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Migration of bisphenol A from polycarbonate baby bottles under real use conditions

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Abstract

Migration of the potential endocrine disrupter bisphenol A (BPA) from 31 polycarbonate (PC) baby bottles into aqueous food simulants was studied under real repetitive use, with a sensitive and fully validated liquid chromatographic method with fluorescence detection. Confirmation of the presence of BPA was performed by liquid chromatography-mass spectrometry (LC-MS). The effects of cleaning with dishwasher or with a brush, sterilization with boiling water and the temperature of migration were examined. It was shown that temperature was the crucial factor for the migration of BPA from the plastic bottles to water. All the samples released BPA in the concentration range of 2.4-14.3 $\mu\text{g kg}^{-1}$, when they were filled with boiled water and left at ambient temperature for 45 min. The decrease of BPA release in the sterilization water and in the food simulant over twelve cycles of use indicated that the hypothesis of polymer degradation in water is rather doubtful. The estimated infantile dietary exposure, regarding the use of PC baby bottles, ranged between 0.2 and 2.2 $\mu\text{g kg}^{-1} \text{ bw d}^{-1}$, which is below the Tolerable Daily Intake of 50 $\mu\text{g kg}^{-1} \text{ bw}$, recently established by EFSA.

Keywords: BPA, xenoestrogens, migration, infants, feeding bottles, HPLC-FLD, LC-MS, polymer hydrolysis

Introduction

It is widely known that bisphenol A (BPA) is used as a monomer in the synthesis of polycarbonates (PC), a type of polymer that is utilized in the manufacture of plastic food containers, such as infant feeding bottles. Exhibiting high transparency, low weight and high heat and impact resistance, polycarbonate plastics are increasingly replacing glass in such applications (Plastics Europe 2007).

In the early 1990s, it was reported that BPA, was released from polycarbonate flasks during autoclaving, exhibits estrogenic activity in *in vitro* assays at concentrations of 10-25 nM (2-5 ng ml⁻¹) (Krishnan et al 1993). Later studies reported that exposure to low doses of BPA causes reproductive toxic effects, such as increases in murine prostate gland and reduced sperm efficiency (Vom Saal et al 1998). Additionally, recent studies state that BPA exposure affects brain development of rats at a very low dose range of 10⁻¹² -10⁻¹⁰ M (0.23 – 23 pg ml⁻¹) (Zsarnovszky et al 2005), and brain development of murine at a higher dose of 3 µg g⁻¹ (Tando et al 2006).

The presence of unreacted BPA in PC baby bottles has been reported, and the residual amount of the monomer was found to range from 7 to 58 µg g⁻¹ in samples purchased in Washington (Biles et al 1997) and from < 3 to 141 µg g⁻¹ in samples purchased in Singapore (Wong et al 2005). The occurrence of BPA in the plastic, the fact that baby bottles are intended to be used repeatedly and the potential adverse effects of BPA at low doses, especially regarding infants, has generated the need for a thorough investigation of BPA migration from this type of food contact material.

Migration studies of BPA from infant feeding bottles have been conducted under diverse treatments and diverse conditions using water (Biles et al 1997, Kawamura et al

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4 1998, Sun et al 2000, D'Antuono et al 2001, Brede et al 2003), ethanol/water mixtures
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6 (Biles et al 1997, Kawamura et al 1998, D'Antuono et al 2001, Wong et al 2005), 3-4%
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8 acetic acid (Kawamura et al 1998, D'Antuono et al 2001), n-heptane (Kawamura et al
9
10 1998) and oil (Biles et al 1997, Kawamura et al 1998, Wong et al 2005) as food simulants.
11
12 In particular, Biles et al (1997) reported that residual BPA observed to migrate from PC
13
14 bottle to 10% aqueous ethanol at 100 °C for 30 min over four cycles of use, decreased
15
16 rapidly after an initial "bloom" and then leveled out. Accordingly, migration tests of PC
17
18 nursing bottles with water at 95 °C for 30 min for four or five repeated cycles revealed a
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20 decrease since the first elution of BPA (Kawamura et al 1998, Sun et al 2000). Moreover it
21
22 has been shown that the amount of BPA eluted to water at 95 °C for 30 min was greatly
23
24 decreased by washing and boiling the bottle (Kawamura et al 1998). In contrast, another
25
26 migration study conducted in twelve PC baby bottles supports that dishwashing, boiling
27
28 and brushing, significantly increased the migration of BPA during incubation with water at
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30 100 °C for 1 h (Brede et al 2003). The authors attributed these findings at possible polymer
31
32 degradation. The increase of BPA migration to water due to PC degradation has not been
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34 verified experimentally by other researchers. Although decomposition of PC has been
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36 reported before, only an alcoholic simulant consisted of 50% ethanol or more in water
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38 could provoke this observation (Biles et al 1997). This obscurity concerning the release of
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40 BPA from reusable PC baby bottles, the possible decomposition of polycarbonate material
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42 and the conditions that could lead to this phenomenon, reveal the need for further
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44 investigation on this subject.
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53 The objective of this study is to contribute to the elucidation of BPA migration from PC
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55 baby bottles during realistic and repeated handling. The material of the baby bottles was
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3 identified with FT-IR analysis and the determination of BPA was performed with a
4 sensitive method based on liquid chromatography with fluorescence detection (HPLC-
5 FLD). After validation of the HPLC-FLD method, the effect of various parameters of
6 simulated use with water and acidic food simulants was investigated. The effects of
7 sterilization in boiling water, the way of cleaning (dishwasher or brush) and the effect of
8 different temperatures to two different brand names, were examined by two experimental
9 procedures. Subsequently, a third experimental procedure of twelve cycles of realistic use
10 of baby bottles was applied to four other brand names, including cleaning of the bottles
11 with brush, sterilization in boiling water for 10 min, filling with hot water and leaving at
12 ambient temperature for 45 min. In addition, the sterilization water of the twelve cycles was
13 also analyzed for BPA in order to monitor the behavior of PC bottles during sterilization,
14 and investigate the possibility of polymer degradation under these exaggerated conditions.
15 The presence of BPA was confirmed by LC-MS analysis.
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38 **Materials and methods**

