Characterization of chemical mixtures migrating from plastic-based materials used in reusable water bottles for children

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Final Report

A collaboration between Tegengif and Dr Stéphane Bayen (Principal Investigator) at McGill University.

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Contributors / roles of the collaborators:

McGill University: Dr Stéphane Bayen: principal investigator, designed and piloted the experiment in collaboration with Tegengif, compiled and interpreted the data, reporting. Dr. **Lei Tian:** postdoctoral fellow: conducted the migration tests, conducted the LC-MS analysis, compiled and interpreted data. **Aining Li:** research assistant, collected information on the samples (pictures, dimensions), conducted the 40 rounds of dishwashing. Dr. **Lan Liu:** research associate, calibrated the LC-MS analysis.

Tegengif: **Annelies den Boer:** collaborated in the design of the experiment and the interpretation of the data, reporting. **Dr. Ewa Skoczynska:** sample collection; FTIR analysis; collaborated in the design of the experiment and the interpretation of the data, reporting.

Regular exchanges between the partners took place over the duration of the project, including a kick-off meeting and three presentations of the results.

1. RESEARCH OBJECTIVES

The **objective** of this study is to characterize the chemical mixtures migrating from plastic-based materials used in reusable water bottles for children.

More specifically, **the objectives of this project were**: (i) obtain reusable water bottles for children purchased and characterize their composition (FTIR), (ii) simulate chemical migration under from bottles before and after cycles of dishwashing, (iii) quantify chemical migration for pre-selected targeted compounds measured in the food simulants, (iv) identify molecular features significantly increasing or decreasing in bottles washed in a dishwasher using non-targeted analysis.

2. METHODOLOGY

2.1 Sampling

A total of 195 drinking bottles (39 sample types, 5 replicates per type) were obtained in 2023 from stores in five European countries. 156 samples were shipped to Montreal for analysis at McGill. A fifth replicate was kept for FTIR analysis in the Netherlands.

Pictures were taken for each type of bottles, and their dimensions were obtained. Bottles were labelled prior to testing.

Table 1: List of bottle samples and initial information.

Code on the packing	Material on bottle	Color	Transparency	Diameter (mm)	Additional Notes
LV 01	PCTG (7)	clear	yes	67	
LV 02	HLPE	deep blue	no	66	
LV 03		clear	yes	64	
LV 04	PP	pink	yes	66	
LV 05	other (7)	clear	yes	64	
LV 07	PP, HDPE	clear	semitransparent	72	
LV 08		blue	no	69	
Plastic CZ 01	PP	grey	no	73	
Plastic CZ 02	PP	pink	no	73	
Plastic CZ 03		pink	no	74	
Plastic CZ 04		blue	yes	66	
Plastic CZ 05	PP	clear	semitransparent	74	
Plastic CZ 06		blue	no	64	unregular shape narrow in the middle (d=53)
Plastic CZ 07	（7）	blue	yes	67	
Plastic CZ 08	PE	black	no	70	
Plastic DK 01		blue	no	64	unregular shape narrow in the middle (d=53)
Plastic DK 02	other (7)	clear	yes	68	
Plastic DK 03	PE-LD (4)	pink	no	69	
Plastic DK 04	（7）	deep blue	yes	66	
Plastic DK 05		clear	yes	66	
Plastic DK 06		turquoise	yes	66,60	unregular shape (ellipse base) narrow in the middle (d=52)
Plastic DK 07	PE-LD (4)	white	no	64	
Plastic DK 08	PE	red and clear	semitransparent	69	
Plastic ESP 01	PE	mustard yellow	no	67	
Plastic ESP 02	PP	blue	yes	72	
Plastic ESP 03	（7）	clear	yes	70	
Plastic ESP 04	PCTG (7）	clear	yes	64	
Plastic ESP 05#		green	semitransparent	64, 58	unregular shape (ellipse base)
Plastic ESP 06		green	no	70	
Plastic ESP 07		clear	yes	70	
Plastic ESP 08	PP	deep blue	no	70	
Plastic NL 01	PP	margenta	no	57	、
Plastic NL 02	PP	pink	yes	66	unregular shape (ellipse base) narrow in the middle (d=60)
Plastic NL 03	PET	clear	yes	62, 59	
Plastic NL 04		yellow	no	68	
Plastic NL 05		clear	yes	64	
Plastic NL 06		pink	yes	63	
Plastic NL 07	PET	clear	yes	50	
Plastic NL 08	PE	green	no	69	

2.2 Dishwashing

The leaching of chemicals was compared for the two washed bottles and their unwashed equivalents (2 replicates). Chemical migration was assessed for the main body of the bottles, so the caps and associated parts (e.g. suction tubes) were removed and not tested.

For each bottle sample type, 2 replicates were washed under normal conditions (normal, not eco-program washes at temperatures around 65°-70°C) in a countertop dishwasher (Figure 1) for 20 cycles to simulate use. A mass of 60±1 g of detergent (Finish® Gel Liquid, Lemon Scent, Reckitt, US - Figure 1) was added in the detergent compartment of the dishwasher.

One replicate of each of the 39 types of bottles were washed together for 20 consecutives cycles of dishwashing. This was repeated for the second replicates in separate 20 consecutive cycles. Bottles positioned at the edge of the tray in the first round were positioned in the center for the second round, and vice versa. Four amber glass jars were washed under the same conditions and were analyzed as control 'washed blank' samples, and compared to another four glass jars not washed in the dishwasher.

