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Experimental investigation on Bisphenol A and its leaching properties in various repositories: real-time analysis

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ABSTRACT

Bisphenol A (BPA) is a chemical reagent used during the molding of various plastics and daily use ware. BPA is a toxic substance rated blue, 3, in fire diamond by National Fire Protection Association 704 standards. This research work mainly views on measurement of quantity of BPA leaching into the liquid stored (here water) under two different temperature conditions. The samples were subjected in a wide field of repositories from plastics to metal tins. Quantification of leached BPA was achieved by employing competitive enzyme immunoassay technique, which is an antigen-specific conjugation process. Herein, the BPA is the antigen, and the horseradish peroxidase (–HRP) is the BPA-specific antibody. The complete assay process was carried out in BPA Enzymed-Linked Immunosorbent Assay kit (12 stripes, 8 wells each). BPA–HRP conjugation was initiated by physical contact of the samples and the antibody. The process was conducted devoid of sunlight. The inhibition of the conjugation process is done by concentrated sulfuric acid. After the subsequent removal of free antibody, 3,3′,5,5′-Tetramethylbenzidine chromogen solution was added to visualize the complex product. BPA–Hypothalamic-Pituitary-Adrenal conjugation product results a colored compound, whose intensity was measured using a photometer at 450 nm to obtain the optical densities of the samples from which the concentration of the BPA leached was calculated. The findings show that water stored in a carbonated tin had the highest amount of BPA leached in comparison with other plastic repositories used. This research concludes that the prolonged use of plastic containers must be avoided and can be replaced with metal containers, which are advisable for health perspective.

Keywords: BPA; HRP; Photometry; Repositories; Leaching; Toxicity

1. Introduction

Bisphenol A (BPA), a popular organic synthetic compound used as a plasticizing material, has a chemical formula $(CH_3)_2C(C_6H_4OH)_2$ (Shown in Fig. 1). First known existence of BPA was from 1891 due to the invention by a Russian chemist Dianin [1]. In 1957, the property of BPA to harden on polymerizing with plastics was discovered, which made it an essential part of plastic industry. This property made of BPA when employed with plastics made it an excellent alternative for steel and glass containers. Within 50 years of this, BPA production in US raised to half a billion pounds [2]. It was only after the Canadian government banning BPA, the real search for its alternative began. Major healthcare companies were scrambling to avoid all the BPA they produced or sell [3]. In August 2009, the Food and Drug Administration (FDA) of United States accepted to review the toxicity posed by BPA and to conduct extensive research [4]. In 2011, FDA released its test results, according to which, it had concluded that 65% of all canned food in US are prone to BPA leaching [5].

BPA is also termed as an obesogenic compound when in high concentration. In 2011, a study was sanctioned by the Institutional Review Boards of the School of Public

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Health, Fudan University, and Shanghai Institute of Planned Parenthood Research in finding the concentration of BPA in urine of school-age children. The study helped in observing the varying concentration in students based on sex. The female students falling in the age bracket of 9–12 years were associated with weight gain and high BPA levels in urine, whereas it was not the same in the case of male students [6]. In another research experiment conducted to study the BPA influence on obesity in adults, it was concluded that adults with a median concentration of 1.5 ng/mL showed more potential to obesity than those with 1.1 ng/mL [1]. In 2009, an article published by American Journal of Health Science inferred that regulations on BPA are political and also argued for unavailability of adequate funding in this field. Most importantly, it recorded that most of the government-funded BPA research in US concluded that concentrations lower to that of the standard values are seen to have some effects [2]. In August 2004, a research group investigated that the migration of stimulants from polycarbonate containers follows an additional diffusion apart from normal Fick's law [7].

BPA has been found to leach into the liquids present inside the polycarbonates through either diffusion of residual BPA or by hydrolysis of the polymer of the item [8]. It has also been noted that the contact time, temperature, and pH of the food simulant are the main parameters impacting the leaching of BPA [9]. In an article published in 2011, the leaching of endocrine disruptors was proved evidently, invariable of their make, made, and selling point. It further adds that those advertised as BPA-free were also found to leach significant quantity of endocrine disruptors. Further, it adds that though there is less evidence to show that the products stored in those containers contribute to leaching, it is not a reason to be ruled off of research [10]. However, a study concluded that estrogenic activity (EA)-free resins are possible based on the precise selection of the EA-free additives. The plastic items made from these EA-free additives have a huge advantage of reusability with leaching any EA [11].

