervelló, Cornellà de Llobregat, Esplugues de Llobregat, Hospitalet de Llobregat, El Prat de Llobregat, Sant Boi de Llobregat, San Joan Despí, San Just Desvern	Abanto-Zierbena, Alonsotegi, Arrigorriaga, Barakaldo, Barrika, Basauri, Berango, Bilbao, Derio, Erandio, Etxebarri, Galdakao, Getxo, Leioa, Lezama, Loiu, Ortuella, Portugalete, Santurtzi, Sestao, Sondika, Sopelana, Trapagaran, Ugao-Miravalles, Urduliz,Zamudio, Zaratamo, Zeberio	Castellón De La Plana	Guadalajara	Lleida, Alpicat	Chamartín, Tetuán, Moncloa-Aravaca, Chamberí, Centro, Arganzuela, Retiro, Ciudad Lineal, Salamanca, Moratalaz, Puente de Vallecas	Chamartín, Tetuán, Moncloa-Aravaca, Fuencarral-El Pardo, Pozuelo de Alarcón, Las Rozas, Majadahonda	Móstoles, Alcorcón, Fuenlabrada (all served also by other WWTPs)	Palma beach, Sant Jordi, El Pí-lari, Son Sant Joan airport, part of Palma city	Main part of Palma city, Son Castelló, Can Valero and Son Rosinyol Industrial States, Marratxí, Esporles and Bunyola	Reus, Castellvell, Almoester	Santiago de Compostela	Tarragona, La Canonja, els Pallaresos	Toledo	Valencia	Albal, Alcasser, Alfafar, Benetusser, Beniparrell, Burjassot, Catarroja, Llocnou de la Corona, Massanassa, Mislata, Paiporta, Paterna, Picanya, Picassent, Sedaví, Silla, Torrent, Valencia,	Alaquàs, Aldaia, Manises, Mislata, Quart de Poblet, Xirivella
% of main city population covered by WWTP(s)³	35%	100%	100%	100%	100%	30%		90%	100%	100%	100%	100%	100%	100%	100%	100%	100%
Location of autosampler	Mechanical bar screens	After coarse screens and pumping	Before fine screen	Before fine screen	Before fine screen	After sieving	After fine screen	After fine screen	After fine screen	After fine screen	After fine screen	After fine screen	Before fine screen	After sieving	After fine screen	After fine screen	After fine screen
Autosampler refrigerated?	Yes	No	No	No	No	Yes	Yes	Yes	No	No	No	No	No	No	Yes	Yes	No
Time of beginning of the sampling	9:00	8:00	8:30	10:00	6:00	8:00	8:00	8:00	10:00	10:00	20:00	9:00	8:00-9:00	8:00	8:00	8:00	8:00
Sampling mode⁴	T (50 mL/10 min)	T (100 mL/60 min)	T (100 mL/15 min)	T (200 mL/60 min)	T (200 mL/60 min)	T (400 mL/30 min)	T (100 mL/60 min)	T (100 mL/60 min)	T (100 mL/15 min)	T (100 mL/15 min)	F	T (150 mL/10 min)	T (450 mL/60 min)	T (100 mL/15 min)	T (100 mL/60 min)	T (100 mL/60 min)	F