Figure 1: Positioning of the bottles in the dishwasher (left) and detergent (right)

2.3 Chemical migration studies

Migration was assessed using food simulants according to guidelines [1], considering drinking bottles may be in contact with juices. Therefore, migration was tested for each item (direct filling)

with simulant B (acetic acid 3%; for hydrophilic food with pH<4.5) [1, 2] at 22°C (±1°C). Aluminum foil was placed on the bottle and the cap was gently positioned to protect the simulant from external contamination. One aliquot (900 µL) was collected in a LC glass amber vial in each bottle after 24 hours and after 10 days. 100 µL of methanol was added in the LC vial (final volume of sample is 1 mL). Samples were stored at -20°C in the dark until analysis. The surface area (dm^2) exposed to the food simulant was assessed based on the height of solvent in the bottles and the dimensions of the bottles (diameter).

2.4 LC-QTOF-MS analysis of food simulants

Food simulants were analyzed on a LC-QTOF-MS system (Agilent 1290 Infinity LC coupled to an Agilent 6545 QTOF mass spectrometer) using separation on a reverse-phase column (Poroshell 120 Phenyl Hexyl from Agilent Technologies). The QTOF (ESI, *m/z* 50-1,700) was operated in both positive and negative polarity modes to investigate the widest range of migrants. Samples were analyzed together with calibration standards (36 targets, 1 to 100 ng/mL, see section 2.5), procedural blanks (4×2), solvent blanks (5) and instrument pooled QCs and reinjections (to validate the mass error, retention time shift, and relative response performances of the instrument). The samples were injected in a random order. Water (mobile phase A) and methanol (mobile B) both in LC-MS grade are purchased from Fisher Scientific (Hampton, USA). 0.1% formic acid (positive polarity) and 5 mM ammonium acetate (negative polarity) are added in both mobile phases under different polarity analysis. The LC gradient starts from 95% A for 0.5 min and the organic mobile phase B increased linearly to 100% from 0.5 to 3 min, then mobile phase B maintained at 100% for 9 minutes and at 12.1 min the gradient came back to the initial condition (5% B). The flow rate was 0.2 mL/min and injection volume was 20 µL. Nitrogen was used as the drying gas (325°C). The gas flow was 5 L/min. Samples were run in the Full scan mode (no collision energy) with a fragmentor voltage of 100 V.

In total, this work resulted in about 800 injections (312 each in ESI+ and ESI- modes, respectively and + 30% for calibration standards and QC reinjections), so about160 hours of LC-QTOF-MS run time.

2.5 Data analysis: Targeted Analysis

The following 36 target analytes (see Table 2 below) were quantified using matrix-matched calibrations in the food simulants.

Table 2: List of target analytes.

Target analytes	**CAS number**
Diethyl phthalate (DEP)	84-66-2
Benzyl butyl phthalate (BBzP)	85-68-7
Di-n-octyl phthalate (DnOP)	117-84-0
Bis(2-ethylhexyl) phthalate (DEHP)	117-81-7
Bis(2-propylheptyl) phthalate (DPHP)	53306-54-0
Dipentyl phthalate (DPP)	131-18-0
Diheptyl phthalate (DHpP)	3648-21-3
Diisononyl phthalate (DiNP)	28553-12-0
Bis(2-ethylhexyl) adipate (DEHA)	103-23-1
Diisobutyl phthalate (DiBP)	84-69-5
Dibutyl phthalate (DBP)	84-74-2
Isodecyl acrylate	1330-61-6
2-Ethylhexanoic acid	149-57-5
2,4,7,9-Tetramethyl-5-decyne-4,7-diol (Surfynol 104)	126-86-3
Mono-2-ethylhexyl phthalate (MEHP)	4376-20-9
Decyl octyl phthalate	119-07-3
Dibutyl adipate	124-04-9
Dibutyl sebacate	109-43-3
DINCH	331673-15-5
DIDA ((Diisodecyl adipate))	27178-16-1
4-Dodecylbenzesulfonic acid	121-65-3
Diisooctyl adipate	1330-86-5
Tris(4-nonylphenyl) phosphite	3050-88-2
Resorcinol	108-46-3
Diisobutyl adipate	141-04-8
Bis(4-methyl-2-pentyl) phthalate	146-50-9
Diisodecyl phthalate	26761-40-0
Didecyl phthalate	84-77-5
Irganox 1076	2082-79-3
Irganox 1030	1709-70-2
Bis(2-ethylhexyl) terephthalate	6422-86-2
Triphenyl phosphate	115-86-6
Triphenyl phosphite	101-02-0
Acetyl tributyl citrate	77-90-7
Tributyl phosphate	126-73-8
4-nonylphenol branched	84852-15-3

For the targeted analysis, data were analyzed using Agilent MassHunter Workstation Software - *Quantitative Analysis* (version 10.0). The quantification method was built using the calibration standards (6 points ranging from 1 to 100 ng/mL) with a mass extraction window of 20 ppm and retention time (RT) window of 0.1 min. The method detection limit (MDL) and the limit of quantification (LOQ) were calculated as three and ten times of standard deviation of the blank signals in the food simulant matrix, respectively.

The concentrations of the targeted compound measured in the food simulants were converted into migration expressed in $\mu g/dm^2$ for each bottle using the surface of contact estimated from the dimensions of the bottles.

2.6 Data analysis: Nontargeted Screening

Data files obtained for each sample (LC-QTOF-MS analysis in full scan mode; section 2.4) were re-investigated to detect other leachables. Non-targeted screening versus available lists of plastic-related chemicals and other computational tools were used to identify some of those leachables.

2.6.1 Molecular feature extraction

An initial list of molecular features (~compounds) was obtained through treatment of the LC-QTOF-MS data (including samples, procedural blanks and QCs, see section 2.5) for each ionization mode using Agilent MassHunter Profinder (version 10.1). This first step is called molecular feature extraction (i.e., finding the compounds in the large data set using data analysis tools), and the detailed parameters for the molecular feature extraction are provided in Table 3.

