2nd Imeko Foods: Metrology Promoting Objective and Measurable Food Quality and Safety

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

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Abstract
The effect of several "real-life use conditions" on the migration of different contaminants was evaluated for plastic baby bottles by means of duration tests and with the legal EU repetitive use conditions. All authorised compounds were detected well below their specific migration limits (SML). 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.

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. 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. 2012; Onghena et al. 2014). Quantification of the identified compounds has been mostly conducted by a semi-quantitative approach (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 (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 H_2O-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. No information regarding the migration under such real-life circumstances is yet available for baby bottles made of materials other than PC. 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.

Chemicals
All the chemicals used in this research were the same as those described in the publication by Onghena et al. 2016.

Methods
Migration testing: EU repetitive use conditions and Duration tests

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 H_2O-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. 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 will serve 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 H_2O-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 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. Again, 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 µL 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 µg kg^{-1}) was included as well. Next, the obtained extracts were analysed by GC-QqQ-MS or LC-QqQ-MS.

Instrumentation

All GC and LC-QqQ analysis were done according to the conditions described in Onghena et al. 2016.

Results and Discussion

EU repetitive use experiment

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). 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 to adequately 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 are used further 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 also 2,2,4-trimethyl-1,3-pentanediol diisobutyrate (TXIB), benzophenone, di(iso)butyl phthalate and 3,4-dimethylbenzaldehyde were identified. Yet, the detected concentrations were relatively low (mostly not-detected or <LOQ (Limit of Quantification).

Duration tests: Microwave heating

Microwave heating of the selected bottles during 100 cycles showed that only a few compounds were released and in low concentrations (µg kg^{-1} level). The detected concentrations were continuously decreasing cycle after cycle and were below LOQ or LOD after 25 cycles. Maximum concentrations of migrating substances were measured in the first cycles and showed a downwards tendency afterwards. When comparing the values obtained in these microwave duration tests to those of the reference experiment, 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). 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,…), though only at low concentrations. When comparing the observed levels to the reference treatment, they were generally slightly higher after using the dishwasher, indicating that the washing program could cause a slight increase in the release of some compounds (e.g azacyclotridecan-2one: 98 vs. 70 µg 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 µg 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 µg kg^{-1} in reference; acetophenone ND vs. 27 µg kg^{-1}). Most probably the elevated temperature (55-60°C) and long washing time (almost 3h) were the main causes of this phenomenon. Anyhow, the detected concentrations for all compounds remained at low levels and far below the SMLs.

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. 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. Generally, the detected concentrations were lower or similar to those seen after the reference treatment. Concentrations remained relatively low and still 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 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 results of this treatment suggested mainly the same conclusions as drawn from the steam sterilisation. Therefore, it might be advised to perform some sterilisation cycles before the first use of new baby bottles. Whereas none of the aforementioned plastics released hardly any compounds, here the detected concentrations were significantly higher than for the steam sterilisation and the reference treatment. 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.

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. Yet, 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.

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