39 *Chemicals*

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41 Standard BPA ($\geq 97\%$) was purchased from Fluka (Buchs, Switzerland). Stock solution
42 (375 mg l⁻¹) was prepared in methanol and used for further dilutions. The stock solution
43 was stored at -15 °C for six months. Methanol of HPLC grade was obtained from LAB
44 SCAN (Dublin, Ireland) and acetic acid of 99.9% purity from Carlo Erba (Rodano, Italy).
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3 BPA standard working solutions, was purified with a Milli-Q water system (Millipore,
4 Bedford, MA, USA).
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11 *Baby bottle samples*

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14 A total of thirty one plastic baby bottles of six different brand names (A-F) were purchased
15 from the Greek market and were used for the migration trials (Table I). The maximum
16 volume of the bottles, up to which they were filled for the migration tests, ranged from 120
17 to 250 ml.
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24 *Identification of baby bottles material with FT-IR*

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27 Three bottles of three different brand names were analysed with a Perkin Elmer FT-IR
28 System 2000 (Bucks, England). A small piece of each sample was finely pulverized and
29 mixed with KBr at a ratio of approximately 1:10. The mixture was compressed into an
30 evacuable pellet die in conjunction with a 15 ton manually operated hydraulic press
31 (Graseby Specac, Kent, UK). The produced KBr pellet was placed in the cell of the
32 spectrometer and scanned within the frequency range of 4000–370 cm^{-1} . The obtained
33 spectra of transmittance were compared by a matching algorithm (Euclidean search) to the
34 spectra of known polymers available in BIO-RAD Sadtler Library (Philadelphia,
35 Pennsylvania). The best match of the Euclidean search list was polycarbonate resin for all
36 the tested baby bottles.
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HPLC-FLD method validation

BPA was determined with a Hewlett-Packard 1050 series HPLC system, equipped with an HP 1046 fluorescent detector and an HP 1100 autosampler. Injections of 25 μl were performed into an Inertsil ODS-2 (250 mm \times 2.1 mm, 5 μm) C18-bonded reverse phase column. The isocratic elution was carried out with a mixture of methanol/water 70:30 v/v at a flow rate of 1.3 ml min^{-1} . The solvents were degassed using helium prior to analysis. The fluorescent detector was set at 235 nm for the excitation and at 317 nm for the emission. The HPLC system was operated by HP Chemstation software.

Standard working solutions were prepared in water and 3% w/v acetic acid. In order to cover a wide range of concentrations including very low levels of BPA expected from the migration tests but also including the European Specific Migration Limit (SML 0.6 $\mu\text{g ml}^{-1}$), two calibration curves were constructed for each simulant, one for low concentrations and one for high concentrations. Low concentration calibration curves were obtained by measuring standard working solutions at five levels in the range 7.5 - 180 ng ml^{-1} , with two replicates per concentration. Similarly, high concentration calibration curves were obtained by measuring standard working solutions at five levels in the range 0.18 - 6.0 $\mu\text{g ml}^{-1}$ with two replicates per concentration. Linear regression analysis was performed using the analyte peak area *versus* analyte concentration.

For the determination of the LOD, standard solutions of the three lowest concentrations were analyzed ten times. For every concentration level the mean concentration, \bar{C} and the standard deviation, SD, of the ten replicates were calculated and linear regression analysis was performed using standard deviations against mean values of concentrations ($\text{SD} = b \times \bar{C} + S_0$). The LOD was estimated using the intercept, S_0 of the obtained equation curve

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3 and it was defined as $3 \times S_0$. The same methodology was applied for the estimation of LOD
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6 for both simulants.
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9 Within run precision was expressed as the relative standard deviation of ten replicate
10 measurements of a standard solution at three different concentrations for water (30, 75 and
11 180 ng ml⁻¹) and at three different levels for 3% w/v acetic acid (15, 30 and 75 ng ml⁻¹).
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13 Between run precision was expressed as the relative standard deviation of six independent
14 replicate analyses (preparation and measurement) of a standard solution of 30 ng ml⁻¹ for
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16 both simulants.
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22 23 *LC-MS analysis*