Table 3. Parameters for molecular feature extraction (Agilent MassHunter Profinder, v.10.1)

Parameters	**ESI+**	**ESI-**
RT window	0.1 min	0.1 min
mass window	20 ppm	20 ppm
Peak filter (counts)	5,000	3,000
Limit assigned charge states	1-3	1-2
Post-processing peak height filter (counts)	10,000	6,000
Minimum filter matches in at least one sample group	30%	30%
Minimum find by ion RT score	80	80
Peak integration threshold (peak height filter in >30% of the files)	2500	2500

2.6.2 List of relevant molecular features for individual bottle samples

After molecular feature extraction (section 2.6.1), the intensities of each individual molecular feature were compared between the samples and the procedural blanks using Excel to establish a list of relevant molecular features for individual bottle samples (list RA and RB). Features detected in actual simulants with an intensity 2-fold greater than a threshold signal set from the corresponding procedural blanks (average of 4 blank replicates plus 3 × standard deviation) were retained for further interpretation as recommended in the literature for non-targeted analysis [3]. The predicted formulae for each of the molecular feature was compared with two lists of plastic-related chemicals, the Agilent EnL PCDL database (1076 compounds) and an in-house list of additional key leachables from re-usable bottles (24 compounds) reported in the literature by Tisler and Christensen [3]. Features with the formula matching any of the lists were further investigated in terms of structural identification.

2.6.3 List of relevant molecular features for specific groups of bottles

A second separate data analysis was performed to establish lists of relevant molecular features relevant to specific groups of bottles. In this case, molecular features (from section 2.6.1) were imported to Agilent MassHunter Mass Profiler Professional (MPP, version 15.1) without any further normalization. The mean intensities in the sample groups and the blanks were compared. Molecular features in a specific group with a mean intensity 3× than the blanks were recorded and further investigated. Samples were also grouped according to their material type (3 groups: “PE”, “PP” and “others”) or their washing status (“unwashed” and “washed”). Features with a relatively higher intensity in any of those groups compared to the blanks were retained and further investigated in terms of structural identification.

2.6.4 Structural identification

The next steps of the structural elucidation included a reinjection of selected samples in LC-QTOF-MS in the targeted MS/MS mode and structure prediction using computational tools. Each candidate feature was manually inspected using Agilent Software - MassHunter *Qualitative Analysis* (version 10.0) to (i) inspect peak shape, and (ii) assess the intensity of the raw intensity among the food simulants. Targeted-MS/MS was achievable only for features with a raw intensity greater than 10^5 in one of the food simulants.

Reference standards of the pure compounds were purchased if the MS/MS fragments of selected features matched the suggested identity in library (2.6.1) or the SIRIUS software (Jena University, Germany. https://bio.informatik.uni-jena.de/software/sirius/) gave a predicted structure based on the MS/MS information of selected feature with a score over 60% (2.6.2).

2.7 Migration rate estimation

The concentration of identified compound in simulant was estimated versus a single point external calibration standard (based on the peak area of the characteristic ion).

The resulting estimated migration rates (per 1 or 10 days) were calculated based on the respective estimated concentration divided by the surface area of contact.

3. RESULTS

3.1 FTIR analyses and sample groups

The polymer type for each bottle was confirmed using FTIR analysis. All the FTIR results matched the label of the bottles, and a distribution is presented (Figure 2).

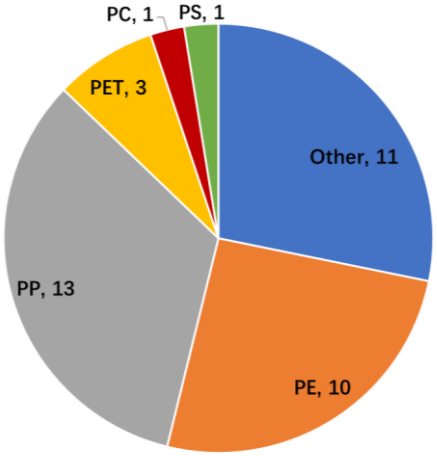

Figure 2: Distribution of materials among the bottle samples based on FTIR analyses. PE: polyethylene. PP: polypropylene. PET: polyethylene terephthalate. PS: polystyrene. PC: polycarbonate. Unspecific polyester was associated to the "Other" category.

It is important to note that one bottle (*Plastic ESP-08*) was labelled "not ideal for dishwasher". Some other bottles were found not dishwasher safe. For example, the shape of bottles (made of PET – Figure 3) labelled as *Plastic NL-03* and *Plastic NL-07* changed after a few cycles of dishwashing.

Figure 3: Example of PET bottle after 20 cycles of dishwasher (left) compared to the same type of bottle initially (right).

3.2 Chemical migration – targeted analysis

3.2.1 QA/QC

The overall method performances of the analytical method were assessed for all the targeted compounds (Table 4). Calibration standards were prepared at six different concentrations (ranging from 1 to 100 ng/mL). The linearity of the instrument response was assessed from these standards for each analyte and instrument response for calibration standards was linear ($R^2 \geq 0.98$). LOQs were below 6 ng/mL for 32 targets, and below 1 ng/mL for 15 targets. The precision of method was assessed by the analysis of three replicates of the same sample injected in different days and the precision (RSD) was lower than 10% for the whole batch.

Table 4: Limit of quantification (LOQ) expressed as a concentration of food simulant injected (ng/mL; injection volume: 20 µL) for each of the individual targeted compounds.

