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Evaluation of the migration of chemicals from baby bottles under standardised and duration testing conditions

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Abstract

After the prohibition of Bisphenol-A-containing polycarbonate baby bottles in the European Union, alternative materials, such as polypropylene, polyethersulphone, Tritan™ copolyester, etc. have appeared on the market. Based on an initial screening and *in vitro* toxicity assessment, the most toxic migrating compounds were selected to be monitored and quantified using validated GC- and LC-QqQ-MS methods. The effect of several “real-life use conditions”, such as microwave, sterilisation and dishwasher, on the migration of different contaminants was evaluated by means of duration tests. These results were compared with a reference treatment (filling 5 times with pre-heated simulant (40°C)) and with the legal EU “repetitive use conditions” (3 migrations, 2h at 70°C). Analysis of the 3th migration step of the EU “repetitive use conditions” (which has to comply with the EU legislative migration limits) showed that several not-authorised compounds were observed in some baby bottles exceeding 10 µg kg⁻¹. However, all authorised compounds were detected well below their respective specific migration limits (SML). The reference experiment confirmed the migration of some of the compounds previously detected in the EU repetitive use experiment, though at lower concentrations. Analysis of extracts from the microwave and dishwasher experiments showed a reduction in the migration during the duration tests. In general, the concentrations found were low and comparable to the reference experiment. Similar observations were made for the two sterilisation types: steam and cooking sterilisation. However, steam sterilisation seemed to be more recommendable for daily use of baby bottles, since it resulted in a lower release of substances afterwards. Repeated use of baby bottles under “real-life” conditions showed no increase in the migration of investigated compounds and, after some time, the migration of these compounds became even negligible.

Keywords: Baby bottles; GC/LC-QqQ-MS; food contact material; real-life conditions; migration

Introduction

In 2011, the European Union banned the use of bisphenol-A (BPA) for the production of polycarbonate (PC) baby bottles (European Union 2011a) due to the wide variety of possible toxic effects of this substance (Vandenberg et al. 2012; Singh & Li 2012; Yang et al. 2013; Vandenberg et al. 2013; Mansilha et al. 2013). Since then, a wide variety of alternative materials has entered the market. Polypropylene (PP) has become the most common material for the manufacturing of baby bottles, followed by polyethersulphone (PES), polyamide (PA), Tritan™, and silicone (Onghena et al. 2014). Until now, research on these alternative polymer materials has focused mainly on the identification of possible migrants, such as additives, monomers, degradation products, etc. (Simoneau et al. 2011; Simoneau et al. 2012; Onghena et al. 2014; Onghena et al. 2015). Quantification of the identified compounds has been mostly conducted by a semi-quantitative approach (Simoneau et al. 2012; Onghena et al. 2014), though lately Onghena et al. (Onghena et al. 2016) proposed a quantification strategy to accurately quantify a selection of priority migrants based on the screening of these alternative materials.

To assess the prioritisation of the detected migrating compounds, several criteria were taken into account. Firstly, information on the genotoxic hazard of the identified substances was collected from the ECHA database, using the *in silico* prediction tools ToxTree and Derek Nexus™, and the Vitotox® test for detecting DNA damage. A decision tree combining the collected genotoxic information was applied to classify the substances into different priority groups (Mertens et al. 2016). Furthermore, receptor gene assays were applied to determine the estrogen, androgen, progesterone, glucocorticoid, thyroid beta, peroxisome proliferator gamma and aryl hydrocarbon receptor mediated transactivational activity, since these nuclear receptors are involved in several regulating processes in the human body (Zoeller et al. 2012; Osimitz et al. 2012; Pereira-Fernandes et al. 2013). Based on this series of bioassays, an overall cumulative toxicity scoring was assigned to each tested compound (Simon et al. 2016). Finally, the semi-quantitatively estimated concentrations of the migrants were also considered for the prioritisation of these substances.

Next, the migration of the selected substances under conventional EU repetitive use conditions (3 migrations during 2h at 70°C with H₂O-EtOH (50:50, v/v, milk simulant))(European Union 2011b) was quantified by gas- and liquid tandem mass spectrometry (GC- and LC-QqQ) (Onghena et al. 2016).

However, the results of the migration using the conventional EU repetitive use conditions can be compared to the migration when daily use conditions are applied. These

conditions can be mimicked by duration tests, which consist in stressing the polymer by applying one specific parameter (e.g. sterilisation) for several repeated cycles to determine its resistance and possible degradation. Several studies already investigated the migration of BPA from PC baby bottles under different circumstances. Not only standardised EU migrating conditions (Wong et al. 2005), but also the mimicking of real-life use conditions of baby bottles, such as microwave heating (Ehlert et al. 2008; Biedermann-Brem & Grob 2009), sterilisation (Mountfort et al. 1997) or use of a dishwasher (Brede et al. 2003; Maragou et al. 2008), have been studied thoroughly. No information regarding the migration under such real-life circumstances is yet available for baby bottles made of materials other than PC.

However, for food contact materials (FCMs) other than PC, this research field has only been explored for the analysis of the influence of microwave heating on PP food containers (Alin & Hakkarainen 2010; Alin & Hakkarainen 2011), which reported an increased release of antioxidants compared to conventional heating. This clearly indicates the need for more research in this field, especially for FCMs that are intended for young children, such as baby bottles.

Therefore, the aim of this work was to determine the migration of the priority substances from baby bottles undergoing duration tests (e.g. microwave, sterilisation and dishwasher). Finally, these results were compared to the previously collected data of the 3rd migration of the EU repetitive use experiments in order to evaluate the appropriateness of the conventionally used migration conditions.