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25 Identification of BPA was carried out according to a method described previously
26 (Maragou et al 2006). A Thermo Finnigan LC-MS system (San Jose, USA) consisted of a
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28 Spectra System P 4000 pump, a Spectra System AS 3000 autosampler with the volume
29 injection set to 10 µl was used with a Surveyor MSQ quadrupole mass spectrometer
30 equipped with an electrospray ionization LC-MS interface (ESI). In brief, the
31 chromatographic separation was performed using a LiChrospher 100-RP18 (250 mm × 4
32 mm, 5 µm) reversed phase LC column and isocratic elution with a mobile phase consisted
33 of methanol-water (70:30 v/v) at a flow rate of 0.9 ml min⁻¹. ESI was applied in the
34 negative ionization mode and the capillary was held at a potential of 3.5 kV. The cone
35 voltage was 70 V and the ionization source was set at a temperature of 500 °C. The full
36 scan spectrum (m/z 100-300) and the single ion monitoring (SIM) chromatograms of the
37 deprotonated molecule of BPA (m/z 227) were acquired at every analysis.
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Migration testing

The study of BPA migration from PC baby bottles was divided into three experimental procedures (I-III), which differ in the sample treatment before the migration test and/or in the migration conditions. In all cases the bottle treatment and the migration conditions were selected so as to be representative of real use. Water and 3% acetic acid (w/v) in aqueous solution were used as food simulants for the migration testing. Water was used as simulant for milk and 3% w/v acetic acid was used as simulant for fruit juices, according to the Directive in force, 85/572/EEC. Regarding the water as milk simulant, it has been noted that milk should not be experimentally modelled by water (Franz 2005), but this argument was based on the results of a migration experiment with a very lipophilic migrant (1,4 – diphenyl butadiene). On the contrary, BPA is a compound with polar characteristics, containing two hydroxyl groups and with a reported water solubility of 120-300 mg l⁻¹ (Staples et al 1998). Additionally, a typical preparation of milk consists of pouring boiling water in the baby bottle, which seems to be the critical point for BPA migration, as it appears from the results of this work. After that, addition of the milk powder follows.

Table I summarizes the number of baby bottles of each brand name, the experimental procedure to which each group was subjected and the simulant used. Before the very first use, all the specimens were sterilized in boiling water for 5 min according to the manufacturer's instructions.

[Insert Table I about here]

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Experimental Procedure I. In the first experimental procedure (I) the impact of the repetitive use of the dishwasher and the kind of the simulant on the migration of BPA were examined. Four new bottles from brand name A and four new bottles from brand name B were subjected to ten cycles of simulated use with water as simulant. In each cycle, the bottles were washed in a dishwasher at 60 °C using a common detergent and distilled water for the final wash. Subsequently the bottles were sterilized for 10 min in boiling water, and then they were filled up to the mark with the simulant. Finally, they were incubated for 2h at 70 °C (Table II). Exactly the same procedure, consisted of ten cycles, was applied to three new bottles of brand name A and three new bottles of brand name B using 3% w/v acetic acid as a simulant.

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Experimental Procedure II. In the second experimental procedure (II) the impact of brushing during cleaning, the impact of sterilization and the effect of the incubation temperature and time were examined. The eight bottles of labels A and B, which had already been subjected to the experimental procedure I with water, were used to this experimental procedure, as well, with the same simulant. For this reason the numbering of cycles starts from eleven. This migration test was also consisted of ten cycles of simulated use. From the eleventh to the fourteenth cycle the bottles were brushed with detergent under tap water and rinsed with distilled water. After the cleaning step the bottles were sterilized for ten minutes in boiling water and then they were filled up to their maximum volume with the simulant and were incubated for 2h at 70 °C. The objective of these four cycles was to examine the impact of brushing during cleaning. The results were compared with those obtained from the experimental procedure I where dishwasher was used. In the fifteenth cycle, the bottles were washed only with distilled water, without brushing and

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3 sterilization, and afterwards they were filled with the simulant and incubated for 2h at 70
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5 °C. The purpose of the fifteenth cycle was to investigate whether brushing and sterilization
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7 remove residual BPA from the plastic surface. During the last five cycles, the bottles were
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9 brushed and sterilized as it has already been described for cycles 11-14. Afterward the baby
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11 bottles were filled with boiling water and left at room temperature for 45 min (Table II).
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13 The conditions of these last five cycles of use were applied as the most representative of
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15 actual preparation of milk.
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20 *Experimental Procedure III.* Based on the findings of the experimental procedures I and
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22 II and with the intention of expanding this study to a larger number of brand names we
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24 proceeded to experimental procedure III. Seventeen more new bottles of the brand names
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26 C, D, E and F were subjected to twelve repeated cycles of simulated use. Each cycle
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28 consisted of brushing, sterilization, filling with boiling water and incubation at room
29
30 temperature for 45 min, as it has already been described for cycles 16-20 of experimental
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32 procedure II (Table II). In this procedure, each group of bottles of the same brand name was
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34 sterilized in one kettle and different kettles were used for the different brand names. The
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36 sterilization water of each group was collected and measured in order to investigate the
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38 release of BPA during this step and the possible degradation of the polymer.
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46 After the expiration of the incubation time of every migration experiment, each simulant
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48 was transferred in an Erlenmeyer flask and homogenized by manual agitation. Then, a sub-
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50 sample of the simulant was measured with the validated method of HPLC-FLD. For the
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52 quantification of BPA in the solutions of the migration experiments and the sterilization
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54 water, external standard calibration was applied.
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4 Table II summarizes the steps of simulated treatment and the migration conditions for
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6 the three experimental procedures (I, II and III) where water was used as simulatant. Acetic
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8 acid was not used to experimental procedures II and III as it is not expected to drink fruit
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10 juices at such high temperatures.
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15 [Insert Table II about here]
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20 21 **Results and discussion**

