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STUDYING OF BISPHENOL A LEVELS IN SOME CANNED FOOD, FEED AND BABY BOTTLES IN EGYPTIAN MARKETS

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ABSTRACT

Bisphenol A (BPA) is an endocrine disrupter which causes negative effects on human health. The aim of the present study is to determine BPA levels leaching of several kinds of canned foods, beverages, baby bottles and feed in local Markets by using gas chromatography-mass spectrum (GC/MS/MS). Furthermore, estimate a relationship between BPA migrations from polycarbonate baby bottles, temperature, and effect of numbers of use. Results showed that in more than 130 different samples taken from Egyptian markets, the highest mean BPA level was in chicken cocktail sausaged (710.59 ppb) which was given highly significant results with regard to all other food samples result, whilst the lowest was for tomato ketchup at (5.75 ppb). BPA levels were ranged between 31.02 and 724.06 for canned meat, 5.57 and 233.78 for vegetables and fruits samples and 30.89 and 312.08 for milk samples. It was found that the levels of BPA ranged between (233.65 to 933.75 ppb) in feed additives. The leaching of BPA from baby bottles to milk was found to be highest contents (123.53 ppb) compare with other baby drinks. The effect of temperature and numbers of use on BPA migration from baby bottles to milk results indicated a positive relationship between them, wheres an increasing of temperatures and numbers of use increased BPA migration levels in milk. The concentration of BPA in milk has reached 1046.79 ppb when baby bottles were used 100 times at 90℃.

KEYWORDS:
Baby bottles, Bisphenol A, Feed, Food, Numbers of use, Temperature.

INTRODUCTION

Years ago endocrine disruptors have caught the attention of the world because of their possible harmful effects on human health. Bisphenol A (BPA) is a substance of worry since it is an endocrine disruptor and has been related to different negative health effects [1].

BPA is an organic synthetic compound consists of two phenol rings associated with a methyl connect, with have two methyl functional groups joined to the bridge. BPA made by mixing acetone and phenol is utilized to produce epoxy resins and polycarbonate (PC) plastics. Epoxy resins are utilized to lining metal cans to avoid touch between the inside can surface and the food, for prevent can erosion and rustiness [2]. BPA can be leaching when canned food is heated to typical can processing temperatures [3]. BPA can also be leaching from deficiently polymerized epoxy resins.

These protective layer are like utilized for metal covering in cap for food in glass bottles [2]. Because of a deficient polymerization operation, remains of BPA monomer in PC container and covering can be leaching into food, mostly throughout storage and manufacturing at elevated temperatures [4].

Polycarbonate plastics are utilized in food and drink canned and reusable jugs. The carbonate linkages are relatively steady however can be hydrolyzed at high temperature and unbiased to antacid pH, bringing about the arrival of BPA [5]. Various variables including pH, salt, oil, and glucose have been appeared to impact the movement of BPA from the lacquer and plastic infant bottle to the continuer content [6, 7].

The major risk of BPA is food exposition for kids and elderly [8]. BPA caused harmful health effect so should domination leaching from container coatings by setting assizes by various agencies. The leaching limit for BPA in food was confirmed by [9] at 600 ng/g whilst the tolerable daily intake (TDI) and maximal reference dose were confirmed at (50 and 25 µg/kg body weight/day) by [10, 11]. As of late, another preparatory 10 time bring down TDI of 5 µg/kg b.w. /day was prescribed by [12].

Inside mice, low measurements presentation has been related to early maturity [13], increased nervous action [14], and changed maternal conduct [15]. Exposition to BPA through different life stages has additionally been related to specific diseases, including mammary gland and prostate tumors; diabetes; and immune system modification [16]. Rising urinary BPA levels have been significantly connected with male sexual dysfunction as depicted by seven files, including reduced sexual lust, erectile, and ejaculatory issues [17, 18]. Many studied on animal indicated BPA to be a regenerative, formative, and systemic toxicant, and lowly estrogenic [19]. Woodruff et al., [20] Examined the number of chemicals to which pregnant ladies in the U.S. are uncovered discovered BPA in 96% of studied ladies. Moreover, drinking water and different drinks from plastic containers made with BPA raised the urinary concentration of the dangerous compound by about 70% [21].

