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New Methodology for the Determination of Phthalate Esters, Bisphenol A, Bisphenol A Diglycidyl Ether, and Nonylphenol in Commercial Whole Milk Samples

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This paper reports a new methodology aimed at determining dimethyl phthalate, diethyl phthalate, di-*n*-butyl phthalate, butylbenzyl phthalate, bis(2-ethylhexyl) phthalate, nonylphenol, bisphenol A, and bisphenol A diglycidyl ether in commercial whole milk. These compounds are used as plastic additives, lacquers, resins, or surfactants and can be found in milk due to contact with plastic materials during food processing and storage. They are all suspected endocrine disrupters or mutagens. A multiresidue method based in solid-phase extraction with C-18 cartridges followed by a cleanup step using disposable cartridges was developed. Detection and quantification were performed by gas chromatography coupled to mass spectrometric (GC-MS) detection using an appropriate surrogate (4-*n*-nonylphenol) and internal standard [deuterated bis(2-ethylhexyl) phthalate]. Limits of detection were from 0.06 to 0.36 μg/kg and intraday variation from 3 and 27%, with recoveries between 73 and 119%. Five brands of commercial whole milk processed and packed in different ways were analyzed. All samples contained target compounds at concentrations between 0.28 and 85.3 μg/kg, and the total concentration ranged between 79.3 and 187.4 μg/kg, the levels being higher in sterilized milks. Nonylphenol, diethyl phthalate, dibutyl phthalate, and bis(2-ethylhexyl) phthalate were the major contributors.

KEYWORDS: Endocrine-disrupting compounds; milk; solid-phase extraction; cleanup; GC-MS

INTRODUCTION

Some epidemiological studies relate different reproduction pathologies in wildlife with the presence of some heterogeneous chemical group of compounds capable of modulating or disrupting the endocrine system (*1*−*3*). Human health effects have also been reported (*2*−*4*). Aware of the problem, the European Union (EU) has set a "priority list of substances for further evaluation of their role in endocrine disruption" (*5*) and indicates the need to assess the levels and effects of endocrine-disrupting compounds (EDCs). Among others, phthalate esters, nonylphenol (NP), bisphenol A (BPA), and bisphenol A diglycidyl ether (BADGE) are all used in the manufacture of plastics, epoxy resins, and lacquers for coating the inside of food containers and are in contact with food products. They are potential active EDCs, whereas BADGE is suspected to have mutagenic properties. Food and feed may contain some of these products as a result of (i) diffuse environmental pollution and direct uptake by animals via food or air and potential bioaccumulation and transfer through the food chain; (ii) food processing by contact with plastics, resins, lacquers, surfactants, and paints from pipes, gaskets, and containers; and (iii) migration from packaging and bottling material, envelopes, and printed tints. Specific migration limits (SML) have been set for the compounds indicated above in food or food simulants (*6*, *7*). Migration testing is often performed with food simulants that can model various categories of foods (*8*), whereas the use of real foods is not so frequent due to the fact that foods are complex mixtures of variable composition and their analysis is more complicated and costly. Among many food samples, cow's milk was chosen in this study because several public health benefits can be obtained: (i) milk constitutes a primary food source, especially for children, and for the majority of the population, it is ingested throughout one's life, and (ii) the presence of mutagenic or potential EDCs in milk reflects the propensity for migration during food processing or storage. Finally, by examining milk, it will be possible to use the proposed method and results to estimate daily intake of emerging contaminants via milk (*9*).

Several studies have been performed to assess the presence of EDCs in milk. NP was determined in milk and infant formula at concentrations from 0.4 to 81 μg/kg and was found to be ubiquitous in different types of food at levels between 0.1 and 19 μg/kg (*10*, *11*). The major source of NP residues in food packaging comes from oxidation of trisnonylphenyl phosphite (TNPP), used as an antioxidant in polymeric materials [poly(vinyl chloride) (PVC), polyolephins, and acrylics] and migration from PVC films and jars to milk (*11*). In addition, NP is

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the main toxic and persistent metabolite of alkylphenol ethoxylates (APEs) (*12*), which are used in the food industry as nonionic surfactants in cleaning agents, disinfectants, and pesticide formulations.