Positive polarity					
Target analyte	**Formula**	**m/z $[M+H]^+$**	**RT (min)**	**MDL (ng/mL)**	**LOQ (ng/mL)**
Diethyl phthalate (DEP)	C12H14O4	223.0970	6.20	0.6	1.9
Dibutyl adipate (DBA)	C14H26O4	259.1909	6.78	0.2	0.7
Tributyl phosphate (TBPP)	C12H27O4P	267.1725	6.54	0.07	0.2
Diisobutyl phthalate (DiBP)	C16H22O4	279.1597	6.69	0.2	0.6
Dipentyl phthalate (DPP)	C18H26O4	307.1910	7.12	0.7	2.4
Benzyl butyl phthalate (BBzP)	C19H20O4	313.1440	6.94	0.3	0.9
Dibutyl sebacate (DBSA)	C18H34O4	315.2535	7.27	0.2	0.6
Triphenyl phosphate (TPPP)	C18H15O4P	327.0786	6.64	0.003	0.01
Bis(4-methyl-2-pentyl) phthalate (BMPP)	C20H30O4	335.2222	7.34	0.03	0.1
Diheptyl phthalate (DHpP)	C22H34O4	363.2536	7.85	1.6	5.3
Bis(2-ethylhexyl) adipate (DEHA)	C22H42O4	371.3162	7.98	1.3	4.3
Di-n-octyl phthalate (DnOP)	C24H38O4	391.2849	8.27	0.3	0.9
decyl octyl phthalate (DOP)	C26H42O4	419.3167	8.75	1.0	3.3
Diisononyl phthalate (DiNP)	C26H42O4	419.3175	8.52	1.0	3.3
Diisononyl hexahydrophthalate (DINCH)	C26H48O4	425.3630	9.19	0.2	0.5
Diisodecyl adipate (DIDA)	C26H50O4	427.3787	8.67	2.0	6.7
Bis(2-propylheptyl) phthalate (DPHP)	C28H46O4	447.3471	8.87	0.3	0.9
Diisodecyl phthalate (DiDP)	C28H46O4	447.3474	9.20	0.3	0.9

2,4,7,9-Tetramethyl-5-decyne-4,7-diol (Surfynol 104)	C14H26O2	227.2006	6.11	9.2	30
Isodecyl acrylate	C13H24O2	213.1845	6.51	0.6	2.0
Resorcinol	C6H6O2	111.0446	6.69	1.5	4.8
Diisobutyl adipate (DiBA)	C14H26O4	259.1909	6.66	0.4	1.2
Dibutyl phthalate (DBP)	C16H22O4	279.1597	6.82	0.2	0.7
Acetyl tributyl citrate (ATC)	C20H34O8	403.2331	6.98	0.03	0.1
Triphenyl phosphite (TPPi)	C18H15O3P	311.0837	7.10	5.4	17.8
Diisooctyl adipate (DiOA)	C22H42O4	371.3161	7.98	1.0	3.3
Bis(2-ethylhexyl) phthalate (DEHP)	C24H38O4	391.2848	8.02	0.1	0.3
Didecyl phthalate (DDP)	C28H46O4	447.3474	9.24	0.6	1.9
Tris(4-nonylphenyl) phosphite (T4NPPi)	C45H69O3P	689.5062	8.56	1515	5000 [a]
bis(2-ethylhexyl) terephthalate (DEHtP)	C24H38O4	391.2848	8.02	0.4	1.5
mono-2-ethylhexyl phthalate (MEHP)	C16H22O4	279.1597	6.52	0.5	1.6
Negative polarity					
Target analyte	**Formula**	**m/z [M-H]$^-$**	**RT (min)**	**MDL (ng/mL)**	**LOQ (ng/mL)**
2-ethylhexanoic acid (2-EHA)	C8H16O2	143.1080	5.72	1.8	6.0
4-nonylphenol branched	C15H24O	219.1749	6.73	7.6	25
4-Dodecylbenzesulfonic acid	C18H30O3S	325.1870	6.33	0.2	0.5
Irganox 1076	C35H62O3	529.4620	9.89	1.0	3.3
Irganox 1330	C54H78O3	773.5872	9.62	1.3	4.2

[a] The LOQ for tris(4-nonylphenyl) phosphite was assessed to be high (5 µg/mL). Better results could have been obtained but it would have required another analytical run for this compound only.

3.2.2 Occurrence of target compounds in real samples

Out of the 36 targets, only diisobutyl phthalate (DiBP) was detected to in the food simulants that had been in contact with the plastic bottles for 1 or 10 days. The levels of DiBP in individual bottles/replicates are presented in Table 5.

Overall, DiBP was detected uniquely in one single sample (*LV 02-1*) of unwashed bottles made of PE. After 20 cycles of dishwashing, DiBP was detected in bottles made of both PP (13 bottle types) and PE (9 bottle types). Levels were always higher in the washed vs. the unwashed equivalent bottles, and levels were higher after 10 days of migration compared to 24 hours (Table 5).

Results were overall reproducible with a mean relative percent difference between duplicates of 30%.

Table 5: Levels (ng /mL) of DiBP detected in food simulants in contact with the bottles.