The point of this survey was to determine BPA levels leaching in several kinds of canned foods, feed, beverages and drinks in baby bottles in Egyptian Markets. Furthermore, estimate a relationship between BPA migrations from polycarbonate baby bottles, temperature, and effect of numbers of use.

MATERIALS AND METHODS

Materials and reagents. Bisphenol A standard (minimum purity ≥ 99%) was purchased from Sigma–Aldrich (UK), n-Heptane (Analytical reagent grade, Fisher chemical), Acetonitrile (J. T. Baker, HPLC grade), Anhydrous sodium sulphate (Sigma-Aldrich), Potassium carbonate (Sigma-Aldrich), Acetic acid anhydrous (Honeywell, 99.8%) and distilled water.

Samples. (1) Canned Food samples. Different types of market packing food, three cans for each sample which include (Beef, sausaged, luncheon, tuna, Beans, Mushroom, Sweetcorn, Tomato ketchup, Tomato paste, oil, pickles, fruit, milk, and Beverages) were purchased from different local markets in Egypt.

(2) Feed samples. Seven feed additives samples (a mixture of amino acid, vitamin and essential oil addition in the diet for animal) were purchased from different countries in different types of plastic bottles.

(3) Water samples. Reusable water bottles. Four brands of mineral water bottles have been chosen.

(4) Drinks in baby bottles. Different liquids given to baby in a baby bottle i.e. anise, milk, and water.

Determination of Bisphenol. A Sample preparation. The whole contents of a can were fully blended utilizing homogenizer. Twenty gram (g) of subsample was taken. All content canned samples were extracted by adding n-heptane and acetonitrile (20 ml + 20 ml) into glass measure utilizing a homogenizer for five min. The extract was remained for15 min then filtrated by utilizing Double rings paper and a Buchner flask. The heptane was taken away, utilizing glass syringe then make another extraction on residue sample in filter paper by adding new acetonitrile 20 ml utilizing the Omni blender homogenizer. Then filtered another extraction as before and removed heptane. The twice acetonitrile was collective and added anhydrous sodium sulphate (30 g, 1 min), then poured the mixture into cylinder 100 ml. Canned samples which content little lipids, such as meat, fish, oil, vegetables, fruits and drinks heptane was decanted. The mixture was steamed to around 5 ml, then dilution by water to 50 ml and put in separating funnel 300 ml. Added potassium carbonate and methanol {10 ml (72% w/v), 10 ml} respectively, then added 10 ml of acidic anhydride to the mixture whirled slowly. Then left the mixture for 15 min and extracted by n-heptane 5 ml. The heptane solvent was gathered and determine by GC/MS/MS.

Baby formula samples were extracted by weighted ten g of sample and added acetonitrile 20 ml into glass beaker 50 ml and shaking it for 5 min. Baby formulae samples were extracted by weighted ten g of sample and added acetonitrile 20 ml into glass beaker 50 ml and shaking it for 5 min. The mixture was permitted to settle, then filtered an acetonitrile by a Double rings paper. The remains of a sample were extracted again by added acetonitrile 10 ml, then shaken and filtered. The remains of sample was extracted again by added acetonitrile 10 ml, then shaken and filtered. The two mixture were collective and steamed such as mentioned above. Drinks samples should open to permit gas to go out before extraction. Fifteen ml of the sample was put into separation funnel 250 ml and extracted as previously mentioned. The experimental of leaching BPA into water samples from the bottles added boiling water to all bottles and left one day [22].