Materials and methods

Materials

Market Survey and Sampling

Samples were selected based on the market study conducted in our previous research (Onghena et al. 2014). Due to the labour intensity of these duration tests, one bottle of each different polymer type (PP, PES, PA, Tritan™ and silicone) was selected. Due to the large market share of PP-bottles, an additional PP bottle exhibiting another variety of migrating compounds was selected for the duration tests. The selected baby bottles corresponded to baby bottles 2 and 9 for PP, PES 1, PA 1, Tritan™ and silicone from our previous research (Onghena et al. 2016).

Chemicals

Ethanol (absolute for analysis EMSURE®, Reag Ph Eur), ethyl acetate (for LC

LiChrosolv®), n-hexane (EDC for GC and FID SupraSolv®), sodium chloride (ACS, ISO, Reag Ph Eur), sodium sulphate (ACS, ISO, Reag Ph Eur), formic acid (for analysis EMSURE® ACS, Reag Ph Eur), acetonitrile (for LC LiChrosolv®), ammonia (for analysis EMSURE® ACS, Reag Ph Eur) and ammonium sulphate (extra pure) were purchased from Merck KGaA (Darmstadt, Germany). Milli Q water was prepared with Elga Purelab flex system of Veolia Water Solutions & Technologies (Tienen, Belgium). Acetophenone ($\geq 99.0\%$), 4-methylbenzaldehyde ($\geq 97.0\%$), 2-butoxyethoxyethyl acetate ($\geq 99.2\%$), 3,4-dimethylbenzaldehyde (98%), 4-propylbenzaldehyde (97%), 2-undecanone (99%), 2,4,6-trimethylbenzaldehyde (98%), 2,6-di-*tert*-butylbenzoquinone (98%), dicyclopentyl-(dimethoxy)silane (98%), 2,4-di-*tert*-butylphenol (99%), oxacyclotridecan-2-one (98%), 2,2,4-trimethyl-1,3-pentanediol di-*iso*-butyrate (TXIB, 98.5%), *p-tert*-octylphenol (98.5%), cedrol (purity not specified by the manufacturer), benzophenone ($\geq 99\%$), 2,6-di-*iso*-propylnaphthalene (purity not specified by the manufacturer), 2,6-di-*tert*-butyl-4-hydroxybenzaldehyde (98%), azacyclotridecan-2-one (98%), di-*iso*-butyl phthalate (99%), dibutyl phthalate (99%), 4-phenylbenzophenone (99%) and methyl oleate ($\geq 99\%$) were purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). The deuterated internal standard (IS) 2,6-di-*tert*-butyl-4-methylphenol-D24 was purchased from Campro Scientific GmbH (Berlin, Germany). Helium (ALPHAGAZTM, 99.999%) and nitrogen (99.99%) were purchased from Air Liquide (Liege, Belgium).

Methods

Migration testing: EU repetitive use conditions

The migration from the selected baby bottles under EU repetitive use conditions was evaluated for selected substances according to the procedure described by Onghena et al. (Onghena et al. 2016). Briefly, baby bottles were sterilised by filling them during 10 min with boiling water and afterwards three consecutive migrations were executed using H₂O-EtOH (50:50, v/v) (milk simulant) for 2 h at 70 °C following EU Regulation No. 10/2011 on plastic materials and articles intended to come into contact with food (European Union 2011b). The third migration solution was analysed.

Migration testing: Duration tests

In order to evaluate the impact of the duration test on the migration, a set of baby bottles was also filled with simulant at 40 °C and kept at room temperature for 30 min. Afterwards, the simulant was transferred into glass containers and stored at +4 °C prior to

analysis; this was repeated 5 times. These experiments served as reference to evaluate whether migration of compounds occurs when the baby bottles have not been subjected to any pre-treatment.

Four different types of duration treatments were applied to determine the migration of targeted compounds when mimicking real-life conditions: microwave heating, dishwasher cleaning, steam and cooking sterilisation.

Firstly, the influence of microwave heating was simulated with the following procedure: bottles were filled with H₂O-EtOH (50:50, v/v) at room temperature (23 °C) up to the indicated volume and sealed. They were then placed individually in the centre of the microwave oven (Whirlpool Gusto GT288WH) and evenly heated to a temperature of 40 °C with their respective heating time by the three-dimensional release of microwave radiation in combination with a rotating baking dish. The power was set at 500 W and the heating time was adjusted depending on the size and volume of each bottle to ensure that a temperature of 40 °C was reached. After heating, bottles were placed at room temperature for 30 min to simulate the real drinking process by infants. Finally, the simulant was transferred into glass containers and stored at +4 °C. After each migration, baby bottles were rinsed with 50 ml of Milli Q water and refilled with new simulant for a new migration test. To simulate aging under the influence of microwave radiation, the bottles were subjected to a total of 100 cycles in the microwave oven and the solutions obtained after cycles 1 to 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90 and 100 were analysed. However, it should be noted that the bottles were not sterilised prior to the experiment.

Secondly, the impact of the repetitive use of the dishwasher was examined. Six new bottles were subjected to a dishwasher treatment. In each cycle, bottles were washed in a dishwasher operated at “eco-mode” (2h55min at 55-60 °C) using a common detergent. The inclination of the bottles in the dishwasher was adjusted such that the whole internal surface was in contact with the water spray. After each cycle, bottles were rinsed with 50 ml of Milli Q water, filled with preheated H₂O-EtOH (50:50, v/v) simulant (40 °C) and left for 30 min. Finally, the simulant was transferred into glass containers and stored at +4 °C. To simulate aging under the influence of the dishwasher, the bottles were subjected to a total of 10 cycles and the migration solutions obtained after 1, 2, 4, 6, 8 and 10 cycles were analysed.

