Phthalates and Bisphenols Migration in Mexican Food Cans and Plastic Food Containers

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Abstract: The presence of endocrine disruptors bisphenol-A, bisphenol-A-dimethacrylate, bisphenol-A-diglicydyl-ether, phthalic-acid, dibutyl-phthalate, diethyl-phthalate and dioctyl-phthalate was determined in vegetable cans, baby bottles and microwaveable containers from the Mexican market. Gas-Chromatography-Mass-Spectrometry was used for the identification and High-Performance-Liquid-Chromatography with UV/Visible light and fluorescence detectors was used for the quantification. Endocrine disruptors were found in all samples. PA and DOP were the substances most commonly found, and maximum concentrations were 9.549 µg/kg and 0.664 µg/kg, respectively from a jalapeno peppers can. Bisphenol A, phthalic-acid, bisphenol-A-dimethacrylate and dibutyl-phthalate were found in baby bottles and microwaveable containers.

Keywords: Baby bottles, cans, endocrine disruptor, plastic.

Synthetic polymers known as epoxy resins contain among their components, bisphenol A (BPA) or its derivatives, which are used to improve the durability of inner varnishes of food cans and in the manufacturing of different kinds of food containers, such as baby bottles, microwaveable containers and plastic dairy containers. Other molecules such as phthalates are used to increase the flexibility of plastics and in the manufacture of teething rings and pacifiers (European Chemicals Bureau 2003). Endocrine disruptors as bisphenols and phtalates have harsh effects on the human reproductive system (Roy et al. 2009). The bisphenols also are associated to endometrial hiperplasia and endometrial cancer in human beings (Hiroi et al. 2004). Several reports have shown the migration of BPA from cans and baby bottles to foods (Cao and Corriveau 2008; Yonekubo et al. 2008). Infants, whose growth and development are highly dependent upon the endocrine system, are particularly vulnerable to endocrine disruptor exposure (Ozaki et al. 2002). However, there are few reports showing the migration of phthalic acid and their esters in food cans or plastic food containers, despite that they also exhibit endocrine disruption activity and may produce hepatocarcinogenesis (Huber 1996; Ozaki et al. 2002).

The aim of this work was to determine the migration of phthalates and bisphenols from Mexican vegetable cans, microwaveable containers, baby bottles and plastic food containers, subjected to high temperatures.

Materials and methods

All chemicals were purchased from Sigma-Aldrich with purity of 99.9%. The analyzed samples included 24 cans of vegetables (sweet corn kernels, jalapeno peppers, mushrooms, peas, and peas and carrots), 4 plastic microwave oven containers, 12 plastic (yoghurt or cream) food containers and 6 baby bottles. All of the sample items were bought in supermarkets of San Luis Potosi, Mexico.

The extraction of bisphenols and phthalates from vegetable cans was done using water as a food simulant liquid according to the following procedure: 300 mL of water (Milli-Q) were added to each sample item and sterilized at 121°C for 15 min. An aliquot of 75 mL was taken and 20 mL of methanol were added. The aliquot was vigorously agitated for 10 min in a decantation funnel, and then 40 mL of organic solvent (see table 1) were added. The aliquot was agitated once again for 10 min and centrifuged at 1200 *g* for 15 min. The

organic phase was taken and concentrated in a rotary evaporator at 40°C and reduced pressure to a final volume of 10 mL. The solvent was further eliminated using a nitrogen stream to a final volume of 1 mL. Then 0.5 mL of sulphuric acid was added and centrifuged at 1200 *g* for 10 min. The organic phase was aspirated and dried through a stream of gaseous nitrogen. The dry remains were solubilised in 1 mL of methanol and analyzed by HPLC and GC-MS as described below. The determination of disruptors migration from baby bottles and microwave containers was based on the methodology reported by Brede et al. (2003) using a microwave oven (Samsung Co., Malaysia).

Compound identification was done with a Gas Chromatograph coupled to a Mass Spectrometry detector Starum 2000 (Varian Instrument, Walnut Creek, CA) with an automatic injector 8200, SPI/1078 (Varian Instrument, Walnut Creek, CA), and using a capillary column DB5-MS (Varian Instrument, Walnut Creek, CA) 30-meters long and 0.25 mm diameter. The injector temperature was 250°C. The GC was operated in a split-less mode using helium as the carrier gas at 1 mL/min. The temperature program started at 50°C for 3 min, and it was increased by 30°C/min rate until 200°C was reached. The temperature was held at 200°C for 5 min. Then the temperature was increased at 30°C/min to 270°C and it held at 270°C for 8 min. Followed by another temperature increase of 30ºC/min to 310ºC. The temperature was held at 310°C for 2 min. The ionization potential was 70 eV and a scan function with 100-300 *m/z*. Compounds were identified by comparing the mass spectrum with the spectra from the NIST library of the Masslab software (Thermo Quest, Manchester, UK).