Sample ID	LC-MS date file	Day 1 (ng/mL)	Day 10 (ng/mL)	Material (IR)	Color
LV 02-1	23092TL-Bottle 005.d	13.65	22.58	PE	Blue
LV 02-2	23092TL-Bottle 006.d	14.68	27.24		
LV 02-3	23092TL-Bottle 007.d	26.77	57.16		
LV 02-4	23092TL-Bottle 008.d	23.38	55.93		
LV 04-1	23092TL-Bottle 013.d	ND	ND	PP	Pink
LV 04-2	23092TL-Bottle 014.d	ND	ND		
LV 04-3	23092TL-Bottle 015.d	0.19	10.07		
LV 04-4	23092TL-Bottle 016.d	6.58	20.54		
LV 07-1	23092TL-Bottle 021.d	ND	ND	PP	Clear
LV 07-2	23092TL-Bottle 022.d	ND	ND		
LV 07-3	23092TL-Bottle 023.d	ND	11.99		
LV 07-4	23092TL-Bottle 024.d	ND	11.57		
LV 08-1	23092TL-Bottle 025.d	ND	ND	PP	Blue
LV 08-2	23092TL-Bottle 026.d	ND	ND		
LV 08-3	23092TL-Bottle 027.d	3.05	14.44		
LV 08-4	23092TL-Bottle 028.d	0.00	22.97		
CZ 01-1	23092TL-Bottle 029.d	ND	ND	PP	Black
CZ 01-2	23092TL-Bottle 030.d	ND	ND		
CZ 01-3	23092TL-Bottle 031.d	ND	12.53		
CZ 01-4	23092TL-Bottle 032.d	ND	20.15		
CZ 02-1	23092TL-Bottle 033.d	ND	ND	PP	Pink
CZ 02-2	23092TL-Bottle 034.d	ND	ND		
CZ 02-3	23092TL-Bottle 035.d	ND	4.97		
CZ 02-4	23092TL-Bottle 036.d	10.86	29.93		
CZ 03-1	23092TL-Bottle 037.d	ND	ND	PP	Pink
CZ 03-2	23092TL-Bottle 038.d	ND	ND		
CZ 03-3	23092TL-Bottle 039.d	ND	7.68		
CZ 03-4	23092TL-Bottle 040.d	ND	3.82		
CZ 05-1	23092TL-Bottle 045.d	ND	ND	PP	Clear
CZ 05-2	23092TL-Bottle 046.d	ND	ND		
CZ 05-3	23092TL-Bottle 047.d	ND	9.13		
CZ 05-4	23092TL-Bottle 048.d	5.36	25.13		
CZ 06-1	23092TL-Bottle 049.d	ND	ND	PE	Blue
CZ 06-2	23092TL-Bottle 050.d	ND	ND		
CZ 06-3	23092TL-Bottle 051.d	7.91	18.39		
CZ 06-4	23092TL-Bottle 052.d	11.67	30.28		
CZ 08-1	23092TL-Bottle 057.d	ND	ND	PE	Black
CZ 08-2	23092TL-Bottle 058.d	ND	ND		
CZ 08-3	23092TL-Bottle 059.d	2.94	20.47		
CZ 08-4	23092TL-Bottle 060.d	5.73	20.65		
DK 01-1	23092TL-Bottle 061.d	ND	ND	PE	Blue
DK 01-2	23092TL-Bottle 062.d	ND	ND		

DK 01-3	23092TL-Bottle 063.d	6.97	16.07		
DK 01-4	23092TL-Bottle 064.d	3.81	14.81		
DK 03-1	23092TL-Bottle 069.d	ND	ND	PE	Pink
DK 03-2	23092TL-Bottle 070.d	ND	ND		
DK 03-3	23092TL-Bottle 071.d	12.85	25.43		
DK 03-4	23092TL-Bottle 072.d	17.56	30.73		
DK 06-1	23092TL-Bottle 081.d	ND	ND	PP	Blue-green
DK 06-2	23092TL-Bottle 082.d	ND	ND		
DK 06-3	23092TL-Bottle 083.d	ND	ND		
DK 06-4	23092TL-Bottle 084.d	ND	5.53		
DK 07-1	23092TL-Bottle 085.d	ND	ND	PE	White
DK 07-2	23092TL-Bottle 086.d	ND	ND		
DK 07-3	23092TL-Bottle 087.d	1.22	12.05		
DK 07-4	23092TL-Bottle 088.d	5.46	14.62		
DK 08-1	23092TL-Bottle 089.d	ND	ND	PE	Red
DK 08-2	23092TL-Bottle 090.d	ND	ND		
DK 08-3	23092TL-Bottle 091.d	ND	ND		
DK 08-4	23092TL-Bottle 092.d	12.87	19.01		
ESP 01-1	23092TL-Bottle 093.d	ND	ND	PE	Yellow
ESP 01-2	23092TL-Bottle 094.d	ND	ND		
ESP 01-3	23092TL-Bottle 095.d	7.74	25.41		
ESP 01-4	23092TL-Bottle 096.d	11.37	27.49		
ESP 02-1	23092TL-Bottle 097.d	ND	ND	PP	Blue
ESP 02-2	23092TL-Bottle 098.d	ND	ND		
ESP 02-3	23092TL-Bottle 099.d	ND	4.13		
ESP 02-4	23092TL-Bottle 100.d	10.44	16.05		
ESP 05-1	23092TL-Bottle 109.d	ND	ND	PP	Green
ESP 05-2	23092TL-Bottle 110.d	ND	ND		
ESP 05-3	23092TL-Bottle 111.d	ND	3.48		
ESP 05-4	23092TL-Bottle 112.d	ND	3.13		
NL 01-1	23092TL-Bottle 125.d	ND	ND	PP	Magenta
NL 01-2	23092TL-Bottle 126.d	ND	ND		
NL 01-3	23092TL-Bottle 127.d	ND	ND		
NL 01-4	23092TL-Bottle 128.d	ND	5.58		
NL 02-1	23092TL-Bottle 129.d	ND	ND	PP	Pink
NL 02-2	23092TL-Bottle 130.d	ND	ND		
NL 02-3	23092TL-Bottle 131.d	1.40	7.51		
NL 02-4	23092TL-Bottle 132.d	8.69	15.99		
NL 04-1	23092TL-Bottle 137.d	ND	ND	PP	Yellow
NL 04-2	23092TL-Bottle 138.d	ND	ND		
NL 04-3	23092TL-Bottle 139.d	8.34	8.74		
NL 04-4	23092TL-Bottle 140.d	8.53	10.28		
NL 08-1	23092TL-Bottle 153.d	ND	ND	PE	Green
NL 08-2	23092TL-Bottle 154.d	ND	ND		
NL 08-3	23092TL-Bottle 155.d	1.86	8.21		
NL 08-4	23092TL-Bottle 156.d	8.42	12.94		

Note: Blue color indicates washed samples, white color indicates unwashed samples. ND=not detected.

3.2.3 Migration per dm^2 and interpretation

The levels of diisobutyl phthalate (DiBP) in the food simulants were converted into migration per unit of contact surface area (µg /dm^2). Results are presented in Table 6.