The objective of the third and fourth treatment was to investigate the influence of different sterilisation techniques on the migration. A typical electric steam steriliser available in specialised baby shops (Philips Avent 3-in-1 electric steam steriliser) was used. Bottles were placed together with 100 ml of tap water and steamed for approximately 10 min

(according to the conditions mentioned in the user manual). Also a cooking sterilisation was applied for which bottles were boiled in tap water for 10 min. After sterilisation, bottles were also rinsed with 50 ml of Milli Q water, filled with simulant (40 °C), and kept for 30 min at room temperature before storage. Again, 10 cycles were performed using new samples for both types of treatment and the migration solutions obtained after 1, 2, 4, 6, 8 and 10 cycles were analysed.

For all types of duration tests, a glass bottle was taken through the entire procedures and afterwards filled with simulant as a blank control sample.

Analysis of the migration solutions

The obtained migration solutions were processed with a previously optimised and validated liquid-liquid extraction (LLE) method with ethyl acetate (EtOAc)-*n*-hexane (50:50, v/v) for GC-QqQ-MS analysis or directly analysed by LC-QqQ-MS (Onghena et al. 2016). Briefly, the LLE consisted in extracting 30 ml of simulant 3 times with 10 ml of EtOAc-*n*-hexane (1:1) and evaporating the organic extract to ± 5 ml of which 200 μ L was taken for injection. In addition to the zero extract sample (processed matrix sample from the glass bottle without analyte, but with IS), a quality control (QC) sample spiked at an intermediate concentration in the expected sample concentration range (50 μ g kg⁻¹) was included as well. Next, the obtained extracts were analysed by GC-QqQ-MS or LC-QqQ-MS.

Instrumentation

GC-QqQ-MS analysis

The EtOAc-*n*-hexane (1:1) extracts were analysed with an Agilent 7890A gas chromatograph (Agilent JW Scientific, Diegem, Belgium). One μ L of extract was injected into a PTV injector in pulsed splitless mode with an injection temperature of 300 °C. Separations were carried out in a 30 m $\times$ 0.25 mm $\times$ 0.25 μ m DB-5ms column (Agilent JW Scientific). The temperature of the oven was initially set at 60 °C for 3 min, was then increased to 115 °C at a rate of 7°C min⁻¹, then increased to 240 °C at a rate of 10 °C min⁻¹, and finally to 300 °C at a rate of 15°C min⁻¹, where it was kept for 15 min. The total run time was 42.36 min. Helium was used as a carrier gas at a constant flow rate of 1.0 ml min⁻¹ during 22 min. Then, the flow was increased to 1.5 ml min⁻¹ for 5 min, after which it was set back to 1 ml min⁻¹ for the remaining analysis time. The GC system was coupled to an Agilent 7000 GC-MS triple quadrupole (QqQ) equipped with an electron impact (EI) ionisation source and operated in multiple reaction monitoring (MRM) mode for the detection and quantification of compounds. The quadrupole

and ion source temperatures were set at 150 and 230 °C, respectively. N₂ was used as collision gas at a flow of 1.5 ml min⁻¹. The multiplier voltage was 1537 V and 1630 V, respectively for a gain of 10 and 20. In order to improve the number of cycles per second, several acquisition segments were created with different dwell times (15 and 20 ms, respectively). The mass spectrometer compound dependent parameters were tailored for each compound individually in previous work (Onghena et al. 2016).

LC-QqQ-MS analysis

The liquid chromatographic system was a Waters Acquity Ultra High Pressure LC (UHPLC) fitted with a degasser, a binary high-pressure gradient pump, a thermostated column compartment and an autosampler module. Chromatographic separation was achieved using a Waters Acquity UPLC C₁₈ BEH column (100 mm x 2.1 mm, 1.7 µm) at a flow rate of 0.4 ml min⁻¹. An injection volume of 10 µL was applied and the column was maintained at a constant temperature of 30 °C during analysis. The mobile phases used for the LC were: for the positive ionization mode: solvent A: H₂O with 0.1 % HCOOH and solvent B: AcN with 0.1% HCOOH; for the negative mode: solvent A: H₂O with 0.1 % NH₃ and solvent B: AcN with 0.1% NH₃. For both ionisation modes, the gradient was as follows: 0 min: 5% B; 0-6 min: 95% B; 6-8 min: 95% B; 8-10 min: 5% B. The LC system was coupled to a Waters XevoTQ-S triple quadrupole mass spectrometer with an electrospray interface (ESI) operated in both positive and negative modes for the detection and quantification of compounds. Source parameters were as follows: a capillary voltage of 3 kV was applied for the ESI(+) mode and 2.5 kV for the negative mode. Cone Voltage (30 V), cone gas flow (50 L h⁻¹), source temperature (150°C), desolvation gas flow (800 L h⁻¹), and collision gas flow (0.15 L h⁻¹) were the same for both polarities. Two compounds were measured in ESI(+) mode and four in ESI(-) mode. Mass spectrometer parameters were optimized for each compound individually (Onghena et al. 2016).

Results and Discussion

EU repetitive use experiment

A harmonised European Regulation is available for plastic food contact materials (European Union, 2011b), specifying both migration conditions (three migrations for repetitive use materials such as baby bottles) and simulants (H₂O-EtOH (50:50, v/v) to simulate milk). The Regulation sets out an EU list of authorised substances that can be intentionally used in the manufacture of plastics, together with restrictions, specifications and

specific migration limits (SMLs). For substances for which no SML or other restrictions are established, Article 11(2) of this Regulation describes that the specific migration of these substances shall not exceed a generic SML of 60 mg kg⁻¹. To assess a materials final compliance, the concentrations of migrating compounds measured in the 3rd migration step should not exceed those specified in the Regulation. Derogation from the latter can be applicable when the substance is separated from the food by a functional barrier. In this case, substances not present on the Union list can be used if they do not migrate into the food at detectable concentrations (with a detection limit of 10 µg kg⁻¹) (Barlow 2009) and if they do not exhibit carcinogenic, mutagenic or reprotoxic (CMR) properties. If the SML of a substance is specified as non-detectable (ND), a detection limit of 10 µg substance per kg food is applicable unless specified differently for the individual substance. In both cases, the material or article must already respect the SML in the first migration.