Effect of temperature and numbers of use on PC baby bottles. The PC baby bottles were brought with various brand from a local pharmacy in Egypt and taken three bottles from each brand. All bottles were rinsed with water. Baby bottles are always washed up and sterilized before being used and filling with water, anise, and milk to each bottle to its capacity and left one hour. Then tested the effect of different temperature and times of use it.

Condition of GC/MS/MS. The determination of the bisphenol A was done utilizing a gas chromatography (Agilent Technologies 7890A) connected by a mass-selective detector (MSD, Agilent 7000). Which content 5% - phenyl methyl polysiloxane (HP-5ms, Agilent) and column (capillary, 30 m × 0.25 mm i. d. and 0.25 µm film thickness Helium was used as carrier gas by velocity (one ml/ min). The program of the oven was (120 ºC / 1 min, 1.5 min at a rate of 30 ºC/ min then 300 ºC /1.7 min at a rate of 35 ºC/min). The temperature of injector and

TABLE 1
Bisphenol A Levels in food from local market

Products	Brand	PH	Pack type	BPA Conc. (ppb)	Average range
Meat					
Corned beef	A	6.1	Can	$255.78^{c} \pm 0.39$	(255.10 - 255.78)
Corned beef	B	6.1	Can	$84.49^{lm} \pm 1.12$	(82.46- 86.31)
Chicken cocktail sausaged	A	6.4	Can	$710.59^{a} \pm 7.97$	(696.51 - 724.06)
Chicken luncheon meat	B	5.3	Can	$32.52^{q} \pm 0.87$	(31.02 - 34.02)
Beef luncheon meat		5.9	Can	$75.50^{n} \pm 0.88$	(74.56 - 77.26)
Mean				231.77±125.41	(31.02 - 724.06)
Fish					
Light meat tuna with chili (shredded)	A	6.5	Can	$94.11^{k} \pm 1.51$	(91.35 - 96.56)
Dark shredded tuna meat skipjack	B	6.1	Can	$60.80^{o} \pm 1.77$	(57.68 - 63.79)
Light meat tuna (fancy solid)	C	5.8	Can	$146.49^{hi} \pm 2.80$	(141.35 -150.96)
Dark shredded tuna	D		Can	$167.94^{g} \pm 0.32$	(167.41- 168.52)
Mean				117.34 ± 28.21	(57.68 - 168.52)
Vegetables and Fruits					
Beans					
Plain Mesdames fava beans	A	6.2	Can	$49.59^{p} \pm 0.85$	(48.66 - 51.28)
Fava beans with Salsa	C	5.8	Can	$114.14^{j} \pm 1.32$	(112.22 - 116.67)
Bean in plastic bag	D	6.6	Plastic	$14.90^{uv} \pm 0.18$	(15.20 - 14.91)
White kidney beans	A	6.2	Can	$164.77^{g} \pm 1.12$	(162.54 - 165.89)