A review article done on BPA and effects on human beings lists a veracity of human disorders ranging from debates to blunted immune function. It argues that infertility can be linked to BPA, and there is a significant raise of infertility in the western world. It also states that for every year, 6 million tons of BPA are produced and apart from household exposure of BPA, occupational hazards too arise, on which an individual needs to be educated [12]. BPA has been studied to have an impact on male reproductive system as well. It is found that BPA is an antiandrogen compound affecting the testosterone count and quality of sperm in human [13]. Various literatures suggest the risk of exposure to BPA for cancer especially the ovarian, breast, cervical, lung, and prostate cancers [14]. Apart from just polycarbonate bottles, paper bills also contribute to BPA exposure. In a research conducted in US, of all working women, cashiers showed 25% high concentration of BPA levels compared with others [15]. BPA has become a ubiquitous existence in the environment. Plastics being burnt in open environment have been found to be the major source of BPA emitted into the atmosphere [16].

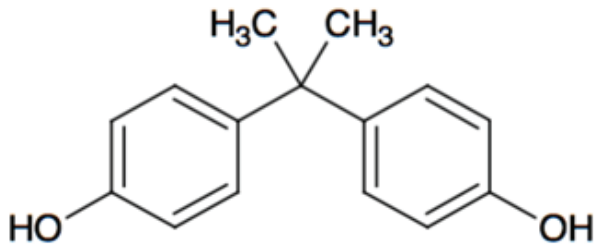

Fig. 1. Chemical structure of BPA.

2. Materials and methods

2.1. Storing procedure

Six different types of materials (two each) made of plastic were taken for the experiment. Each of the materials was numbered as per our wish. The materials were separated into two groups of six. One group was used for storage of water which was kept cold at a temperature of 10°C, and the other group was used for storage of water which was heated to a temperature of 80°C. 250 mL of sample water was taken and filled in each of the given materials. The set of plastic repositories filled with cold water was stored inside a room where a temperature of 15°C was maintained throughout, and the other set containing the hot water was placed in an open terrace with direct influence of sunlight to gain a higher temperature. The samples were kept under these conditions for 28 d before the experiment was to be performed.

2.2. Sample preparation (water samples)

Water samples were analyzed by two-step dilution tests. Using the direct method, the measuring range of BPA was found out. 250 µL of the sample water was pipetted out into a glass tube. 50 µL of 100% MeOH was added to the glass tube. 200 µL of the dilution buffer mix was added to the tube. An aliquot of 50 µL was used for testing in the Enzymed-Linked immunosorbent assay (ELISA) kit. The above procedure was repeated for all the 12 samples of water. The experimental flowchart for sample preparation is given in Fig. 2.

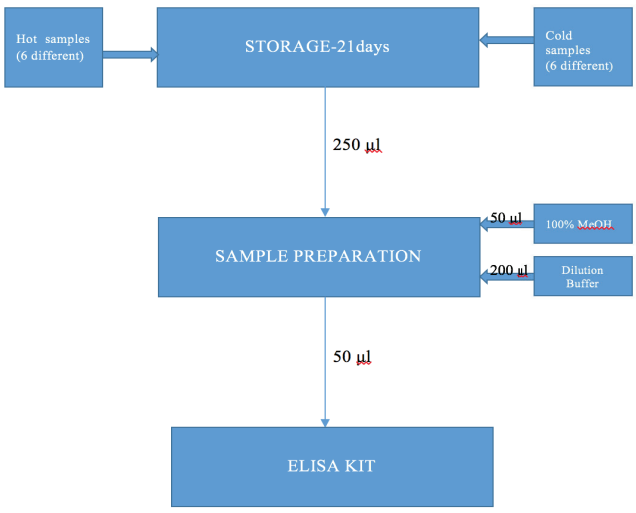

Fig. 2. Experimental flow chart.