City ¹	Barcelona	Bilbao	Castellón	Guadalajara	Lleida	Madrid	Madrid	Móstoles	Palma de Mallorca	Palma de Mallorca	Reus	Santiago de Compostela	Tarragona	Toledo	Valencia	Valencia	Valencia
Sampling date⁵ - 1	2018.03.19	2018.04.23	2018.04.16	2018.05.07	2018.03.12	2018.05.21	2018.06.25	2018.05.21	2018.04.16	2018.04.23	2018.04.17	2018.03.19	2018.04.17	2018.04.23	2018.04.16	2018.04.16	2018.04.16
Sampling date - 2	2018.03.20	2018.04.17	2018.04.17	2018.05.08	2018.03.13	2018.05.22	2018.06.26	2018.05.22	2018.04.10	2018.04.24	2018.04.18	2018.03.13	2018.04.18	2018.04.17	2018.04.10	2018.04.10	2018.04.10
Sampling date - 3	2018.03.14	2018.04.18	2018.04.11	2018.05.02	2018.03.07	2018.05.16	2018.06.20	2018.05.23	2018.04.11	2018.04.18	2018.04.19	2018.03.14	2018.04.19	2018.04.18	2018.04.11	2018.04.11	2018.04.11
Sampling date - 4	2018.03.15	2018.04.19	2018.04.12	2018.05.03	2018.03.08	2018.05.17	2018.06.21	2018.05.17	2018.04.12	2018.04.19	2018.04.20	2018.03.15	2018.04.20	2018.04.19	2018.04.12	2018.04.12	2018.04.12
Flow (m³/day) - 1	253120	312461	29597	29849	34500	107309	47045	29649	44849	50180	17980	99917	25713	15450	118934	272420	38055
Flow (m³/day) - 2	400037	287205	33363	30342	44720	109814	46726	25420	45739	48840	17720	102672	24426	14935	131003	213799	36143
Flow (m³/day) - 3	255858	268352	46317	30246	40700	108173	41622	27738	49850	51905	18100	111999	24673	13713	136262	248374	29789
Flow (m³/day) - 4	244204	256858	37625	29853	41410	112666	42978	26379	44579	50979	17742	122643	23748	13627	121017	205501	26932

¹ Name of the main city served by the WWTP (some WWTPs receive wastewater from other towns included in the capital metropolitan area)

² C: census (year); BOD: biochemical oxygen demand; COD: chemical oxygen demand; H: number of homes connected to the sewage system

³ WWTPs serving parts of the same main city were considered all together for this calculation

⁴ T: time proportional (volume sampled/frequency of sampling); F: flow proportional

⁵ Sampling date/day 1-4: Mo-Th for all cities but Reus and Tarragona (Tu-Fri)

Table S2. Analytes and deuterated analogs used as surrogate/internal standards (IS), retention times (RT), cone voltage (CV) and collision energy (CE) values, quantifier (Q) and qualifier (q) transitions, ratio between them, method detection limits (MDL) and method quantification limits (MQL).

Compounds	[M-H] ⁻ Formula	RT (min)	IS	Precursor m/z	CV (V)	Quantifier (Q)		qualifier (q)		Ratio q/Q	MDL (ng/L)	MQL (ng/L)
						m/z	CE	m/z	CE			
MMP	C ₉ H ₇ O ₄	3.3	MMP-D ₄	179	27	107	8	77	17	1.33	10	32
MEP	C ₁₀ H ₉ O ₄	3.8	MBP-D ₄	193	22	77	15	121	10	1.28	1.3	4.2
MiBP	C ₁₂ H ₁₃ O ₄	5.9	MBP-D ₄	221	27	77	16	134	12	0.81	1.5	4.9
MnBP	C ₁₂ H ₁₃ O ₄	6.2	MBP-D ₄	221	23	77	17	177	9	0.38	1.1	3.5
MBzP	C ₁₅ H ₁₁ O ₄	7.0	MBP-D ₄	255	27	77	20	183	10	0.86	0.69	2.3
MEOHP	C ₁₆ H ₁₉ O ₅	6.4	MEHHP-D ₄	291	27	143	12	121	16	0.84	2.9	9.5
MEHHP	C ₁₆ H ₂₁ O ₅	6.2	MEHHP-D ₄	293	32	145	13	121	20	1.03	4.0	13
MMP-D₄	C ₉ H ₃ D ₄ O ₄	3.3	—	183	27	111	8	—	—	—	—	—
MBP-D₄	C ₁₂ H ₉ D ₄ O ₄	6.1	—	225	23	81	17	—	—	—	—	—
MEHHP-D₄	C ₁₆ H ₁₇ D ₄ O ₅	6.1	—	297	32	125	20	—	—	—	—	—