Red kidney beans dark	B	5.8	Can	$60.66^{o} \pm 0.89$	(59.01 - 60.92)
Mushroom					
Mushroom	A	5.7	Can	$23.48^{st} \pm 0.11$	(23.3 - 23.69)
Mushroom	B	5.7	Can	$17.61^{tu} \pm 0.21$	(17.18 - 17.82)
Sweet corn					
Whole kernel golden sweet corn	A	6.5	Can	$6.14^{w} \pm 0.23$	(5.68 - 6.38)
Kernel sweet corn	B	5.7	Can	$7.46^{w} \pm 0.13$	(7.24 - 7.70)
Tomato					
Tomato paste	A	4.1	Can	$83.20^{m} \pm 1.07$	(81.91 - 85.33)
Tomato paste	B	4.0	Can	$19.62^{tu} \pm 0.23$	(19.29 - 20.06)
Tomato ketchup	A	3.7	Plastic	$8.69^{vw} \pm 0.09$	(8.51 - 8.81)
Tomato ketchup	B	3.6	Plastic	$9.75^{vw} \pm 0.52$	(8.72 -10.30)
Tomato ketchup	C	3.7	Plastic	$5.75^{w} \pm 0.09$	(5.57 - 5.88)
Local tomato sauce of koushari	D	4.3	Plastic	$231.59^{e} \pm 1.32$	(229.21 - 233.78)
Pickles					
Pickles (olive)	A	3.4	Plastic	$29.42^{qr} \pm 0.39$	(28.69 - 30.04)
Pickles (lemon)	B	2.3	Plastic	$10.46^{vw} \pm 0.37$	(10.02 - 11.19)
Oil					
Local market oil	A		Plastic	$79.44^{mn} \pm 3.87$	(72.1 - 85.22)
Fruits					
Peach (halves of peaches in light syrup)	A	3.2	Can	$74.63^{n} \pm 0.17$	(74.31 - 74.89)
Peaches (halves in light syrup)	B	3.8	Can	$22.24^{st} \pm 0.99$	(21.14 - 24.21)
Mean of vegetable and fruits				51.53 ± 12.79	(5.57 - 233.78)
Milk					
Cream cheese spread	A	6.5	Plastic	$31.38^{q} \pm 0.24$	(30.89 - 31.62)
Cream	B	6.5	Can	$219.85^{f} \pm 0.80$	(218.33 - 221.04)
Milk (infant milk formula)	A	5.9	Can	$89.35^{l} \pm 1.06$	(87.34 - 90.95)
Milk (sweetened condensed milk)	B	6.6	Can	$33.43^{q} \pm 0.48$	(32.71 - 34.35)
Yoghurt	A		Plastic	$236.76^{d} \pm 1.67$	(234.95 - 240.1)
Dannet chocolate	A	6.7	Plastic	$79.28^{mn} \pm 0.64$	(78.21 - 80.43)
Dannet vanilla	B	6.5	Plastic	$311.74^{b} \pm 0.17$	(311.50 - 312.08)
Mean				129.38 ± 55.30	(30.89 - 312.08)
Beverages					
Orange juice	A	3.9	Plastic	$149.36^{h} \pm 3.67$	(142.2 - 154.34)
Soft drink Orange juice	B	3.3	Plastic	$35.53^{q} \pm 0.15$	(35.36 - 35.83)
Guava juice	A	4.0	Plastic	$17.06^{tu} \pm 0.26$	(16.72 - 17.58)
Guava juice	B	4.0	Plastic	$32.63^{q} \pm 0.28$	(32.24 - 33.17)
Nectar mango	A	3.7	Plastic	$25.79^{rs} \pm 0.27$	(25.36 - 26.28)
Mango float	B	3.6	Can	$14.50^{uv} \pm 0.28$	(13.96 - 14.87)
Mean				45.81 ± 29.71	(13.96 -154.34)
L.S.D 0.05				4.6280	