2.3. Reagents preparation procedure

The reagents were brought to ambient temperature before the experiment was started. The reagents were freshly prepared and made sure that there were no contaminants in them. For sample dilution buffer preparation, 9 mL of dilution buffer was added to 1 mL of 10% methanol. 5 μL of the conjugate is added to 495 μL dilution buffer as the conjugate is delivered 100 times concentrated. 400 μL was required for every 2 × 8 wells. The antibody is delivered 100 times concentrated; hence, 5 μL of the antibody is added to 495 μL dilution buffer to make it into a diluted mix. 400 μL was required for every 2 × 8 wells. The remaining conjugate and antibody solution was stored at 2°C –8°C for further use. Dilutions were freshly prepared. The rinsing buffer was diluted as it was 20 times concentrated. 40 mL of rinsing buffer was used for each well (2 mL rinsing buffer + 38 mL distilled water). The substrate solution precipitates at 4°C and hence the substrate solution was stored in the dark and only taken before usage.

2.4. Testing procedure (ELISA kit)

In ELISA kit, between each immunological incubation step, unbound components have to be removed efficiently. This was achieved by appropriate rinsing. Rinsing was carried out in such a way that good inter- and intra-assay results were produced.

2.5. Manual rinsing

The contents of each well were emptied by turning the microtiter plate upside down, and the residual liquid was removed by striking the plate against a paper tissue towel. The wells were filled up to the rim by the rinsing buffer. The rinsing cycle was carried out three times. The plates were turned upside down and emptied by a vertical movement. The inverted plate was placed on absorbent paper towels and tapped firmly to remove residual washing solution if any present. It was also made sure none of the wells dried out before the next reagent was dispensed.

2.6. Assay protocol

The samples and reagents were prepared and kept ready for the experiment. 100 μL of zero standard was pipetted in duplicates into the blank wells H1 and H2. 50 μL of zero standard was pipetted in duplicates in wells A1 and A2. 50 μL of each standard dilution was pipetted in duplicate from wells B1 and B2 to G1 and G2. 25 μL of diluted conjugate was added to all the wells except H1 and H2. 25 μL of diluted antibody solution was added to all the wells except H1 and H2. The microtiter plate was sealed and shaken in a microtiter plate shaker for a few minutes. It was incubated in the dark for an hour at 4°C. The solution was discarded from the microtiter plate and washed three times with rinsing buffer. 100 μL of substrate solution was pipetted into each well as shown in Fig. 3. It was incubated for 30 min at 20°C–25°C. 100 μL of stop solution was added to each well as shown in Fig. 4. The optical density (OD) values were recorded immediately at 450 nm using an ELISA kit reader [17–19].

3. Results and discussions

The readings from the ELISA reader were tabulated, and the procedure for calculating the BPA concentration is discussed below. The OD of the sample wells was found using an ELISA reader and was noted down as shown in Table 1. The absorbance value of sample in each well was taken at 450 nm in an ELISA reader. Subtract the mean OD of the wells H1 and H2 (blank) from the individual OD of the wells containing the standards and the samples. The OD values of the six standards and the samples (mean values of the duplicates) are divided by the mean OD value of the zero standard (wells A1 and A2) and multiplied by 100, mathematically represented as maximal absorbance = (OD of sample/OD of zero standard) × 100. The zero standard (B_{max}) is thus made equal to 100% (maximal absorbance), and the other OD values are quoted in percentages of the maximal absorbance as

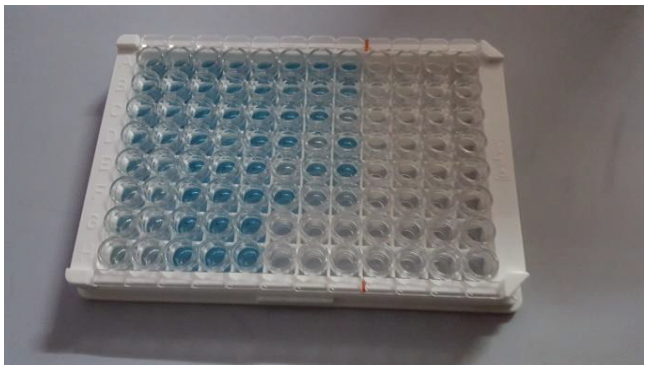

Fig. 3. ELISA kit added with substrate solution.