Table S3. Oral Reference Doses (RfD) and Tolerable Daily Intakes (TDI) set by the U.S. Environmental Protection Agency (EPA) and the European Food Safety Authority (EFSA) for the five phthalate esters considered for risk assessment. Safe Reference Values (SRV) calculated from RfD and TDI considering average body weights of 70.8 kg for adults and 11.5 kg for toddlers (Walpole et al., 2012, WHO et al., 2006). Toxic Equivalents (Tox EqPAE), i.e. factors expressing the individual toxicity of a single phthalate relative to the most toxic derivative, calculated based on the RfDs provided by the EPA.

Phthalate esters	RfD-EPA ($\mu\text{g}/(\text{kg}\cdot\text{day})$)	TDI-EFSA ($\mu\text{g}/(\text{kg}\cdot\text{day})$)	SRV ($\mu\text{g}/(\text{day}\cdot\text{adult})$) 70.8 kg adult		SRV ($\mu\text{g}/(\text{day}\cdot\text{toddler})$) 11.5 kg toddler		Tox EqPAE
			RfD-EPA	TDI-EFSA	RfD-EPA	TDI-EFSA	
DEP	800	—	56640	—	9200	—	0.025
DiBP	100	10	7080	708	1150	115	0.2
DnBP	100	10	7080	708	1150	115	0.2
BzBP	200	500	14160	35400	2300	5750	0.1
DEHP	20	50	1416	3540	230	575	1

Walpole, S.C., Prieto-Merino, D., Edwards, P., Cleland, J., Stevens, G., Roberts, I., 2012. The weight of nations: an estimation of adult human biomass. *BMC Public Health*, 12, 439-444.

World Health Organization (WHO 2006). WHO Child Growth Standards Length/height-for-age, weight-for-age, weight-for-length, weight-for-height and body mass index-for-age: methods and development.

Table S4. Population-normalized metabolite loads (in $\mu\text{g}/(\text{day}\cdot\text{inh})$) for every location and day. Simple average, standard deviation (SD), median, 5th and 95th percentiles.