The prescribed migration conditions were applied on the plastic baby bottles as well as on the stainless steel and silicone bottles (Onghena et al. 2016). Table 1 summarises the results of the 3rd migration step of the EU repetitive use experiment for the six bottles that were selected to be subjected to duration tests as well. None of the authorised compounds exceeded the SML, yet some unlisted compounds were detected above the proposed threshold of 10 µg kg⁻¹ (Onghena et al. 2016).

Reference migration testing

To be able adequately to compare the influence of the duration tests on the selected baby bottles of different polymer types available on the Belgian market, a set of bottles was first subjected to a reference treatment. This consisted in filling the bottles 5 times with pre-heated simulant (40 °C) and leaving them at room temperature for 30 min to simulate the direct use of the bottles without any pre-treatment by the consumer. The results of this experiment are given in Table 2 and are used further through this manuscript as a reference for comparison with the other treatments. The encountered concentrations reached a maximum in the first migration step and showed a decreasing tendency towards the consecutive migration steps. Azacyclotridecan-2-one (PA), dicyclodipentyl(dimethoxy)silane (Tritan™) and acetophenone (PES and silicone) were some of the compounds detected in the reference treatment. Furthermore, for the silicone bottle 2,2,4-trimethyl-1,3-pentanediol di-*iso*-butyrate (TXIB), benzophenone, di-*iso*-butyl phthalate and 3,4-dimethylbenzaldehyde were also identified. Yet, the detected concentrations were relatively low (mostly not-detected or <LOQ (Limit of Quantification)).

Duration tests: Microwave heating

Microwaves are often used to warm infant feeding formula in a short time. The processes involved in heat transfer during microwave heating are different from those during conduction or convection heating, and consequently unpredictable migration behaviours could be expected (Letellier & Budzinski 1999; Alin & Hakkarainen 2012).

Microwave heating of the selected bottles during 100 cycles showed that only a few compounds were released and in low concentrations ($\mu\text{g kg}^{-1}$ level). The detected concentrations were continuously decreasing cycle after cycle and were below LOQ or LOD after 25 cycles.

Some of the targeted substances previously seen in the 3rd migration of the EU repetitive use and reference experiment were released also here. Generally, the concentrations detected were significantly lower (Table 2) than those of the 3rd migration step, most probably due to the higher temperature (70 °C instead of 40 °C) applied in the repetitive experiment. Concentrations of migrating compounds were more in the range of those observed in the reference experiment.

Maximum concentrations of migrating substances were measured in the first cycles and showed a downwards tendency afterwards. For both PP bottles, none of the targeted compounds were detected or they were present at levels < LOQ (data not shown), whereas the concentration of some compounds (e.g. 3,4-dimethylbenzaldehyde: 59 $\mu\text{g kg}^{-1}$) were significantly higher in the EU repetitive use experiment indicating their actual presence in the polymer and the possibility to be released. The PA bottle only exhibited the presence of azacyclotridecan-2-one at 124 $\mu\text{g kg}^{-1}$ after the 1st microwave treatment, though in the subsequent steps this concentration rapidly decreased to 31 after the 2nd microwave treatment (Table 3). Yet, it was noteworthy that this was the only bottle which demonstrated a continuous release (concentrations > 5 $\mu\text{g kg}^{-1}$) of one of its migrants (azacyclotridecan-2-one) throughout the entire 100 cycles. For the Tritan™ bottle, only few compounds were detected at measurable concentrations (e.g. 4-propylbenzaldehyde: 8 $\mu\text{g kg}^{-1}$ and disappearing after 8 cycles; dicyclopentyl(dimethoxy)silane: 1 $\mu\text{g kg}^{-1}$). TXIB was released continuously at levels between the LOQ and 13 $\mu\text{g kg}^{-1}$ during the first 25 cycles after which its concentration dropped below the LOQ (Table 3). For the silicone bottle, TXIB was found between LOQ and 11 $\mu\text{g kg}^{-1}$ during the first 25 cycles, although for the reference experiment, the concentration reached more than 118 $\mu\text{g kg}^{-1}$. Dibutyl phthalate was not detected in the silicone bottle in the microwave experiments, whereas in the reference treatment it was detected starting at 9 μg

kg⁻¹. Yet generally, the other components detected in the reference experiment of the silicone bottle displayed higher concentrations in the microwave extracts (e.g. acetophenone 43 µg kg⁻¹ (1st cycle microwave-experiment) vs. 27 µg kg⁻¹ (1st reference-experiment)). Although detected in the microwave and reference treatment, acetophenone was not seen in the 3rd migration of the EU repetitive experiment of the silicone bottle. This is most probably due to the fact that it already disappeared entirely after the first two EU repetitive use migrations. For the PES material, acetophenone was the only detected compound at a concentration of 6 µg kg⁻¹ and disappeared after 8 cycles.