22 23 24 *HPLC-FLD method performance*

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27 Table III shows the equations of the low and high concentration calibration curves
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29 generated from standards prepared in water and in 3% w/v acetic acid. The standard
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31 deviation of the slope and the intercept, and the correlation coefficient of every equation are
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33 also given. It is shown that linear calibration curves were obtained with correlation
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35 coefficients always exceeding 0.999 for both simulants. The method LOD for water and 3%
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37 acetic acid simulatant were found to be 2.4 and 1.8 ng ml⁻¹, respectively, without any
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39 preconcentration applied. These values are sufficient for safety control of the materials
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41 intended to come to contact with food since they are much lower than the specific
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43 migration legislative limit of BPA (0.6 mg kg⁻¹ or µg ml⁻¹, assuming that the density of
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45 aqueous simulants is 1 g ml⁻¹) (Directive 2004/19/EEC). In addition, the LOD of the
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47 method is at a concentration level that permits the study of BPA migration and at
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49 concentration levels at which it has been reported that BPA can exhibit estrogenic activity
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51 (Krishnan et al 1993, Vom Saal 1998). The within-run precision, expressed as % RSD
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(N=10), at 30, 75 and 180 ng ml⁻¹ of water simulant was 2.2, 1.7 and 1.0, respectively. The corresponding values for 3% w/v acetic acid at 15, 30 and 75 ng ml⁻¹ were 4.4, 3.0 and 2.2, respectively. The between run precision at 30 ng ml⁻¹, expressed as % RSD (N=6), was 2.9 for water and 4.2 for 3% w/v acetic acid. These values indicate the satisfactory performance of the method.

[Insert Table III about here]

BPA release during migration testing and sterilization

Table IV shows the migration of BPA, as amount of substance (ng) per simulant volume (ml), from the eight baby bottles (brand names A and B) that were subjected to experimental procedure I and subsequently to experimental procedure II, where the used simulant was water. Table V illustrates the corresponding migration results from the seventeen new feeding bottles of brand names C-F that were subjected to procedure (III). In Tables IV and V, BPA migration is expressed as the mean value of N identical migration experiments (N bottles of the same brand name) \pm the standard error. Standard error (S.E.) is defined as SD / \sqrt{N} , where SD stands for the standard deviation of the N values.

Concerning the migration from PC bottles to the acidic simulant, none of the six specimens released detectable amounts of BPA into 3% w/v acetic acid at 70 °C for 2h, throughout the ten cycles of the experimental procedure I (< 1.8 ng ml⁻¹). Our results are in agreement with previous work that reported non detectable BPA (< 0.5 ng ml⁻¹) after migration test of nursing bottle with 4% acetic acid at 60 °C for 30 min (Kawamura et al 1998). The temperature applied at our migration test (70 °C) rather exceeds realistic handling, provided that 3% w/v acetic acid is representative of fruit juices, a food which is

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3 not expected to be heated. Therefore subjection of the baby bottles to more exaggerated
4 conditions with this simulant was not considered essential. However, migration of BPA
5 from baby bottles to 3% acetic acid, at a level of 2.2 ng ml^{-1} , has been reported when a
6 higher temperature of $80 \text{ }^\circ\text{C}$ was applied for a shorter time of 2 min (D'Antuono et al
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13 2001).

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22 [Insert Table V about here]

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27 The results of the migration tests of cycles 1-10 and 11-14 (Table IV) demonstrate that
28 the repetitive cleaning of bottles either with dishwasher or with brushing (Table II), do not
29 lead to detectable BPA migration ($< 2.4 \text{ ng ml}^{-1}$) when incubation is taking place with
30 water at $70 \text{ }^\circ\text{C}$ for 2h. From the comparison of the two sets of cycles it can be concluded
31 that brushing, which is supposed to increase the wear of bottles, is not likely to influence
32 BPA migration remarkably. The objective of the bottle treatment of the fifteenth cycle was
33 to investigate whether the absence of BPA migration in the previous cycles was ascribed to
34 the fact that the available BPA in the plastic was washed away during brushing and
35 sterilization, and therefore there was no substance left to migrate during incubation. The
36 results indicate that this was not the case, since detectable BPA migration was not observed
37 when neither brushing nor sterilization had preceded. Migration was observed only when
38 the bottles were filled with boiling water ($100 \text{ }^\circ\text{C}$) in the sixteenth cycle and afterwards.
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60 From the results of the migration tests of experimental procedure I and II (Table IV) it