- Each value represents the mean ± S.E (Standard Error) and mean of three replicates.
-Values in the same column with the same letter are not significant at $p \leq 0.05$.
- Brand (A, B, C and D) means different company.
- PET (Polyethylene Terephthalate).

detector were 250 ºC. The mode of injection was (split to split, rate 1: 20) and one µl of volume. The mass has parameter ionization potential and interface temperature (70 eV, 280 ºC). The system of selected ion monitoring (SIM) was utilized (213 and 228 m/z). Quantitative results were acquired utilizing the GC-MS outcomes by comparing with external standards.

Statistical analysis. Statistical analysis (standard error ''SE'') was done by [23]. LSD (Least significant difference) analysis was utilized to contrasts any significant differences between the means of treatment [24]. All static analysis was done by Costat program.

RESULTS AND DISCUSSION

Canned food results. BPA contents in food samples under study are appeared in Table 1. It was seen that chicken cocktail sausaged (710.59 ppb) which have highest average BPA concentration result contrasted with other samples results, trailed by dannet vanilla (311.74 ppb); whilst the lowest were for tomato ketchup at (5.75ppb).

Our results agree with [25] indicated for BPA levels in canned corned beef and chicken ranged (17 - 602 ppb). Also, [22] reported for infant formula and milk (9 - 384 ppb) which agree with present results.

The appearance of BPA in canned food due to that the canned foods were sterilized during the production process. Goodson et al. [26] reported that storage factor of the can such as time, temperature and state of the can may affect the levels of BPA in food.

Besides to food in paper containers, the release of BPA into food due to mostly reused this container and can lining materials [27]. BPA will probably migrate into oily, fatty foods or high protein contents food such as meat, fish and coconut cream [28].

Results of baby bottles samples. Table 2 gives the summarized results about BPA levels in two different types baby bottles filling with various kinds of liquid. These data indicate the levels of BPA at PC baby feeding bottles and water drinking bottles. As can be seen, the highest mean BPA level values was in milk in baby bottle brand (A) (123.53 ppb) which was a highly significant result with respect to all drinks samples results, trailed by anise in baby bottle brand (B) (114.94 ppb); whilst the lowest in water in bottle (3.88 ppb).

From above result, it could make seen that the BPA was leaching from the PC bottles to the drinking water they contain. The highest level of BPA in milk due to milk content amines like putrescine and 1,4-diaminobutane which two amines that produce from degradation of protein in milk that simulants migration of BPA and that has been a statement by [29, 30].

The high level may be due to the exposure of baby bottles to the high temperature of the anise or milk while that is not happening in water samples.

Results of feed additives samples. The concentrations of BPA in feed additives are shown in Table 3. It was noted that the levels of BPA ranged between (233.65 to 933.75 ppb) in feed additives bottles which made from PC sample (A, B and C), which agree with [31, 25] which reported that levels of BPA in canned pet foods ranged (179 and 602 ppb). While the levels of BPA ranged between (2.03 to 4.24 ppb) in feed additives bottles which made from HDPE samples (D, E, F, and G).

BPA contents in packing material, like as, HDPE, Tetra Pak, glass, and PET were very smaller than PC canned foods and drinks. This showed that low-level presence of BPA through food processing and before to filling into the container which as reported by [32]. As the presences of BPA in feed additives probably will be found in animal milk which could be risked on human as a result.

TABLE 2
Bisphenol A Levels in two baby bottles after 1h from filling

Products	Brand	PH	Pack type	BPA Conc. (ppb)	Range
Anise in baby bottle	A	8.50	PC	6.53 ^{ef} ± 0.18	(6.21- 6.84)
Anise in baby bottle	B	8.50	PC	114.94 ^{b} ± 1.17	(112.68 - 116.56)
Milk in baby bottle	A	7.00	PC	123.53 ^{a} ± 1.27	(121.28 - 125.66)
Milk in baby bottle	B	7.00	PC	70.27 ^{c} ± 0.28	(69.83 - 70.78)
Water in baby bottle	A	8.50	PC	4.60 ^{fg} ± 0.39	(3.83 - 5.13)
Water in baby bottle	B	8.60	PC	16.55 ^{d} ± 0.42	(16.13 - 17.39)
Water in bottle	A	8.70	PC	5.38 ^{efg} ± 0.46	(4.47 - 5.93)
Water in bottle	B	8.80	PC	3.88 ^{g} ± 0.08	(3.79 - 4.04)
Water in bottle (reused mineral water bottles)	C	8.70	PET	7.05 ^{e} ± 0.18	(6.69 - 7.23)
Water in bottle (water bottle)	D	8.30	PET	4.48 ^{fg} ± 0.16	(4.26 - 4.80)
Mean				35.72 ± 27.99	(3.88 - 123.53)
L.S.D 0.05				1.7883	

- Each value represents the mean ± S.E (Standard Error) and mean of three replicates.
-Values in the same column with the same letter are not significant at p≤0.05.
- Brand (A, B, C and D) means different company.
- PET (Polyethylene Terephthalate), PC (Polycarbonate).