When comparing the values obtained in these microwave duration tests to those of the reference experiment (Table 2), two conclusions could be drawn. Firstly, the concentrations detected during the microwave treatments were higher for almost all compounds compared to the reference experiment. For example, 4-propylbenzaldehyde, which was not detected in the reference treatment of the Tritan™ bottle, was measured after the first microwave cycle at 8 µg kg⁻¹. Although the increase induced by the microwave treatment was rather modest, the same phenomenon was generally perceived also for the other bottle types, e.g. azacyclotridecan-2-one from the PA bottle: 124 µg kg⁻¹ after the first microwave heating vs. 70 µg kg⁻¹ in the first reference-experiment. Secondly, the few compounds that were detected in the reference treatment at higher or similar concentrations, dropped below the LOQ within a few cycles, whereas for the microwave treatment, their release was longer, such as for benzophenone (silicone, 8 cycles before <LOQ) or TXIB (Tritan™, up to 25 cycles). The latter compound was rather particular, as it was the only one that was present at higher concentrations in the reference treatment compared to the microwave duration test (10 vs. 118 µg kg⁻¹). However, this behaviour was only noticed for the silicone bottle, while the concentration of TXIB after the first microwave heating and the first reference experiment was very similar for the Tritan material (9 vs 11 µg kg⁻¹).

In conclusion, the microwave treatment not only systematically increased the release of substances in general, but it also substantially prolonged the number of cycles in which the target compounds were detected.

Duration tests: Dishwasher cleaning

After treatment of the baby bottles with a dishwasher program between 55-60°C during almost 3 h, hardly any of the target compounds could be detected. For the PP and PES bottles, no compounds were detected and for PA, only the monomer azacyclotridecan-2-one was seen at decreasing concentrations (from 98 to 39 µg kg⁻¹ in 10 cycles). Silicone and

Tritan™ exhibited the presence of some of the targeted compounds (TXIB, benzophenone, di-*iso*-butyl phthalate etc.), though only at low concentrations. When comparing the observed levels to the reference treatment, they were generally slightly higher after using the dishwasher (Table 4), indicating that the washing program could cause a slight increase in the release of some compounds (e.g. azacyclotridecan-2-one: 98 vs. 70 $\mu\text{g kg}^{-1}$). For compounds such as benzophenone or di-*iso*-butyl phthalate, the detected levels remained also higher during more cycles than compared to the reference treatment. Moreover, dibutyl phthalate even exhibited a small increase in concentrations after several dishwasher treatments (from 7 to 13 $\mu\text{g kg}^{-1}$). Nevertheless, other target compounds were already partially removed and therefore migrated in lower concentrations in the subsequent migration experiment (e.g. in the silicone bottle: TXIB 36 vs. 118 $\mu\text{g kg}^{-1}$ in reference; acetophenone ND vs. 27 $\mu\text{g kg}^{-1}$). Most probably the elevated temperature (55-60 °C) and long washing time (almost 3 h) were the main causes of this phenomenon. Anyhow, the detected concentrations for all compounds remained at low levels and far below the SMLs. It should be considered though that only the levels of previously selected target compounds were monitored here, and that no information was available on any possible polymer degradation products formed by this treatment. Therefore, this should be further studied into detail.

Duration test: Steam and cooking sterilisation

Steam sterilisation of the selected bottles clearly resulted in a quick elimination of the monitored compounds, since both PP and PES showed no migration. For PA, the detected concentrations were considerably lower than compared to the reference treatment (starting at 59 instead of 70 $\mu\text{g kg}^{-1}$ and rapidly decreasing), as well as for Tritan™ for which only dicyclopentyl(dimethoxy)silane was released at low concentrations. The same was observed for acetophenone, which migrated at very low concentrations from Tritan™ before it disappeared (2 $\mu\text{g kg}^{-1}$ after the first cycle). For the silicone bottle, all detected compounds were seen in decreasing concentrations and most of them also disappeared after a few sterilisation cycles (e.g. 3,4-dimethylbenzaldehyde; di-*iso*-butyl phthalate; acetophenone; 2,4-di-*tert*-butylphenol) (Table 5). Generally, the detected concentrations were lower (e.g. TXIB 28 vs. 118 $\mu\text{g kg}^{-1}$) or similar (di-*iso*-butyl phthalate) to those seen after the reference treatment. Benzophenone was initially released at higher concentrations after steam sterilisation, and it was detected during the entire 10 treatments, whereas for the reference treatment, it disappeared after 2 cycles. Moreover, other compounds were not released (2,4-di-*tert*-butylphenol) or at much lower concentrations (3,4-dimethylbenzaldehyde) during the

reference treatment. This might indicate that the high temperatures applied during sterilisation could influence (and even increase) the release of some particular compounds. Yet, concentrations remained relatively low and exhibited a decreasing tendency. Therefore, when overlooking the general tendency for the majority of the targeted compounds and considering also the other tested baby bottles, it could clearly be concluded that sterilisation is generally recommended to be performed before using a baby bottle in order to remove residual chemicals after production, but also to suppress the microbial contamination.

The cooking sterilisation generally showed the same pattern as seen in the steam sterilisation. The PES and both PP bottles did not display any migration of the selected compounds after they were subjected to a sterilisation in boiling water during 10 min whereas for PA the tendency was similar to the steam sterilisation as well. The Tritan™ bottle showed the release of 4-propylbenzaldehyde (previously seen here in the EU experiments as well) at $12 \mu\text{g kg}^{-1}$ in the 1st migration step, but it was not detected any more after the 2nd sterilisation. Cooking sterilisation of the Tritan™ plastic resulted also in the migration of 4-n-nonylphenol, which was not detected after any of the other treatments, starting at $6 \mu\text{g kg}^{-1}$ after the first cycle and decreasing afterwards. The results of this treatment on the latter plastics therefore suggested mainly the same conclusions as drawn from the steam sterilisation, namely that the application of harsh conditions, such as high temperatures, and in this case even physical movement of the bottles during the cooking, results in temporary release of compounds. Therefore, it might be advised to perform some sterilisation cycles before the first use of new baby bottles. Yet again, only the previously selected compounds were monitored and no data were available on the possible degradation products formed during this cooking sterilisation.