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3 becomes evident that temperature is the only critical factor for the increase of BPA
4 migration from PC baby bottles to water. In particular the contact of the surface of
5 polycarbonate bottles with boiling water (100 °C) seems to affect significantly the
6 migration of BPA.
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13 The data from the cycles 16-20 of procedure II (Table IV) and the data from the cycles
14 1-12 of procedure III (Table V) show that the very first contact of the PC material with
15 boiling water causes leaching of BPA to the aquatic medium at a concentration ranging
16 from 3.9 to 13.8 ng ml⁻¹. After four to eight subsequent cycles (depending on the brand
17 name), migration decreases to a level below 2.4 ng ml⁻¹. In experimental procedure III no
18 migration was observed during the last four cycles 9-12, and for this reason the procedure
19 was not continued further. Considering that the treatment of baby bottles during cycles 16-
20 20 (Exp.Pr. II) and cycles 1-12 (Exp.Pr. III) (Table II) is representative of typical
21 preparation of milk, it could be assumed that BPA migration occurs during actual domestic
22 use of PC baby bottles at least at the first four to eight cycles of use.
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36 Moreover, sterilization water from the cycles of procedure (III) was analyzed and
37 measurable BPA was present in all the samples (Figure 1a,b). The amount of BPA released
38 per bottle during sterilization was calculated by multiplying the concentration of the
39 measured subsample with the actual water volume in the kettle (1000 ml), and then
40 dividing with the number of bottles put in the kettle.
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51 [Insert Figure 1 about here]
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4 It can be observed that in all cases (Figure 1a,b) the release of BPA into sterilization
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6 water declines with the extension of handling. The data clearly reveal that no degradation
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8 occurs during sterilization by boiling the bottles in water for 10 min. Besides, due to the
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10 insolubility of PC in water, the aqueous depolymerization requires severe conditions such
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12 as long reaction times, high temperature and pressure, whereas in alcali-catalyzed
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14 methanol-toluene medium, complete PC degradation occurs at 60 °C in 70 min (Hu et al
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16 1998).
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21 Based on the findings of Table V and those of Fig.1, it could be assumed that there is a
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23 certain amount of residual BPA available on the surface of the PC bottles which is released
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25 during sterilization and during incubation with hot water, but it is not likely that additional
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27 BPA is yielded throughout the repeated treatment. These findings are in contrast with a
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29 previous work (Brede et al 2003), which reported significant increase of BPA migration
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31 from baby bottles to water due to use. Polymer degradation was proposed for the
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33 explanation of those results. However, it should be noted that migration of BPA was
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35 measured only three times in that work, after the first use, after 51 cycles and after 169
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37 cycles of washing, and that the increase of migration was observed between first use (0.23
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39 ng ml⁻¹) and 51st cycle (8.4 ng ml⁻¹) but there was no trend between 51st and 169th cycle
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41 (6.7 ng ml⁻¹).
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47 On the other hand, it has been reported that migration up to 368% of the available BPA
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49 occurred when 50% ethanol in water was used as simulant at 65 °C for 240 h, indicating
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51 hydrolysis of the polymer (Biles et al 1997). It should be mentioned that in the 4th
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53 amendment of the European Directive 2002/72/EC for plastics, which is expected to be
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55 published soon, 50% ethanol in water replaces water in simulation tests for milk contact. In
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3 that case BPA migration may be higher and further investigation on this issue may be
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5 required.
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8 The presence of BPA in sterilization water does not cause any concern regarding the
9 dietary exposure of infants, since the residual water is rejected at domestic use. However,
10 this data can give us an indication for the domestic contribution to environmental pollution,
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12 as this water would normally result in an urban sewerage.
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20 21 *Confirmation of the presence of BPA with LC-MS*

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23 All the BPA peaks detected by HPLC-FLD were confirmed by LC-MS. The presence of
24 BPA was confirmed when the most abundant ion of the eluted peak corresponded to the
25 ratio m/z 227, which has been assigned as the deprotonated molecule $[M-H]^-$ of BPA (MW
26 228). Figure 2 presents the SIM chromatograms (m/z 227) and the full mass spectra at the
27 retention time of the elution of BPA peak, of a standard solution containing 80 ng ml^{-1} of
28 BPA (Fig 2a) and of a water simulant containing approximately 2 ng ml^{-1} after migration
29 testing (Fig 2b).
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43 [Insert Figure 2 about here]
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48 *Infantile dietary exposure and safety*

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50 Our findings demonstrate a measurable migration of BPA to water simulant in the range of
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52 $2.4 - 14.3 \text{ } \mu\text{g kg}^{-1}$ (Tables IV and V, assuming that the density of aqueous simulants is 1 g
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54 ml^{-1}). Considering this migration level and that an infant of 0 to 4 months old, weighs 4.5
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3 kg and consumes 0.7 l of formula each day, which is the highest ratio of food intake to
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6 body weight for infants, according to the opinion of the Scientific Committee on Food
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8 (SCF 2002), the estimated intake is 0.4-2.2 $\mu\text{g kg}^{-1} \text{bw d}^{-1}$. In the same way the estimated
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10 intake for an infant of 6 to 12 months old, that weighs 8.8 kg and consumes 0.7 l, ranges
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12 between 0.2 and 1.1 $\mu\text{g kg}^{-1} \text{bw d}^{-1}$. This assessment on infant dietary exposure to BPA
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14 from polycarbonate baby bottles is slightly lower than the dietary estimates presented in the
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16 latest opinion of EFSA (2006). EFSA proposed that a conservative estimate of total dietary
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18 exposure to BPA at 3 month old infant, who is fed formula with PC bottle, is 11 $\mu\text{g kg}^{-1} \text{bw}$
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20 d^{-1} based on the upper value of 50 $\mu\text{g BPA l}^{-1}$ of infant formula, and 4 $\mu\text{g kg}^{-1} \text{bw d}^{-1}$ based
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22 on the typical value 10 $\mu\text{g BPA l}^{-1}$ of infant formula.
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27 Our results suggest that BPA migration from polycarbonate baby bottles are unlikely to
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29 be of concern, according to the recently established full TDI (Tolerable Daily Intake) of
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31 0.05 $\text{mg kg}^{-1} \text{bw}$ by EFSA (2006), derived by applying a 100-fold uncertainty factor to the
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33 overall NOAEL (No Observed Adverse Effect Level) of 5 $\text{mg kg}^{-1} \text{bw d}^{-1}$.
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37 On the other hand, the up-to-date findings of scientific investigations on the adverse
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39 effects of BPA *in vitro* and *in vivo* have led to an intense controversy regarding the levels
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41 of BPA that can be hazardous to humans. In a recent review, concerning low-dose effects
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43 of BPA, it is reported that in 31 publications with vertebrate and invertebrate animals,
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45 significant effects occurred below the reference dose of 50 $\mu\text{g kg}^{-1} \text{bw d}^{-1}$ (Vom Saal and
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47 Hughes 2005), while in another recent review the updated weight of evidence does not
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49 support the hypothesis that low-doses of $\leq 5 \text{ mg kg}^{-1} \text{bw d}^{-1}$ adversely affects human
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51 reproductive and developmental health (Goodman et al 2006). However, it should be
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53 mentioned that harmful effects on fertility have been reported after dietary exposure of
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3 BPA to 10 member groups of male mice at doses of 0.025 and 0.1 $\mu\text{g kg}^{-1} \text{bw d}^{-1}$ (Al-
4 Hiyasat et al 2002), and to 6 member groups of male rats at doses of 0.2, 2 and 20 $\mu\text{g kg}^{-1}$
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6 bw d^{-1} (Chitra et al 2003). These levels of BPA doses enclose the estimated infant intake of
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8 the present work (0.2 – 2.2 $\mu\text{g kg}^{-1} \text{bw d}^{-1}$). Additionally, it should be taken into
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10 consideration that infants are a susceptible group, possibly exposed to other xenoestrogens
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12 except for BPA (Kuo and Ding 2004).
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18 Taken together, further research on the low dose effect of bisphenol A and on the
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20 combined impact of different endocrine disrupters, potentially present in baby feeding
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22 bottles or baby food, would be essential for a clearer and more realistic estimation of
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24 hazard.
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30 **Conclusions**