TABLE 3
Bisphenol A levels in Feed additives

Products	Brand	PH	Pack type	BPA Conc. (ppb)	Range
Feed additives	A	3.80	PC	$933.75^{a} \pm 2.49$	(929.03 - 937.49)
Feed additives	B	7.70	PC	$414.48^{b} \pm 2.51$	(410.45 - 419.07)
Feed additives	C	6.30	PC	$271.09^{c} \pm 3.93$	(265.86 - 278.78)
Feed additives	D	6.80	HDPE	$7.05^{d} \pm 0.38$	(6.41- 7.71)
Feed additives	E	2.70	HDPE	$4.24^{d} \pm 0.28$	(3.69 - 4.62)
Feed additives	F	4.70	HDPE	$2.91^{d} \pm 0.02$	(2.87 - 2.95)
Feed additives	G	2.30	HDPE	$2.03^{d} \pm 0.04$	(1.96 - 2.07)
Mean				233.65 ± 202.33	(2.87 - 937.49)
L.S.D 0.05				14.1729	

- Each value represents the mean ± S.E (Standard Error) and mean of three replicates.
-Values in the same column with the same letter are not significant at p≤0.05.
- Brand (A, B, C, D and G) means different company.
- HDPE (High density polyethylene), PC (Polycarbonate).

TABLE 4
Bisphenol A levels in milk from PC baby bottles as affected by PC source, temperature and numbers of use

Numbers of use	Type	Temperature / Conc. (ppb)			Range
		25 °C	60 °C	90°C	
	A	$48.50^{g} \pm 0.89$	$65.62^{h} \pm 0.40$	$105.90^{i} \pm 0.33$	$65.62^{h} \pm 0.40$
New bottles	B	$38.04^{h} \pm 0.28$	$165.02^{g} \pm 0.42$	$222.90^{g} \pm 1.47$	$165.02^{g} \pm 0.42$
	C	$67.61^{f} \pm 1.09$	$161.94^{g} \pm 1.35$	$205.71^{h} \pm 1.33$	$161.94^{g} \pm 1.35$
Mean		51.38 ± 8.66	130.86 ± 32.63	178.17 ± 63.48	
	A	$137.80^{e} \pm 0.39$	$190.28^{f} \pm 4.07$	$399.38^{f} \pm 2.01$	$190.28^{f} \pm 4.07$
50	B	$291.57^{d} \pm 1.14$	$436.40^{d} \pm 4.33$	$623.81^{d} \pm 3.02$	$436.40^{d} \pm 4.33$
	C	$132.54^{e} \pm 1.36$	$265.70^{e} \pm 2.00$	$524.85^{e} \pm 3.50$	$265.70^{e} \pm 2.00$
Mean		187.30 ± 52.16	297.46 ± 72.80	516.01 ± 64.94	
	A	$405.01^{c} \pm 0.78$	$527.37^{c} \pm 1.75$	$842.15^{c} \pm 1.31$	$527.374^{c} \pm 1.75$
100	B	$614.04^{a} \pm 3.98$	$918.25^{a} \pm 3.72$	$1022.92^{b} \pm 2.05$	$918.25^{a} \pm 3.72$
	C	$471.32^{b} \pm 4.32$	$883.53^{b} \pm 3.61$	$1046.79^{a} \pm 4.81$	$883.53^{b} \pm 3.61$
Mean		497.12 ± 61.06	776.38 ± 124.19	970.62 ± 64.61	
L.S.D 0.05		6.2920	8.3568	7.5659	

- Each value represents the mean ± S.E (Standard Error) and mean of three replicates.
-Values in the same column with the same letter are not significant at p≤0.05.
- Brand (A, B and C) means different company.
- PC (Polycarbonate).