Compounds -day ¹	Barcelona	Bilbao	Castellón	Guadalajara	Lleida	Madrid Centre	Madrid North	Móstoles	Reus	Santiago de Compostela	Tarragona	Toledo	Valencia PI	Valencia PII	Valencia QB	Palma de Mallorca ³	Average ($\mu\text{g}/(\text{day}\cdot\text{inh})$)	SD ($\mu\text{g}/(\text{day}\cdot\text{inh})$)	Median ($\mu\text{g}/(\text{day}\cdot\text{inh})$)	5 th percentile	95 th percentile
MMP - 1	100	1013	40	117	171	82	153	64	58	203	73	83	66	69	55	225	175	220	91	42	717
MMP - 2	224	784	170	44	238	76	282	79	48	54	42	44	69	134	269	151					
MMP - 3	295	867	76	235	119	55	92	61	53	86	35	59	58	90	98	333					
MMP - 4	197	1137	294	188	182	35	223	172	55	74	59	42	48	159	121	326					
MEP - 1	1041	585	139	953	527	514	699	611	534	742	192	138	533	765	304	1287	660	439	536	138	1465
MEP - 2	616	776	394	787	565	473	539	221	407	367	356	87	1011	1476	1101	686					
MEP - 3	2087	455	284	1015	439	432	568	366	418	275	338	115	895	1062	1139	1403					
MEP - 4	1039	658	857	1095	381	254	427	367	275	355	288	101	751	1553	2108	1044					
MiBP - 1	70	118	23	106	83	52	114	122	29	1445	6.9	25	51	42	22	79	97	180	69	11	188
MiBP - 2	96	108	52	90	128	70	141	12	11	295	61	14	119	112	103	48					
MiBP - 3	148	72	43	121	69	38	169	68	11	60	9.5	12	87	110	61	70					
MiBP - 4	78	97	68	218	39	41	67	51	6.8	192	15	15	52	109	101	54					
MnBP - 1	32	89	33	70	46	35	132	135	6.2	635	3.4	11	22	20	6.9	65	63	82	47	3.1	131
MnBP - 2	63	74	44	82	68	39	67	3.6	3.1	153	46	7.1	124	120	120	48					
MnBP - 3	66	34	66	88	44	29	41	57	3.2	73	4.2	4.9	70	88	44	70					
MnBP - 4	55	90	112	83	38	20	24	27	1.1	117	2.2	2.9	46	105	83	51					
MBzP - 1 ²	0.00-0.15	0.00-0.25	0.00-0.12	9.2	0.00-0.17	0.00-0.10	0.00-0.14	7.0	0.00-0.11	6.9	0.00-0.12	0.00-0.13	0.00-0.16	0.00-0.24	0.00-0.16	1.7-1.8	1.6-1.7	2.5-2.4	–	–	–
MBzP - 2 ²	0.00-0.24	3.7	0.00-0.13	3.1	0.00-0.21	2.4	0.00-0.14	0.00-0.094	0.00-0.11	7.4	2.6	0.00-0.13	3.1	0.00-0.19	0.00-0.15	1.9-2.0					
MBzP - 3 ²	0.00-0.15	0.00-0.22	0.00-0.19	5.3	0.00-0.20	0.00-0.10	0.00-0.13	2.1	0.00-0.11	8.9	0.00-0.12	0.00-0.12	1.8	2.3	0.00-0.13	3.2-3.3					
MBzP - 4 ²	0.00-0.14	0.00-0.21	4.3	3.4	4.1	0.00-0.11	0.00-0.13	0.00-0.10	0.00-0.11	6.0	0.00-0.11	0.00-0.12	0.00-0.16	2.6	3.8	5.1-5.1					
MEOHP - 1 ²	0.00-0.63	5.0	0.50-1.6	9.6	0.00-0.70	0.00-0.43	0.00-0.60	20	0.00-0.45	2.1-7.0	0.00-0.52	0.00-0.56	0.00-0.65	0.00-1.0	0.00-0.68	4.4-4.7	3.1-3.8	4.9-4.8	–	–	–
MEOHP - 2 ²	0.00-1.0	0.00-0.97	6.7	10	0.00-0.90	0.00-0.44	0.00-0.59	0.00-0.39	0.00-0.45	2.2-7.1	0.00-0.50	0.00-0.54	16	6.8	20	3.0-3.3					
MEOHP - 3 ²	4.6	0.00-0.90	4.1	3.3	0.00-0.82	0.00-0.43	0.00-0.53	6.6	0.00-0.46	2.4-7.8	0.00-0.50	0.00-0.50	8.7	11	0.00-0.53	7.1-7.4					
MEOHP - 4 ²	2.2	3.3	13	0.00-0.91	0.00-0.84	0.00-0.45	0.00-0.55	0.00-0.41	0.00-0.45	2.6-8.5	0.00-0.48	0.00-0.50	4.1	8.9	0.00-0.48	9.6-9.9					
MEHHP - 1 ²	0.00-0.87	6.7	0.00-0.69	8.9	0.00-0.96	0.00-0.59	0.00-0.82	27	0.00-0.62	2.9-9.7	0.00-0.72	0.00-0.77	0.00-0.90	0.00-1.4	0.00-0.94	0.00-0.83	3.0-4.0	5.9-5.9	–	–	–
MEHHP - 2 ²	0.00-1.4	0.00-1.3	9.0	11	0.00-1.2	0.00-0.60	0.00-0.82	0.00-0.54	0.00-0.61	3.0-10	6.9	0.00-0.75	13	7.1	0.00-0.89	0.00-0.83					
MEHHP - 3 ²	0.00-0.88	0.00-1.2	0.00-1.1	0.00-1.3	0.00-1.1	0.00-0.59	0.00-0.73	10.6	0.00-0.63	3.3-11	0.00-0.69	0.00-0.69	24	21	0.00-0.73	0.00-0.89					
MEHHP - 4 ²	0.00-0.84	0.00-1.2	9.5	7.3	0.00-1.2	0.00-0.62	0.00-0.75	0.00-0.56	0.00-0.62	3.6-12	0.00-0.66	0.00-0.68	4.8	11	0.00-0.66	0.00-0.84					