Table 6: Migration (µg /dm^2) of DiBP from PP and PE bottles after 1 and 10 days.

Material	Type	1-day (µg /dm^2)				10-day (µg /dm^2)			
		Minimum	Max	Mean	STDV	Minimum	Max	Mean	STDV
PP (N=12)	not washed	ND	ND	N/A	N/A	ND	ND	N/A	N/A
	washed	ND	1.60	0.92 (n=10)	0.5	ND	4.40	1.72 (n=24)	1.1
PE (N=10)	not washed	ND	2.10	2.03 (n=2)	0.1	ND	3.90	3.56 (n=2)	4.7
	washed	ND	3.83	1.43 (n=17)	1.0	ND	8.17	3.28 (n=19)	1.9

Note: ND=note detected; N/A=not available; N=number of bottle type in the same material group and each N has 4 replicates; n=individual bottle number.

According to Commission Regulation (EU) N° 10/2011, and according to good manufacturing practice, plastic materials should be manufactured in such a way that they are not releasing more than 10 mg of substances (total constituents) per 1 dm^2 of surface area of the plastic material, or 60 mg/kg of food or food simulant.

DiBP is a substance of very high concern (SVHC) and is classified as toxic to reproduction and an endocrine disruptor according to ECHA. According to the latest amendment (2023/1442) of the Plastics FCM Regulation (10/2011) DiBP use is not authorised as an additive for plastic FCMs, but may be present in smaller amounts therein as an impurity or as a consequence of its use as a technical support agent in the manufacturing process of certain types of plastic. The Commission Regulation (EU) N° 10/2011 also defines a Specific Migration Limit (SML) of 0.6 mg/kg for the mixture of phthalic acid diesters incl. DBP, DiBP, BBP & DEHP.

Based on individual MDLs, it can be concluded that specific migration for each of all the non-detected target compounds (except tris(4-nonylphenyl) phosphite) was therefore below 1.3 µg/dm^2 (or even lower) after 10 days. As these MDLs of the method were satisfactory, this result suggests that the targeted compounds may not be used in the manufacturing of the collected plastic bottles, or that their migration is very low. The relatively higher MDL for tris(4 nonylphenyl) phosphite

(1.5 µg/mL) would correspond to migration levels below 0.22 mg/dm^2, which is still significantly lower than the general migration limit of 10 mg/dm^2.

Maxima of 3.90 (no dishwashing) and 8.17 µg /dm^2 (dishwashing) were recorded for the chemical migration of diisobutyl phthalate (DiBP) in after 10 days.

3.3 Chemical migration –Nontargeted Screening

3.3.1 Numbers of molecular features

The number of features are detailed in Table 7 below for various data analysis strategies implemented in this project (see section 2.6.1 and 2.6.2).

When combining data for the two ionization modes, several hundreds of molecular features were detected among all the simulants in contact with the 39 types of bottles (section 2.6.1). This number can be considered equivalent to a number of chemical compounds as a first approximation, but it is possible that, on some occasions, several molecular features arise from the same compound (e.g. in-source fragmentation). This large number of chemical entities is in line with what others have reported, i.e. >400 plastic related compounds migrating into drinking water for three types of reusable plastic bottles collected in Denmark [3].

Many of these molecular features are likely to be leachables from the plastic bottles (as further demonstrated in this section). Considering the use of glass jars as ‘blanks’ for both washed and unwashed conditions, the above molecular features are unlikely to be random traces of environmental contamination or traces of chemical residues from the dishwashing step (water, dishwashing liquid, dishwasher materials). This is also further supported by observations on individual replicates for DiBP (see Table 5) and other non-targeted compounds (e.g. dibutyl maleate), as the detection of those leachables was generally consistent among replicates.

As observed in Table 7, the number of molecular features dropped when we raised the intensity threshold from greater to 2× greater than the blanks (e.g. 227 down to 73 features in ESI+). This further supports that these compounds were present at trace levels in the simulants.

Another data analysis approach was tested based on mean intensities for specific groups of bottles, or specific washing conditions (section 2.6.2). Several dozens of molecular features were recorded as reported in Table 7.

Table 7. Number of molecular features detected in food simulants in contact with the bottles for 10 days.

	ESI+	**ESI-**
Relevant molecular features for individual bottle samples (section 2.6.1)		
Total number of features	322	529
Features with intensity greater than the blanks in at least 1 bottle sample	227	238
Features with intensity 2× greater than the blanks in at least 1 bottle sample	73 (List RA1)	83 (List RB1)
Relevant molecular features for specific groups of bottles (section 2.6.2)		
Features with mean intensity 3× greater than the blanks (washed vs. unwashed; and PE-PP vs other materials)	69 (List MA1)	126 (List MB1)
Features with mean intensity 3× greater than the blanks (washed vs. unwashed)	28	73

3.3.2 Suspect screening using the EnL and inhouse lists

Formulas could be matched for 20 out of the 73 features from ESI+ (List RA1) and 14 of the 83 features from ESI- (List RB1) with compounds from the EnL and inhouse lists. The tentative identity for these features is listed in Table 8. The identity of some of these features was further confirmed (see section 3.3.4), but in the absence of additional structural information, the confidence in the identification for the other features remain at level 4 according to the Schymansky et al. scale [4]. Mono-methyl terephthalate reported in reusable plastic bottles in Canada by Tian et al. [5] was not detected in this study, probably because none of the tested bottles were made of Tritan™. Bisphenol A was not detected, even in the one PC bottle sample. This result appears to be in line with a 2008 study, in which BPA migration from PC baby bottles to water was shown to be very low at room temperature, and repeated dishwashing led to a decrease in the average concentration of BPA leached from baby bottles using water as a food simulant [6].

Table 8. Tentative identities (confidence level 4, [4]) of the features with an intensity 2× greater than the blanks in at least 1 bottle sample.