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32 The present study indicates that temperature is the critical factor that favours BPA
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34 migration from PC bottles to water. It was shown that migration of residual BPA takes
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36 place at a concentration range of 2.4 - 14.3 $\mu\text{g kg}^{-1}$, when boiling water is poured into PC
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38 baby bottles, mainly during the first eight cycles of such use. Moreover, it was
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40 demonstrated that BPA release during sterilization decreases throughout twelve cycles of
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42 use, indicating that polymer degradation does not occur during boiling in water for 10 min.
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44 The estimated dietary exposure for infants aged up to one year old ranges between 0.2 and
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46 2.2 $\mu\text{g kg}^{-1} \text{bw d}^{-1}$. Even though these values are far below the recently established TDI
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48 (EFSA 2006), it should not be ignored that there are indications for hazardous effects at
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50 these low doses (Al-Hiyasat et al 2002, Chitra et al 2003).
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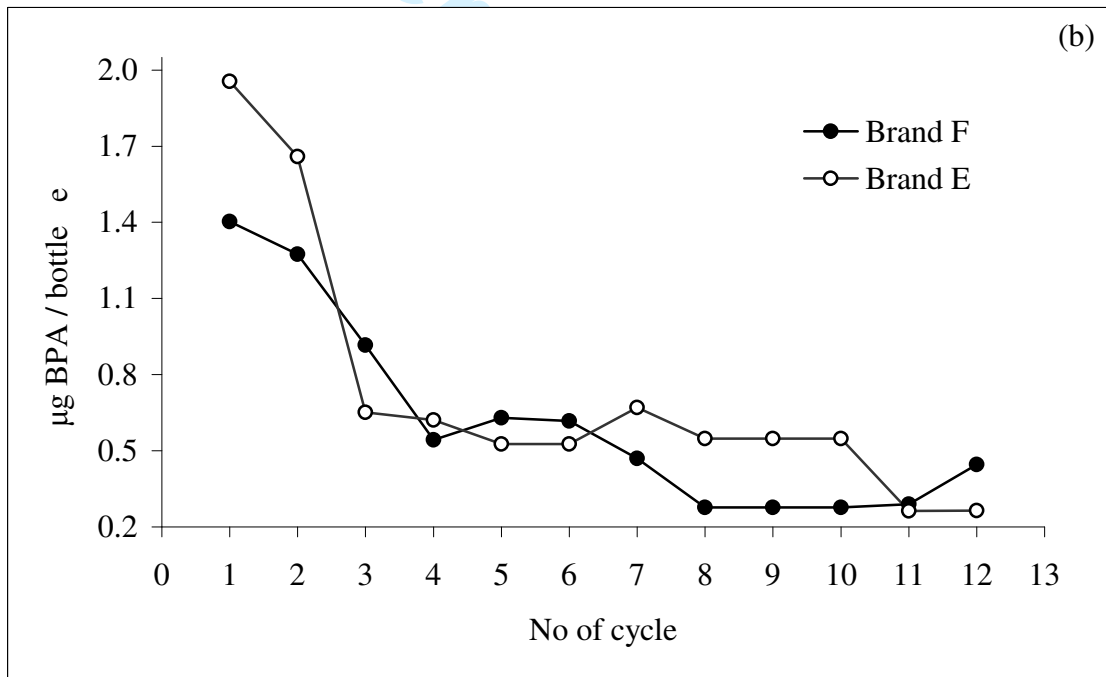
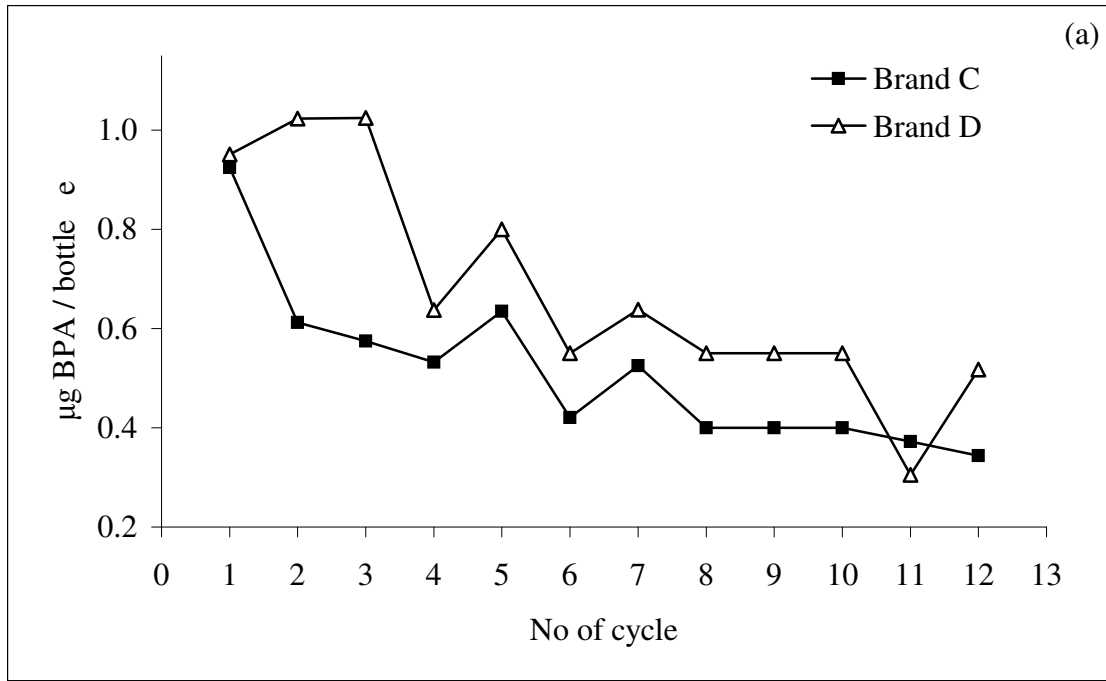