Whereas none of the aforementioned plastics released hardly any substances after the cooking treatment, the silicone bottle showed a totally different migration pattern. Here, an increase in concentrations of the previously observed substances was seen, and even some compounds that were not detected in the reference treatment were released (e.g. cedrol: $13 \mu\text{g kg}^{-1}$; 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde: $4 \mu\text{g kg}^{-1}$), although they disappeared after 2 cycles. Benzophenone, acetophenone, and dibutyl phthalate exhibited a similar migration pattern compared to the steam sterilisation, though for di-*iso*-butyl phthalate (starting at $29 \mu\text{g kg}^{-1}$ and maintaining higher concentrations during more cycles) and TXIB ($247 \mu\text{g kg}^{-1}$ after the first treatment), the detected concentrations were significantly higher than for the steam sterilisation and the reference treatment (Table 5). Cooking sterilisation might be less suitable than steam sterilisation for silicone bottles due to the physical contact with boiling water which seems to enhance the release of some compounds. Therefore, the rather aggressive

conditions of the cooking sterilisation are not recommendable for the silicone bottle. Generally, considering the lower concentrations and number of compounds released during steam sterilisation, this type of sterilisation would be preferable. Further non-target analyses of migrants of the other plastics still needs to be done to draw appropriate conclusions.

When comparing the different duration tests to each other, the lowest release of substances was generally exhibited after the microwave process, showing generally slightly higher concentrations than those of the reference treatment. Figure 1A & B shows the examples of benzophenone from the silicone bottle and azacyclotridecan-2-one from the PA. Furthermore, the concentrations observed after the dishwasher treatment were slightly higher than the microwave, whereas (for some of the compounds) the sterilisation treatments displayed the highest concentrations released.

All targeted compounds detected after application of the different duration tests were in accordance with the EU repetitive use experiment. Yet, this did not necessarily mean that these target compounds were still present in the 3rd step of the repetitive use experiment (Table 1), as they could have migrated already entirely from the polymer material during the first two migrations (Onghena et al. 2014). When comparing the concentrations observed in the duration tests (e.g. after 10 cycles) to those of the EU repetitive use experiment, most compounds detected in the latter experiments clearly displayed higher levels than any of the individual experiments (e.g. TXIB, 2-10 times higher compared to the 10th cycle of the duration tests). Therefore, the EU repetitive use experiment seemed to overestimate the migration originating from the normal daily use of baby bottles for some compounds, sometimes with even more than a factor 10. Yet, for others such as benzophenone (Figure 1A) or butyl phthalate, the concentrations after 10 duration cycles were in the same range as those of the EU repetitive use experiment. Most importantly, the EU repetitive experiment did not made an underestimation of the concentrations released in the duration tests for any of the target compounds. However, it has to be taken into account that the conditions applied for the EU repetitive experiment represented a worst-case scenario for direct migration from the polymer, whereas during the duration tests (dishwashing and sterilisation), potential migrants could already been washed away, resulting in lower actual migration to the simulant solution afterwards. Furthermore, all duration tests were conducted individually and therefore the influence of a combination of these treatments on the migration still has to be investigated.

Conclusions

Migration experiments on the polymer alternatives to PC baby bottles were done following the conditions specified in EU Regulation No. 10/2011 and by performing duration tests to simulate “real-life use” conditions. The experiments following the EU conditions showed that for compounds authorised by EU Regulation, none of the specified SMLs were exceeded. The duration tests showed the release of the same substances as detected in the EU repetitive use experiment, though the detected concentrations were lower (mostly <LOQ or ND) and a downwards tendency of migrant concentrations towards the subsequent treatment cycles was seen. The use of the microwave led to a slightly increased and/or prolonged release of migrating substances compared to a “regular-use reference treatment”. However after 30 microwave cycles, migration was below detection limits. The concentrations detected after dishwasher and sterilisation treatments remained considerably low. These experiments give more insight in the migration behaviour of baby bottles and show that repeated use of baby bottles under “real-life” conditions will not increase the migration of relevant compounds. On the contrary, the migration of these compounds became insignificant after a number of cycles. The results are indeed somewhat contra-intuitive, but the current work monitors only a selection of compounds, and further research on other possible degradation products formed during these treatments needs to be conducted. To further reduce the exposure of young children, the consumer could be advised to perform some cycles of steam sterilisation before the first use of baby bottles.

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Table captions:

Table 1: Concentrations (in $\mu\text{g kg}^{-1}$) of migrating compounds (with their respective SMLs) in the 3rd migration when applying the conventional EU repetitive use migration conditions. “-“ means not-detected.

Table 2: Concentrations of targeted migrating compounds ($\mu\text{g kg}^{-1}$) from six selected baby bottles of the Belgian market when filled five times with preheated simulant (40°C) during the reference treatment. * means < LOQ; “-“ means not-detected.

Table 3: Summary of the concentrations (in $\mu\text{g kg}^{-1}$) of the detected migrating compounds in different polymer types during migration after microwave heating. * means < LOQ; “-“ means not-detected.

Table 4: Summary of the concentrations (in $\mu\text{g kg}^{-1}$) of the detected migrating compounds in different polymer types during migration after dishwasher treatment. * means < LOQ; “-“ means not-detected.

Table 5: Summary of the concentrations (in $\mu\text{g kg}^{-1}$) of the detected migrating compounds in the different polymer types during migration after steam or cook sterilisation treatment. * means < LOQ; “-“ means not-detected. X means data lost during experiment.