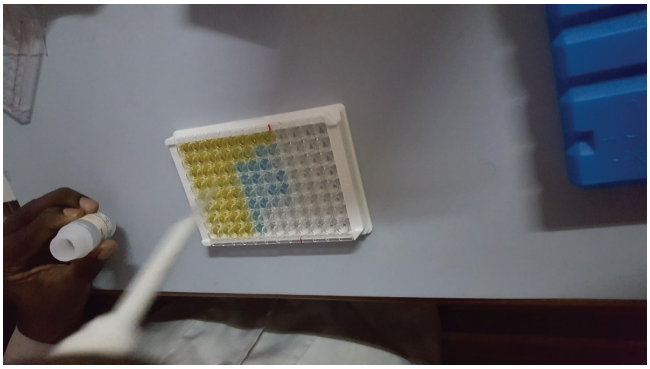

Fig. 4. ELISA kit added with stop solution.

Table 1
Optical density value for each samples in well

	1	2	3	4	5	6	7	8
A	1.275	1.283	1.478	1.194	1.235	1.273	1.219	1.249
B	1.19	1.188	1.249	1.15	1.246	0.942	0.945	0.926
C	1.133	1.033	1.209	1.236	1.231	1.145	1.159	0.577
D	1.016	0.95	1.226	0.874	1.236	1.244	0.579	1.218
E	0.883	0.715	1.252	1.241	1.232	0.563	1.071	1.232
F	0.789	0.77	1.253	1.184	1.252			
G	0.689	0.71	1.223	1.242	1.298			
H	0.583	0.541	1.235	1.224	1.285			

shown in Table 2. The mean OD of H1 and H2 is calculated and is found to be 0.562 from Table 1.

The maximum absorbance (B_{max}) for standard concentration wells was calculated, and the standard curve plotted with concentration in X-axis and B_{max} in Y-axis is shown in Fig. 5. The B_{max} of the different repositories used has been calculated and tabulated in Table 3 on the basis of the temperature under which they were stored using the standard curve plotted. Fig. 6 gives a graphical comparison of the B_{max} of each repository on varying temperature of the sample kept under. Using Fig. 7 as the standard calibration curve, we have found out the concentration of the BPA leached in each repository having a specific B_{max} value and the concentration in ng/mL. Each line has been marked with a number corresponding to that of the serial number of the repository (Table 4). From Fig. 7 and Table 4, we are able to find the concentration of BPA in each repository tested. The leaching of BPA dependence on temperature varies from sample to sample. But on an average scale, the difference between the hot and cold condition was about 0.02–0.025 ng/mL. Besides, carbonated drinks have showed a hike in difference of varying temperatures amounting to 1.09 ng/mL; this shows that temperature has a sound effect on BPA leaching. While other samples were found to agree well to this mean value, artificial juice bottles showed a deviant characteristic, temperature variation creating a difference of about 0.2 which is about 10 times that of the average value.

The various repositories have been found to have leached BPA of concentration greater than 0.1 ng/mL. The various studies in different countries have come up with different Tolerable Daily Intake (TDI) values, varying from 0.1 to 1.5 μg/kg body weight. The investigation carried out shows

Table 2
Subtracting mean OD of H1 and H2 from all the wells

	1	2	3	4	5	6	7	8
A	0.713	0.721	0.916	0.632	0.673	0.711	0.657	0.687
B	0.628	0.626	0.687	0.588	0.684	0.38	0.383	0.364
C	0.571	0.471	0.647	0.674	0.669	0.583	0.597	0.015
D	0.454	0.388	0.664	0.312	0.674	0.682	0.017	0.656
E	0.321	0.153	0.69	0.679	0.67	0.001	0.509	0.67
F	0.227	0.208	0.691	0.622	0.69			
G	0.127	0.148	0.661	0.68	0.736			
H	–	–	0.673	0.662	0.723			

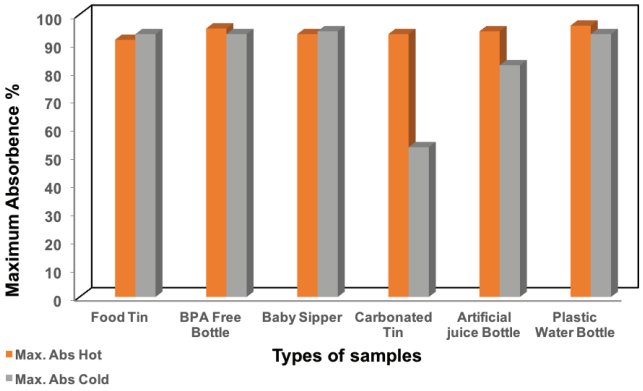

Fig. 6. Maximum absorbance (%) comparison between hot and cold condition.