Figure 1.

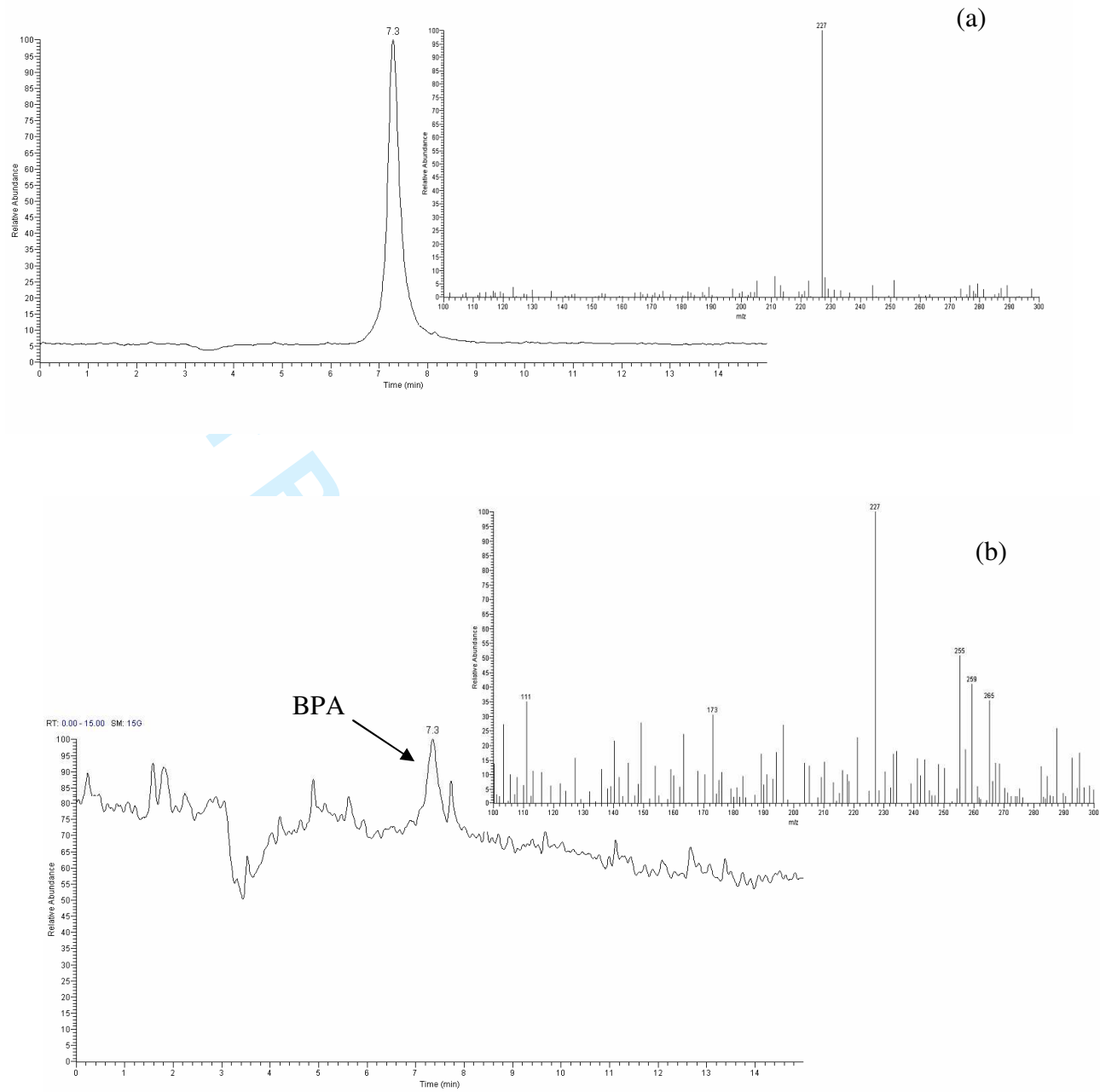


Figure 2.

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Figures captions

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Figure 1. Amount of BPA (μg) released per bottle in the sterilization water in experimental procedure (III) for the brand names (a) C and D and (b) E and F

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Figure 2. SIM chromatogram (m/z 227) and full scan spectrum at the retention time of BPA of (a) a standard solution containing 80 ng ml^{-1} of BPA and (b) a simulant solution after migration test containing approximately 2.0 ng ml^{-1} of BPA

Table I. Number of baby bottles of different brand names used for each experimental procedure

Brand name	Number of samples	Experimental Procedure	Simulant
A	3	I	3% w/v acetic acid
B	3	I	3% w/v acetic acid
A	4	I + II	water
B	4	I + II	water
C	4	III	water
D	4	III	water
E	6	III	water
F	3	III	water

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Table II. Sample treatment and migration conditions of the three experimental procedures using water as simulant

Experimental Procedure	Cycle	Cleaning	Sterilization*	Migration conditions
I	1-10	Dishwasher, detergent, 60 °C	Yes	70 °C, 120 min
	11-14	Brushing with detergent	Yes	70 °C, 120 min
II	15	Wash only with water	No	70 °C, 120 min
	16-20	Brushing with detergent	Yes	Filling with boiling water and left at ambient temperature for 45 min
III	1-12	Brushing with detergent	Yes	Filling with boiling water and left at ambient temperature for 45 min

* Sterilization in boiling water for 10 min