Figure 1: Summary of the detected concentrations ($\mu\text{g kg}^{-1}$) after the different treatments for A) benzophenone in the silicone bottle and B) azacyclotridecan-2-one in the polyamide bottle.

Table 1: Concentrations (in $\mu\text{g kg}^{-1}$) of migrating compounds (with their respective SMLs) in the 3rd migration when applying the conventional EU repetitive use migration conditions. - means not-detected.

Compound name	SML ($\mu\text{g kg}^{-1}$)	PP Brand A	PP Brand B	PES	PA	Tri	Sil
2,2,4-trimethyl-1,3-pentanediol diisobutyrate (TXIB)	5000 ^b	-	-	-	-	-	348
2,4,6-Trimethylbenzaldehyde	^a	-	-	-	-	-	-
2,4-Di- <i>tert</i> -butylphenol	^a	12	-	-	-	8	-
2,6-Diisopropylnaphthalene	^a	-	-	-	-	-	-
2,6-Di- <i>tert</i> -butylbenzoquinone	^a	-	-	-	-	-	8
2-Butoxyethyl acetate	^a	15	-	-	-	-	-
Methyl oleate	^a	-	-	-	-	-	-
Benzophenone	600	-	-	-	-	-	9
Diisobutyl phthalate	^a	-	-	-	-	-	15
Dibutyl phthalate	300	-	-	-	-	-	11
Acetophenone	^a	-	-	3	-	-	-
4-Methylbenzaldehyde	^a	-	-	-	-	-	-
4-Propylbenzaldehyde	^a	-	-	-	-	27	1
2-Undecanone	^a	-	-	-	-	-	-
Oxacyclotridecan-2-one	^a	-	-	-	-	-	-
Cedrol	^a	-	-	-	-	-	-
3,5-Di- <i>tert</i> -butyl-4-hydroxybenzaldehyde	^a	-	-	-	-	-	-
Dicyclopentyl(dimethoxy)silane	^a	-	-	-	-	10	-
3,4-dimethylbenzaldehyde	^a	59	11	-	-	-	15
Bis(2-ethylhexyl)phthalate (DEHP)	1500	-	-	-	-	-	-
Azacyclotridecan-2-one	5000	-	-	-	924	-	-
4-Phenylbenzophenone	^a	-	-	-	-	-	-
Bisphenol-S	50	-	-	-	-	-	-
Bisphenol-A	600	-	-	-	-	-	-
<i>p-tert</i> -Octylphenol	^a	-	-	-	-	-	-
4-n-Nonylphenol	^a	-	-	-	-	-	-

Table 2: Concentrations of targeted migrating compounds ($\mu\text{g kg}^{-1}$) from 6 selected baby bottles of the Belgian market when filled 5 times with preheated simulant (40°C) (Reference treatment). * means < LOQ, - means not-detected.

	Filling	2,2,4-trimethyl-1,3-pentanediol diisobutyrate (TXIB)	2,4,6-Trimethylbenzaldehyde	2,4-Di- <i>tert</i> -butylphenol	2,6-Diisopropylnaphthalene	2,6-Di- <i>tert</i> -butylbenzoquinone	2-Butoxyethyl acetate	Methyl oleate	Benzophenone	Diisobutyl phthalate	Dibutyl phthalate	Acetophenone	4-Methylbenzaldehyde	4-Propylbenzaldehyde	2-U-ecanone	Oxacyclotridecan-2-one	Cedrol	3,5-Di- <i>tert</i> -butyl-4-hydroxybenzaldehyde	Dicyclopentyl(dimethoxysilane)	3,4-dimethylbenzaldehyde	Bis(2-ethylhexyl)phthalate (DEHP)	Azacyclotridecan-2-one	Benzophenone, 4-phenyl-	Bisphenol-S	Bisphenol-A	p- <i>tert</i> -Octylphenol	4-n-Nonylphenol
PP brand-A	1	-	-	*	-	*	-	-	-	-	-	*	-	-	-	-	-	-	-	-	-	-	*	-	-	-	-
	2	-	-	*	-	*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	3	-	-	*	-	*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	4	-	-	*	-	*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	5	-	-	*	-	*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PA	1	-	-	*	-	*	-	-	-	-	-	*	-	-	-	-	-	-	-	-	-	70	-	-	-	-	-
	2	-	-	*	-	*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	32	-	-	-	-	-
	3	-	-	*	-	*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	10	-	-	-	-	-
	4	-	-	*	-	*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	31	-	-	-	-	-
	5	-	-	*	-	*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	15	-	-	-	-	-
PES	1	-	-	*	-	*	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	2	-	-	*	-	*	-	-	-	-	-	*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
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	4	-	-	*	-	*	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	5	-	-	*	-	*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PP brand-B	1	-	-	*	-	*	-	-	-	-	-	*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	2	-	-	*	-	*	-	-	-	-	-	*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
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	5	-	-	*	-	*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Silicone	1	118	-	*	-	*	-	-	12	15	9	27	-	-	-	-	*	*	-	6	-	-	-	-	-	-	-
	2	102	-	*	-	*	-	-	4	12	7	11	-	-	-	-	-	*	-	-	-	-	-	-	-	-	-
	3	64	-	*	-	*	-	-	*	*	*	5	-	-	-	-	-	*	-	-	-	-	-	-	-	-	-
	4	71	-	*	-	*	-	-	*	*	*	4	-	-	-	-	-	*	-	-	-	-	-	-	-	-	-
	5	67	-	*	-	*	-	-	-	*	*	4	-	-	-	-	-	*	-	-	-	-	-	-	-	-	-
Tritan	1	11	-	*	-	*	-	-	-	-	-	*	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-
	2	10	-	*	-	*	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-
	3	7	-	*	-	*	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-
	4	8	-	*	-	*	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-
	5	-	-	*	-	*	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-

Table 3: Summary of the concentrations (in $\mu\text{g kg}^{-1}$) of the detected migrating compounds in the different polymer types during migration after microwave heating. * means < LOQ, - means not-detected.