¹ Day 1-4: Mo-Th for all cities but Reus and Tarragona (Tu-Fri)

² Two values provided in those cases where concentrations were <MDL or fell between the MDL and the MQL: load obtained from the underestimating scenario - load obtained from the overestimating scenario. Median and percentiles were not calculated due to detection frequency (% samples > MDL) < 50%

³ WWTPs at Palma de Mallorca (two): combined and considered as a single one

Table S5. Regression coefficients (R), sample size (n) and p-values of the Pearson pairwise correlation study performed between 4-days average loads for MMP, MEP, MiBP, and MnBP.

		MMP	MEP	MiBP	MnBP
MMP	R		0.1315	0.0311	0.0881
	n		16	16	16
	p-value		0.6273	0.9088	0.7457
MEP	R	0.1315		0.0236	0.1513
	n	16		16	16
	p-value	0.6273		0.9308	0.5758
MiBP	R	0.0311	0.0236		0.9658
	n	16	16		16
	p-value	0.9088	0.9308		<0.0001
MnBP	R	0.0881	0.1513	0.9658	
	n	16	16	16	
	p-value	0.7457	0.5758	<0.0001	

Table S6. Estimated metabolite concentrations in urine (in ng/mL). Average values of 4 days in the different locations assessed, simple average for all locations and all days, standard deviation (SD), median, 5th and 95th percentiles.

Compounds	Barcelona	Bilbao	Castellón	Guadalajara	Lleida	Madrid Centre	Madrid North	Móstoles	Reus	Santiago de Compostela	Tarragona	Toledo	Valencia PI	Valencia PII	Valencia QB	Palma de Mallorca	Average (µg/(day·inh))	SD (µg/(day·inh))	Median (µg/(day·inh))	5 th percentile	95 th percentile
MMP	130	608	92	93	113	40	119	60	34	66	33	36	38	72	86	165	112	141	58	27	459
MEP	762	396	267	613	305	267	356	249	260	277	187	70	508	773	741	704	421	280	342	88	933
MiBP	62	63	30	85	51	32	78	40	9.2	317	15	10	49	59	46	40	62	114	44	7.0	120
MnBP	34	46	41	52	31	20	42	36	2.2	156	8.9	4.2	42	53	40	37	40	52	30	2.0	83
MBzP ¹	0.00-0.11	0.59-0.69	0.69-0.76	3.3	0.65-0.74	0.39-0.44	0.00-0.09	1.5	0.00-0.07	4.7	0.42-0.48	0.00-0.08	0.78-0.83	0.78-0.85	0.61-0.68	1.9	1.0-1.1	1.6-1.5	–	–	–
MEOHP ¹	1.1-1.3	1.3-1.6	3.9-4.1	3.7-3.8	0.00-0.52	0.00-0.28	0.00-0.36	4.3-4.4	0.00-0.29	1.5-4.8	0.00-0.32	0.00-0.33	4.5-4.6	4.2-4.3	3.2-3.5	3.8-4.0	2.0-2.4	3.1-3.0	–	–	–
MEHHP ¹	0.00-0.63	1.1-1.7	2.9-3.2	4.3-4.5	0.00-0.71	0.00-0.38	0.00-0.50	6.0-6.1	0.00-0.40	2.0-6.8	1.1-1.4	0.00-0.46	6.6-6.8	6.2-6.4	0.00-0.51	0.00-0.54	1.9-2.6	3.8-3.7	–	–	–

¹ Two values provided in those cases where concentrations were <MDL or fell between the MDL and the MQL: value from the underestimating scenario - value from the overestimating scenario. Median and percentiles were not calculated due to detection frequency < 50%