Effect of temperature and numbers of use of PC baby bottles on BPA levels. Table 4 including the numbers of use and temperature factors in following the leaching of baby food from baby bottles. The result clearly that there was an appositive relation between the release of BPA in milk, numbers of use and temperature. The BPA concentration was found to increase with raise the temperature of milk and increasing the numbers of using. Where found the higher concentration of BPA baby milk bottles when used 100 times at 90 °C (1046.79 ppb) and the lower concentration BPA when using new bottles at 25 °C (38.04 ppb). Fig. (1) Show that there was a linear relation between temperature, number of use and concentration of BPA.

Environment Canada [33] indicated that leaching from new bottles is very low, but can increase with repeated use, due to cleaning. Hoekstra and Simoneau [34] reported that the appearance of BPA into water due to increasing time and temperature. Le et al., [35] investigated that the concentration of BPA in boiling water which leaching from new polycarbonate drinks bottles higher than water at room temperature by factor 15 - 55.

CONCLUSION

Many various types of canned food and baby bottles could be a hazard factor for human and infant health because of the presence of variable levels of BPA. After analysis of more than 180 different sample which including meat, vegetable, baby bottles and feed additives. It was found different levels of BPA between (2.03 and 1046.79 ppb) which depend on their circumstances of production, transportation, storage, and migration process of the sample itself. Much care should be taken when chosen the material of baby bottles because of there was found that appositive relation between numbers of use and temperature with a concentration of BPA leaching in milk from PC infant bottles.

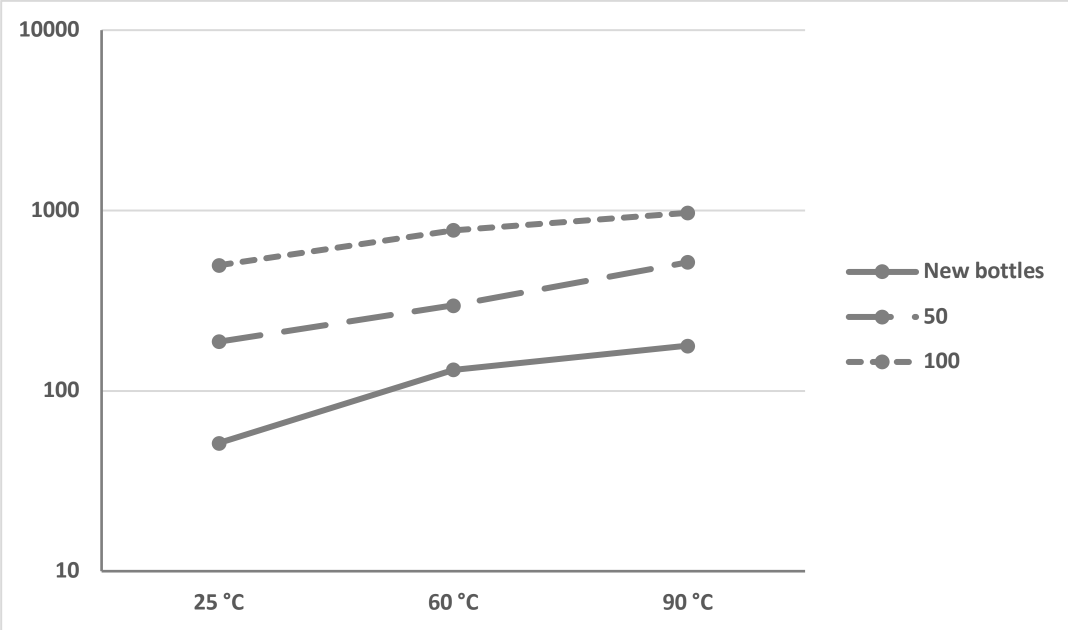

FIGURE 1
Bisphenol A levels in milk from PC baby bottles as affected by PC source, temperature and numbers of use.

To avoid exposure to contamination of food with BPA it was recommended to use more safety material in food packaging such as PET, glass, HDPE, Tetra Pack and Polypropylene (PP).

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