Polymer type	Compound	Microwave heating cycle																						LOQ (μg kg ⁻¹ simulant)
		1	2	3	4	5	6	7	8	9	10	15	20	25	30	40	50	60	70	80	90	100		
PES	Acetophenone	6	2	2	2	2	3	2	2	*	*	*	*	*	-	-	-	-	-	-	-	-	1.7	
PA	Azacyclotridecan-2-one	124	31	19	22	26	25	14	19	34	17	15	7	22	5	5	8	7	6	11	14	7	9.6	
Tritan	2,2,4-trimethyl-1,3-pentanediol diisobutyrate (TXIB)	9	9	7	9	8	*	*	10	9	10	8	9	13	*	*	*	*	*	*	*	*	6.4	
	4-propylbenzaldehyde	8	4	3	3	2	2	1	*	-	-	-	-	-	-	-	-	-	-	-	-	-	0.6	
	Dicyclopentyl(dimethoxy)silane	1	1	1	1	1	1	*	1	1	1	1	*	1	*	*	*	*	*	*	*	*	0.8	
Silicone	TXIB	10	8	*	*	*	*	7	9	8	*	*	8	11	*	*	*	*	*	*	*	*	6.4	
	Benzophenone	14	10	6	6	6	4	5	6	*	*	*	*	*	*	-	-	-	-	-	-	-	3.6	
	Diisobutyl phthalate	24	19	14	15	15	10	14	19	12	15	*	17	17	*	*	*	*	*	*	*	*	8	
	Acetophenone	43	24	14	14	13	7	7	8	5	6	2	*	*	*	*	*	*	*	*	*	*	1.7	
	3,4-dimethylbenzaldehyde	10	6	*	*	*	*	*	*	*	*	-	-	-	-	-	-	-	-	-	-	-	5.6	
PP Brand A & B	No compounds detected																							

Table 4: Summary of the concentrations (in $\mu\text{g kg}^{-1}$) of the detected migrating compounds in the different polymer types during migration after dishwasher treatment. * means < LOQ, - means not-detected.

Polymer type	Compound	Dishwasher cycle						LOQ ($\mu\text{g kg}^{-1}$ simulant)
		1	2	4	6	8	10	
PA	Azacyclotridecan-2-one	98	18	13	55	22	39	9.6
Tritan	2,2,4-trimethyl-1,3-pentanediol diisobutyrate (TXIB)	-	13	8	11	8	8	6.4
	Dicyclopentyl(dimethoxy)silane	1	3	3	3	3	3	0.8
Silicone	TXIB	-	36	34	27	23	22	6.4
	Benzophenone	-	20	13	12	7	7	3.6
	Diisobutyl phthalate	-	13	16	17	14	16	8.0
	Dibutyl phthalate	-	7	10	11	11	13	4.4
	2,4-Di- <i>tert</i> -butylphenol	*	*	*	7	*	*	6.2
PP Brand A & B	No compounds detected							
PES	No compounds detected							

Table 5: Summary of the concentrations (in $\mu\text{g kg}^{-1}$) of the detected migrating compounds in the different polymer types during migration after steam or cook sterilisation treatment. * means < LOQ, - means not-detected. X means data lost during experiment.

Polymer type	Compound	Steam sterilisation cycle						Cook sterilisation cycle						LOQ ($\mu\text{g kg}^{-1}$ simulant)
		1	2	4	6	8	10	1	2	4	6	8	10	
PA	Azacyclotridecan-2-one	59	33	10	4	24	6	51	13	9	19	7	21	9.6
Tritan	2,2,4-trimethyl-1,3-pentanediol diisobutyrate (TXIB)	-	13	8	11	8	8	2	*	*	*	*	*	6.4
	4-n-Nonylphenol	-	-	-	-	-	-	6	X	5	2	2	1	1.0
	Dicyclopentyl(dimethoxy)silane	1	3	3	3	3	3	2	X	1	1	1	1	0.8
	Acetophenone	2	-	-	-	-	-	-	-	-	-	-	-	1.7
	4-propylbenzaldehyde	-	-	-	-	-	-	12	-	-	-	-	-	0.6
Silicone	TXIB	28	27	23	15	16	9	247	201	178	145	127	128	6.4
	Benzophenone	53	66	26	14	9	12	58	36	25	12	7	7	3.6
	Diisobutyl phthalate	10	9	*	*	*	*	29	29	24	19	17	15	8.0
	Dibutyl phthalate	11	14	7	7	-	-	10	12	11	9	8	8	4.4
	2,4-Di- <i>tert</i> -butylphenol	7	*	*	*	*	*	12	7	*	*	*	*	6.2
	2-Butoxyethyl acetate	-	-	-	-	-	-	6	-	-	-	-	-	5.6
	Acetophenone	14	8	*	*	-	-	14	4	*	*	*	-	1.7
	Cedrol	*	*	*	*	*	*	13	11	*	*	*	*	9.7
	3,5-di- <i>tert</i> -Butyl-4-hydroxybenzaldehyde	-	-	-	-	-	-	4	4	*	*	3	*	3.1
	3,4-dimethylbenzaldehyde	18	15	-	-	-	-	6	-	-	-	-	-	5.6
PP Brand A & B	No compounds detected													
PES	No compounds detected													