Analysis by High Performance Liquid Chromatography was performed using a C-18 column 4.6 x 150 mm with a particle size of 5 µm of octadesylsilicate (Merck, Darmstadt, Germany) in a HPLC System 600 (Waters) connected to an UV/Vis 2487 detector (Waters) and a fluorescence 2475 detector (Waters). The UV/Vis detector was set at 280 nm, and the fluorescence detector had an excitation wavelength of 275 nm and an emission wavelength of 300 nm. The solvents were acetonitrile 50% (phase A) and acetonitrile 100% (phase B), at 1 mL/min of flow rate with a gradient 0-100% of B during 20 min, and a temperature of 25°C. The volume of the injected sample was 20 µL. To verify the retention times of analytes, ten samples, dissolved in methanol were injected, and arithmetic means were calculated.

Detection and quantification limits were measured by the signal/noise method and using the standard deviation of the response and the slope (Long and Winfordner 1983). Linear response of detectors was determined by straight calibration curves from the analysis of five standards with increasing analyte concentrations (Thompson et al. 2002).

Results and Discussion

The percentage of endocrine disruptors recovered using hexane, hexane:ethylic ether or chloroform during the extraction protocol is shown in table 1. Chloroform showed the highest recovery percentage with values ranging from 75.0±8.8% to 95.0±5.4% for PA and DBP respectively, whereas hexane had the lowest values. Based on these results chloroform was used in subsequent experiments.

The endocrine disruptors were identified by CG-MS as described in materials and methods. Meanwhile the quantitative analysis was carried out by HPLC. The retention

times, detection and quantification limits are shown in the table 2. The detection limit for BPA in this study was less than that reported by others authors (Lim et al. 2009).

Table 1 Percentage of endocrine disruptors recovered after the extraction using different organic solvents.

Compounds*	Quantity (ng)	Hexane (%)	Hexane: Ethylic ether 1:1 (%)	Chloroform (%)
PA	200.0	12.0±3.4	45.0±5.0	85.0±7.9
	400.0	20.0±2.9	65.0±6.4	88.0±8.7
	600.0	24.0±2.9	66.0±7.9	75.0±8.8
BPA	30.0	-	63.1±4.1	88.4±6.4
	60.0		60.2±5.6	88.1±4.1
	100.0		62.8±2.1	89.2±4.5
BADGE	3.0	-	54.0±6.6	90.0±2.3
	5.0	-	69.0±7.3	90.0±5.3
	7.0	-	64.0±2.9	92.5±3.2
BisDMA	2.0	-	67.3±4.6	88.0±5.3
	4.0	-	62.2±5.2	80.0±5.5
	6.0	-	60.7±4.5	82.0±3.8
DOP	4.0	22.3±1.8	31.4±2.2	87.5±7.6
	6.0	24.5±2.2	33.2±4.1	83.9±6.5
	8.0	21.7±3.1	30.2±2.9	84.7±5.3
DBP	0.40	45.0±4.9	58.2±4.1	90.0±7.3
	0.60	50.0±4.9	55.2±3.3	95.0±5.4
	0.80	40.0±3.9	52.2±4.2	88.0±5.7
DEP	0.40	33.2±3.1	43.2±2.5	92.0±5.4
	0.60	31.4±3.3	40.8±3.8	92.0±5.3
	0.80	30.4±2.8	40.5±5.2	90.0±5.6

*PA: phthalic-acid, BPA: bisphenol-A, BADGE: bisphenol-A-diglicydyl-ether, BisDMA: bisphenol-A-dimethacrylate, DOP: dioctyl- phthalate, DBP: dibutyl-phthalate and DEP: diethyl-phthalate.

Table 2 Retention time, detection and quantification limits of the endocrine disruptors analyzed by HPLC and using the signal/noise method.

Compounds*	Retention time (min.)**	Detection limit ($x10^{-4}$ µg/kg)	Quantification limit ($x10^{-4}$µg/kg)
PA	1.60 ± 0.20	1.0	3.0
BPA	3.20 ± 0.01	0.01	0.02
DEP	5.61 ± 0.05	10.0	60.0
BADGE	10.70 ± 0.04	30.0	80.0
BisDMA	11.70 ± 0.05	30.0	90.0
DBP	12.70 ± 0.01	50.0	20.0
DOP	16.50 ± 0.60	1.5	4.0

*PA: phthalic-acid, BPA: bisphenol-A, BADGE: bisphenol-A-diglicydyl-ether, BisDMA: bisphenol-A-dimethacrylate, DOP: dioctyl- phthalate, DBP: dibutyl-

phthalate and DEP: diethyl-phthalate. BPA was analyzed using a fluorescence detector and for the other compounds were analyzed by UV/Vis detector.
**Means and standard deviations were calculated from n=10 independent experiments.

The results for the analysis of the endocrine disruptors in the samples of vegetable cans are shown in table 3. PA, BPA, DEP, DBP and DOP were found, while BADGE and BisDMA were not detected. PA and DOP were the compounds most frequently found, and the maximum concentrations were 9.549 µg/kg and 0.664 µg/kg, respectively in a can of jalapeno peppers. However, Thomson et al. (2005) found levels of 10 to 29 µg/kg of BPA in 80 different samples of food cans of New Zealand. BPA concentrations between 29.9 and 80 µg/kg were found in 20 samples of vegetable cans from Brazil, France, Spain, Turkey and USA (Brotons et al. 1995). Whereas, Kang et al. (2003) found BPA levels of 5 µg/kg in food cans. The migration of bisphenol derivatives obtained in our work, was less than those reported by Yonekubo et al. (2008) in 38 canned foods bought in Japan.