ESI+

m/z	Library suggested ID	Formula based on accurate mass
213.1457	1-[(1-Butoxy-2-propanyl)oxy]-2-propanol	C10 H22 O3
313.2343	2,5-Dimethyl-2,5-di-(tert-butylperoxy)hexane	C16 H34 O4
130.1586	Dibutylamine	C8 H19 N
173.078	Acetic acid—3-methoxypropan-1-ol (1/1)	C6 H14 O4
301.1408	Butyl isobutyl phthalate	C16 H22 O4
198.1272	DBA / Dibenzylamine	C14 H15 N
229.1421	Dibutyl maleate	C12H20O4
83.9519	Dichloromethane	C H2 Cl2
157.0831	Dipropylene glycol	C6 H14 O3
157.0830	Dipropylene glycol	C6 H14 O3
171.0988	Dipropylene glycol monomethyl ether	C7 H16 O3
323.2116	N-((4-Phenyl-1-(phenylmethyl)-4-piperidinyl)methyl)acetamide	C21 H26 N2 O
239.1256	N-Butyl-N,N-dimethyl-((difluoromethyl)difluoromethyl)methan-1-aminium	C9 H18 F4 N
274.2736	N-Lauryldiethanolamine	C16 H35 N O2
182.9846	Octabromodiphenyl ether	C12 O
393.2089	Octaethylene glycol	C16 H34 O9
149.0227	Phthalic anhydride	C8 H4 O3
250.1143	TAC / Triallyl cyanurate	C12 H15 N3 O3
217.1041	Tetraethyleneglycol	C8 H18 O5
383.2039	Tributyl citrate/ Tributyl citrate	C18 H32 O7
ESI-		
m/z	**Library suggested ID**	**Formula based on accurate mass**
165.0404	1,3,5,7-Tetroxane	C4 H8 O4
417.3214	1-Stearoylglycerol (1-Monostearin)	C21 H42 O4
293.1755	(6)-Gingerol	C17 H26 O4
582.9797	Amaranth (E123) (FD&C Red No. 2) (C.I. 16185)	C20 H14 N2 O10 S3
121.0294	Benzoic acid	C7 H6 O2
299.2006	Dehydroabietic acid	C20 H28 O2
213.0535	DHB / 2,4-Dihydroxybenzophenone (Benzophenone-1)	C13 H10 O3
389.29	Glycerol monopalmitate	C19 H38 O4
89.0242	Lactic acid	C3 H6 O3
133.0139	Malic acid	C4 H6 O5
157.1233	Nonanoic acid	C9 H18 O2
277.1804	NPE / 4-Nonylphenoxyacetic acid	C17 H26 O3
137.0242	Salicylic acid	C7 H6 O3
170.0279	Toluene-2-sulfonamide	C7 H9 N O2 S

3.3.3 Structural predictions using SIRIUS

All the features with a mean intensity greater in one of the sample groups (see Table 7, 69 features in list MA1 and 126 features in list MB1) were further manually inspected and MS/MS was collected for features with a sufficient intensity (9 features in ESI+ and 7 features in ESI- with an intensity $\geq 10^5$). The MS/MS information was examined with SIRIUS (structural correlation based on machine learning) to predict the identity of the features (Table 9).

Table 9. Tentative identity suggested by SIRIUS for selected features with peak intensity greater than 10^5 from lists MA1 and MB1

ESI+		
m/z	**SIRIUS predicted ID**	**Formula based on accurate mass**
191.1636	N/A	C10H22O3
274.2736	N-lauryldiethanolamine	C16H35NO2
284.295	Octadecanamide	C18H37NO
198.1272	N/A	C14H15N
279.1587	DIBP	C16H22O4
130.1586	Dibutylamine	C8H19N
361.2216	Tributyl citrate	C18H32O7
367.3315	3-[(Cyanomethyl)(hexadecyl)amino]butanoic acid	C22H42N2O2
323.2116	N-[(1-benzyl-4-phenyl-4-piperidyl)methyl]acetamide	C21H26N2O
ESI-		
m/z	**SIRIUS predicted ID**	**Formula based on accurate mass**
217.0028	N/A	C7H5FNO6
293.1755	Gingerol	C17H26O4
377.2230	N/A	C24H30N2O2
649.5521	9-Octadecanamide,N'N'-(2-hydroxy-1,3-propaned)	C39H74N2O5
614.9837	N/A	C17H10F8N3O11S
425.3376	N/A	C22H44N5O3
242.1756	13-Amino-13-oxotridecanoic acid	C13H25NO3

3.3.4 Identified leachables and estimated migration

Analytical standards were purchased when commercially available and the structure of seven features could be confirmed (Table 10). Except diisobutyl phthalate (DIBP) which was also a target compound, the migration rate of other compounds was estimated based on one-point external calibration standard.

Table 10. Confirmed chemicals in the food simulants in contact with the bottles.

Method	Feature (mass @RT)	Frequency (out of 39 bottle types)	Compound	CAS	Estimated migration
Suspect screening	228.1317@6.49	1/39	Dibutyl maleate	105-76-0	0.07 μg/dm^2 per 10days
	129.151@5.19	14/39	Dibutylamine	111-92-2	0.10-1.45 μg/dm^2 per 10 days
Non-targeted analysis	283.287@7.24	4/39	Octadecanamide	124-26-5	0.01-0.45 μg/dm^2 per 10 days
	273.2658@7.19	4/39	N-lauryldiethanolamine	1541-67-9	0.10-0.33 μg/dm^2 per 10 days
	294.1822@5.84	9/39	Gingerol isomer*	23513-14-6	N/A
	360.2142@6.68	14/39	Tributyl citrate	77-94-1	0.06-0.19 μg/dm^2 per 10 days
	278.0928@6.69	22/39	Diisobutyl phthalate	84-69-5	up to 8.17 μg /dm^2 per 10 days

*Note: feature 294.1822@5.84 was identified as an isomer of gingerol with targeted MS/MS match and RT match, however the exact structure of isomer is unknown due to too many possible structures.