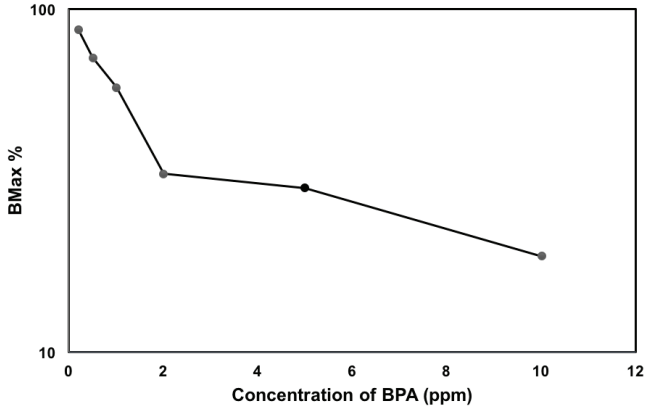

Fig. 5. Standard calibration curve.

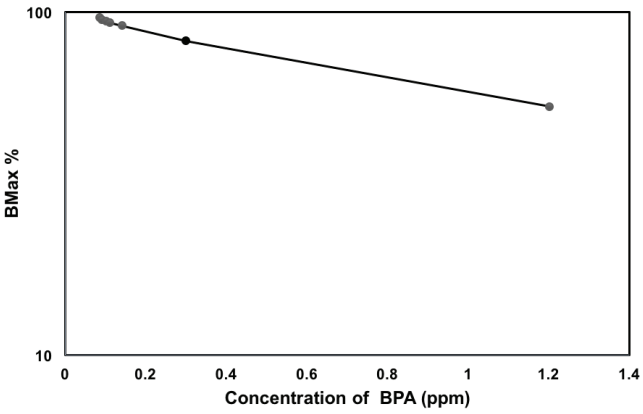

Fig. 7. Analysis of BPA.

Table 3
Calculation of maximum absorbance (%) for hot samples

S. no.	Sample	Maximum absorbance, B_{max}, of hot samples (%)	Maximum absorbance, B_{max}, of cold samples (%)
1	Food tin	91	93
2	BPA free bottle	95	93
3	Baby sipper	93	94
4	Carbonated tin	93	53
5	Artificial juice bottle	94	82
6	Plastic water bottle	96	93

Table 4
Calculation of concentration of BPA

S. no.	Maximum absorbance, B_{max}, (%)	Concentration (ng/mL)
1	96	0.085
2	95	0.09
3	94	0.10
4	93	0.11
5	91	0.14
6	82	0.30
7	53	1.2

that the majority of the samples have exceeded the average TDI limit. There is a need for further studies on individual repositories in daily basis to understand the kinetics of BPA leaching.

4. Conclusion

A systematic investigation of BPA and its leaching properties in different repositories and conditions were studied. The six repositories were chosen in specific due to their regular usage in day-to-day life. Leaching of BPA into specimen samples were confirmed using BPA ELISA kit. The concentration of BPA in the repositories was quantified by studying their absorbance behavior to light at 450 nm. The OD values for each sample in the wells were found to differ from that of its blank solution. This deviation is due to the unique reaction of horseradish peroxidase conjugate with the BPA-leached samples. The concentration of each samples was found using the standard curve plotted between B_{max} and concentration. The obtained results imply that B_{max} and concentration of BPA leached are inversely proportional to each other. The leaching of BPA was found significantly higher in cold and room temperature. Among the repositories used, carbonated tin showed the highest concentration of BPA, which clearly implies that a considerably larger amount of BPA is used in the tin linings and its manufacturing processes. It is advised to use metal containers (brass and copper) and BPA free bottles which are less prone to health risks. The plastic bottles are advised to be thrown away and made sure no further storage was done. From the results, it can be concluded that carbonated tins and artificial juice bottles have the worst effect on human health and were advised to be avoided. The overall research work indicates that storing of water in plastic wares in refrigerators or under room temperatures for a long period of time must be avoided.