Table 3 Endocrine disruptors concentration (µg/kg) found in Mexican vegetable cans.

Sample	PA	BPA ($x10^{-3}$)	DEP	DBP	DOP
Sweet corn kernel	0.001	3.400	0.023	0.006	0.082
n=6	0.398	0.010	-	-	0.262
	0.339	-	0.030	0.001	-
	0.034	-	0.042	-	0.038
	2.187	-	-	0.008	0.060
	0.099	-	0.035	0.006	0.080
Mushrooms	0.068	2.300	-	-	0.031
n=2	1.697	1.200	-	-	0.060
Jalapeno peppers	9.549	15.900	-	-	0.664
n=6	0.082	-	-	0.023	-
	0.484	-	-	0.018	-
	0.016	0.400	-	-	0.030
	1.558	21.300	-	-	0.060
	0.034	-	0.032	-	0.070
Peas	0.056	-	0.022	0.008	-
n=5	0.018	-	-	0.006	0.090
	0.014	2.500	-	0.007	0.100
	-	4.700	0.033	-	0.086
	0.037	2.100	0.018	-	0.076
Peas and carrots	0.030	-	-	-	0.024
n=5	5.876	1.700	-	-	0.034
	5.172	-	-	-	0.510
	0.013	0.400	-	-	0.071
	0.088	-	0.013	-	0.060

The analysis of monomers in the plastic food containers, microwaveable containers and baby bottles is shown in table 4. PA, BPA, DEP, DBP, DOP, BADGE and BisDMA were

detected. PA and DOP were the compounds most frequently found, and the maximum concentrations were 3.866 and 0.228 µg/kg, respectively. The maximum PA concentrations were found in a cream container and a yoghurt container, respectively. BADGE, BisDMA and DBP were not detected as frequently. They were found in baby bottles, yoghurt and microwaveable containers. The importance of these findings is that plastic food containers such as yoghurt and the cream are re-used in Mexican households to keep and to reheat food in microwave ovens, exposing the food to high temperatures and increasing the risk of migration. Kang and Kondo (2003) found BPA in 40 plastic food containers (yoghurt, butter, cream, puddings and condensed milk) in a range of 21-43 µg/kg. Brede et al. (2003) analyzed twelve different polycarbonate baby bottles both new and second hand from Norway using water as food simulant by solid phase extraction, followed by CG-MS analysis. They found bisphenols migration of 0.23 µg/L in the new baby bottles, 8.4 µg/L in bottles dishwashed 51-times, and 6.7 µg/L in bottles dishwashed 169-times. Cao and Corriveau (2008) found bisphenols in baby bottles purchased in Canada, ranging from 228 to 521 µg/kg after warming them up to 70°C for 6 days. Maragou et al. (2008) found levels of bisphenols migration from 2.4 to 14.3 µg/kg in 31 polycarbonate (PC) baby bottles purchased in Greece.

Table 4 Endocrine disruptors concentration (µg/kg) found in food plastic containers purchased in Mexico.

Sample	PA	BPA	DEP	BADGE	BisDMA	DBP	DOP
Microwaveable	-	0.001	-	-	0.024	0.001	-
n=4	0.023	0.002	-	-	-	-	0.076
	0.577	-	-	-	-	0.003	-
	3.251	0.002	-	-	-	-	0.057
Yoghurt	0.011	0.001	-	0.002	0.147	-	-
n=7	-	-	-	-	0.001	-	0.078
	0.432	-	0.012	-	-	-	0.063
	0.251	-	-	-	-	-	0.215
	2.890	0.002	-	-	-	-	0.107
	0.491	-	0.011	-	-	-	0.041
	0.090	0.001	-	-	-	-	0.228
Cream	1.036	-	0.011	-	-	-	0.200
n=5	0.963	-	-	-	-	-	0.115
	0.019	-	0.015	-	-	-	0.077
	1.330	-	0.012	-	-	-	0.004
	3.866	-	0.096	-	-	-	-
Baby bottles	0.012	0.001	-	0.001	0.003	-	-
n=6	1.400	0.500	-	1.100	0.008	0.001	0.063
	1.773	-	-	-	-	-	-
	0.022	-	-	-	-	-	0.037
	0.915	0.001	-	-	-	-	0.063
	0.003	-	-	-	-	-	0.078

The concentrations of bisphenols and phthalates detected in our work did not exceed the limit of 0.6 mg BPA/kg accepted by the European Union (European Community 2004).

All samples analyzed had at least two substances classified as endocrines disruptors. PA and DOP were the compounds most frequently found, followed by BPA. Our results demonstrate the risk of human exposure to endocrine disruptors by migration from food containers.

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