Feature 228.1317@6.49 was identified as **dibutyl maleate** and was detected in all the replicates for the bottles **DK-08**. There was no statistical difference in peak intensity between washed and unwashed samples ($p>0.1$). Dibutyl maleate is mainly used as a plasticizer and it was reported to leach out from PE plastic bottles after dishwashing [3]. Available toxicity data is limited for dibutyl maleate, but it showed no effects on cell viability conducted on mammalian cell lines [7].

Feature 129.1510@5.19 was identified as **dibutylamine** and it was detected only in PE and PP samples and mostly in washed group. Dibutylamine has been reported as plastic additive and was shown to leach out from PE food contact materials [8]. Dibutylamine was also detected in baby-bottle nipples in 1987 [9]. The toxicity about dibutylamine in literature is limited and some study applied it as non-toxic catalyst [10]. To the best of our knowledge, this is the first time that reported dibutylamine in food simulants in contact with children water bottles.

Feature 283.287@7.24 was detected in a few samples and blanks, and only 4 bottle types (out of 39) showed peaks higher than blanks. There is no significant difference ($p>0.1$) in peak intensity between washed and unwashed groups. It was identified as **octadecanamide** and it is reported as a lubricant or a release agent for plastic processing such as polyvinyl chloride, polystyrene, urea-formaldehyde resin, etc. Octadecanamide was detected in food contact

material (starch-based biopolymer) extracted using methanol [11] but this is the first time that reported in food simulants in contact with children water bottles. No toxicological data were found available for this compound.

Feature 273.2658@7.19 was identified as **n-lauryldiethanolamine**. It was detected only in **PE and PP samples** and there was no significant difference ($p>0.1$) in the peak intensity between washed and unwashed groups. The n-lauryldiethanolamine is also a polymer additive, commonly used as plastic antistatic agent. It was reported to leach out from plastic products but never reported in food contact material. N-Lauryldiethanolamine was reported to have a strong inhibitory effect on cell growth, starting from 30 µM. An induction of 61% was found at the lowest test concentration of 3 µM (additional lower concentration tested) with a predicted effect on cholesterol homeostasis [12].

Feature 294.1822@5.84 was predicted as **gingerol**, a natural compound. The reference standard with a 95% purity confirmed that the feature is an isomer of gingerol. Because it matches the RT of the small peak in standard (two peaks in standard solution), the MS/MS information (parent ion and two daughter ions) all matched. There are many possible isomers of gingerol and we could not identify further the specific structure of this feature and the semi-quantification was not conducted.

Feature 360.2124@6.68 was detected only in washed bottles made of PP and PE and it was identified as **tributyl citrate**. Tributyl citrate acts as a plasticizer, enhancing the properties of polymers and resins. It can be the degradation product of acetyl tributyl citrate. Acetyl tributyl citrate was not detected in any simulant in the present study. There is no migration information about tributyl citrate from any food contact materials, and it was only detected in different food stuff [13]. Tributyl citrate has been reported as Cramer Class III (High toxicity) [3] and is currently under assessment for endocrine disruption [14].

Diisobutyl phthalate (DiBP) was detected in targeted screening and it was also identified using the non-targeted workflow (list MA1). Its concentration was higher in "PP and PE group" than "others" and migration rate up to 8.17 µg /dm^2 per 10 days were recorded.

4. CONCLUSION

Based on the present results, it can be concluded that:

- Of the chemicals detected in the food simulant, the concentrations of diisobutyl phthalate (DiBP) were the highest. DiBP is a substance of very high concern (SVHC) and is classified as toxic to reproduction and an endocrine disruptor according to ECHA. According to the latest amendment (2023/1442) of the Plastics FCM Regulation (10/2011), its use is not authorised as an additive for plastic FCM, but may be present in smaller amounts therein as an impurity or as a consequence of its use as a technical support agent in the manufacturing process of certain types of plastic. There is no SML for DiBP in EU. In the EU regulation (Regulation (EU) 2023/1442 amending Annex I to Regulation (EU) 10/2011) is stated that the SML for the group phthalates (DBP+BBP+DEHP+DiBP) is 0.6 mg/kg (600 μg /kg).
- Non-targeted analysis was deployed to assess if unexpected contaminants were also leaching from the reusable water bottles for children, particularly after cycles of dishwashing. Signals corresponding to 100's of chemicals were recorded in the food simulants in contact with children water bottles. Several of these signals were tentatively identified as plastic-related compounds. Using mass spectrometry, the largest signals were further investigated and it was demonstrated that the identified compounds are known for their usage as plastic production. For several of them, this study is the first report on their detection in food simulants in contact with children water bottles. To the best of our knowledge, the literature is scarce on the toxicological profile of these compounds, and a comprehensive assessment of the leached chemical mixtures is not feasible.
- Based on the results obtained for diisobutyl phthalate and other non-targeted signals, dishwashing had an effect on chemical migration from reusable plastic bottles. In many samples, DiBP migration was not detectable in the unwashed bottles, but was shown to leach after 20 rounds of dishwashing.
- Food simulant B (acetic acid 3%) was used in this study to mimic hydrophilic food with pH<4.5. It is important to mention that bottles are recommended by the manufacturers for specific usage (e.g. water), and non-recommended usages should be avoided. In particular, fatty foods or alcoholic beverages should not be stored in plastic water bottles, as migration is expected to be higher for many leachables based on results with ethanol mixtures as simulants [6, 15].

- Daily usage (notably leading to scratches on the material) was not considered in this study but is known to impact chemical migration [3].
- Only a fraction of the hundreds of chemicals recorded in the food simulants could be identified, highlighting the complexity of the observed chemical mixtures. This is one of the current challenge in the understanding of the human exposome, notably during childhood, a critical window period.

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