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References

[1] B. Liu, H.-J. Lehmler, Y. Sun, G. Xu, Y. Liu, G. Zong, Q. Sun, F.B. Hu, R.B. Wallace, W. Bao, Bisphenol A substitutes and obesity in US adults: analysis of a population-based, cross-sectional study, Lancet Planet. Health, 1 (2017) 114–122.
[2] E. Greiner, T. Kaelin, G. Toki, Bisphenol A, Chemical Economics Handbook, SRI Consulting, Menlo Park, CA, 2004.
[3] Draft Assessment of Bisphenol A for Use in Food Contact Applications, Food and Drug Administration, Washington, D.C., USA, 2008.
[4] M. Perrone, Sunoco Restricts Sales of Chemicals Used in Bottles, The Washington Post, 2009.
[5] G.O. Noonan, L.K. Ackerman, T.H. Begley, Concentration of bisphenol A in highly consumed canned foods on the U.S. market, J. Agric. Food Chem., 59 (2011) 7178–7185.
[6] D.-K. Li, M. Miao, Z. Zhou, C. Wu, H. Shi, X. Liu, S. Wang, W. Yuan, Urine Bisphenol-A level in relation to obesity and overweight in school-age children, PLoS One, 8 (2013) e65399.
[7] T. Begley, L. Castle, A. Feigenbaum, R. Franz, K. Hinrichs, T. Lickly, P. Mercea, M. Milana, A. O'Brien, S. Rebre, R. Rijk, O. Piringer, Evaluation of migration models that might be used in support of regulations for food-contact plastics, Food Addit. Contam., 22 (2005) 73–90.
[8] K.A. Ehlert, C.W. Beumer, M.C. Groot, Migration of bisphenol A into water from polycarbonate baby bottles during microwave heating, Food Addit. Contam., 25 (2008) 904–910.
[9] E.J. Hoekstra, C. Simoneau, Release of Bisphenol A from polycarbonate—a review, Crit. Rev. Food Sci. Nutr., 53 (2013) 386–402.
[10] C.Z. Yang, S.I. Yaniger, V.C. Jordan, D.J. Klein, G.D. Bittner, Most plastic products release estrogenic chemicals: a potential health problem that can be solved, Environ. Health Perspect., 119 (2011) 989–996.
[11] G.D. Bittner, M.S. Denison, C. Z. Yang, M.A. Stoner, G. He, Chemicals having estrogenic activity can be released from some bisphenol A-free, hard and clear, thermoplastic resins, Environ. Health, 13 (2014) 1–18.
[12] J.R. Rochester, Bisphenol A and human health: a review of the literature, Reprod. Toxicol., 42 (2013) 132–155.
[13] C. Desdoits-Lethimonier, L. Lesne, P. Gaudriault, D. Zalko, J.P. Antignac, Y. Deceuninck, C. Platel, N. Dejucq-Rainsford, S. Mazaud-Guittot, B. Jegou, Parallel assessment of the effects of bisphenol A and several of its analogs on the adult human testis, Human Reprod., 32 (2017) 1465–1473.
[14] A. Shafei, M.M. Ramzy, A.I. Hegazy, A.K. Husseny, U.G. El-Hadary, M.M. Taha, A.A. Mosa, The molecular mechanisms of action of the endocrine disrupting chemical bisphenol A in the development of cancer, Gene, 647 (2018) 235–243.
[15] A.L. Wozniak, N.N. Bulayeva, C.S. Watson, Xenoestrogens at picomolar to nanomolar concentrations trigger membrane estrogen receptor-alpha-mediated Ca^{2+} fluxes and prolactin release in GH3/B6 pituitary tumor cells, Environ. Health Perspect., 113 (2005) 431–439.
[16] P. Fu, K. Kawamura, Ubiquity of bisphenol A in the atmosphere, Environ. Pollut., 158 (2010) 3138–3143.
[17] Commission Directive relating to plastic materials and articles intended to come into contact with foodstuffs, Off. J. Eur. Union, 220 (2002) 18–58.
[18] Commission Directive amending Directive as regards the restriction of use of Bisphenol A in plastic infant feeding bottles, Off. J. Eur. Union, 26 (2011) 11–14.
[19] EFSA Draft opinion on BPA exposure, 2013.