Table III. Calibration curves of BPA standard solutions in water and in 3% aqueous acetic acid for low and high concentrations. C represents the concentration of the measured solution (ng ml^{-1} or $\mu\text{g ml}^{-1}$). Calibration points: 5

Simulant	Concentration range	Calibration Curve	r
water	$7.5 - 180 \text{ ng ml}^{-1}$	$y = (-0.18 \pm 0.05) + (36.8 \pm 0.5) \times 10^{-3} C$	0.9994
	$0.18 - 6.0 \mu\text{g ml}^{-1}$	$y = (-2.1 \pm 1.5) + (44.3 \pm 0.4) C$	0.9998
3% acetic acid	$7.5 - 180 \text{ ng ml}^{-1}$	$y = (-0.01 \pm 0.04) + (38.5 \pm 0.4) \times 10^{-3} C$	0.9998
	$0.18 - 6.0 \mu\text{g ml}^{-1}$	$y = (2.6 \pm 4.3) + (46.1 \pm 1.2) C$	0.9991

Table IV. Migration of BPA to water during experimental procedure I (cycles 1-10) and experimental procedure II (cycles 11-20) for brand names A and B. \bar{C}_{BPA} : mean concentration, $S.E.$: standard error

No of cycle	$\bar{C}_{BPA} \pm S.E. (ng\ ml^{-1})$	
	A (N=4)	B (N=4)
1-10	< LOD	< LOD
11-14	< LOD	< LOD
15	< LOD	< LOD
16	13.3 \pm 0.2	13.8 \pm 0.5
17	13.3 \pm 0.2	10.6 \pm 2.7
18	10.3 \pm 2.6	14.3 \pm 0.4
19	13.3 \pm 0.5	4.2 \pm 2.6
20	4.4 \pm 2.8	< LOD

Table V. Migration of BPA to water during the twelve cycles of procedure III for brand names C, D E and F

No of cycle	$\bar{C}_{BPA} \pm S.E. (ng ml^{-1})$			
	C (N=4)	D (N=4)	E (N=6)	F (N=3)
1	3.9 ± 2.3	10.6 ± 2.7	5.8 ± 2.7	5.5 ± 3.5
2	4.7 ± 1.0	2.6 ± 1.2	3.7 ± 1.0	3.2 ± 1.6
3	3.3 ± 1.0	5.2 ± 3.5	< LOD	2.6 ± 1.2
4	< LOD	2.7 ± 1.3	4.8 ± 1.5	2.8 ± 1.3
5	2.4 ± 1.0	3.6 ± 1.2	2.8 ± 0.9	2.8 ± 1.3
6	3.9 ± 1.3	5.3 ± 1.2	3.8 ± 1.1	3.1 ± 1.6
7	3.9 ± 1.3	< LOD	2.4 ± 0.7	< LOD
8	2.7 ± 1.2	< LOD	4.8 ± 2.2	< LOD
9 - 12	< LOD	< LOD	< LOD	< LOD