Phthalate esters are plastizicers used in food handling and storage, and some of them are considered to be ubiquitous pollutants but present slight endocrine-disrupting properties (*13*). Contamination during food processing confirmed that phthalates levels increased in milk upon machine milking, in comparison to hand milking, due to contact of milk with the rubber parts of the milking machine (*14*). Reported values of phthtalate esters in commercialized milk are quite variable, depending on the methodology used and the origin of samples. McNeal et al. indicated levels of bis(2-ethylhexyl) phthalate (BEHP) and dibutyl phthalate (DBP) from nondetected to 51 and 11 μg/kg, respectively, in six brands of infant formula from the United States (*11*). Castel et al. reported values of 20−35 μg/kg of BEHP in commercial milk samples from Norway (*15*). Sharman et al. (*16*) reported phthalate levels of whole milk from different countries: in Norway, BEHP ranged from 60 to 380 μg/kg and total phthalates from 200 to 2260 μg/kg, whereas in the United Kingdom and Spain, levels ranged from <10 to 90 μg/kg for BEHP and from 40 to 720 μg/kg for total phthalates. Infant formula from the United Kingdom showed levels from 200 to 400 μg/kg for BEHP and total phthalate levels between 400 and 3000 μg/kg (*16*).

BPA is used in the synthesis of polycarbonated plastics, epoxy adhesives, and can-coating material and is known to migrate from can coatings into food (*17*). Levels from <0.1 to 13.2 μg/kg were determined in canned infant formula samples (*18*), or another study indicated concentrations from 45 to 113 μg/kg (*19*). BADGE is the synthesis product from the reaction between BPA and epichlorhydrin and is used as an intermediate in the manufacture of epoxy resins and paints and is used as a lacquer coating on food cans and food storage vessels, acting as a heat stabilizer. BADGE has been classified as a tumorigen, mutagen, and primary irritant by the National Institute for Occupational Safety and Health (*20*). In addition, BADGE and its degradation products are able to induce cytotoxic and genotoxic effects in vitro in cultured human lymphocytes; thus, its presence in food constitutes a health risk to consumers (*21*). BADGE has a high propensity for migration into fats, but it is hydrolyzed in the presence of aqueous simulants with a half-life of <2 days at 40 °C (*22*). Summerfield et al. considered milk to be a vulnerable matrix due to its high fat content and likely consumption without cooking (*23*). However, in a study aimed at determining the migration of BADGE from can coatings into foods, BADGE was not detected in canned milk (*23*).

European legislation limits the concentration of potential mutagen or EDCs in food in contact with plastic (Directive 2001/62/CE) (*24*). For BPA the limit is 3 mg/kg; for BADGE and derivates, the limit is 1 mg/kg; phthalates limits are between 20 and 40 mg/kg; and the total phthalate ester leached level from polymeric material that may have contact with food is 60 mg/kg. Despite the fact that these compounds are included in the legislation, not much work has been done on the determination of potential EDCs or BADGE in milk or any other type of foods.

Most analytical protocols dealing with the determination of the migration potential of organic compounds in milk involve single analyte or chemical class. The analysis of NP in milk is based on steam distillation/solvent extraction or solid-phase extraction (SPE) followed by a cleanup step and gas chromatography coupled to mass spectrometry (GC-MS) quantification (*10*, *11*). BPA is analyzed by SPE and quantification and confirmation by GC-MS (*18*) or by liquid−liquid extraction, cleanup with C-18 cartridges, and GC-MS quantification (*19*). BADGE is liquid−liquid extracted and analyzed by HPLC-UV with GC-MS confirmation (*23*). The detection of phthalates is based, mainly, on modifications of multiresidue methods from the pesticide analysis manual of the U.S. Food and Drug Administration (*25*), consisting of a process with many steps: denaturization of protein (methanol, potassium hydroxide, potassium oxalate), sequential liquid−liquid extraction with different solvents (hexane, ethyl ether, acetonitrile, water, etc.), cleanup (Florisil, size exclusion and gel permeation chromatography), and quantification by GC-MS (*15*, *16*, *26*−*28*). Other authors use a modified protocol that follows the pesticide manual of the Canadian Health Protection Branch (Health and Welfare Canada 1986) (*29*, *30*).

The main objective of this work was to develop a simplified multiresidue method to detect, at micrograms per kilogram levels of BPA, its derivative BADGE, NP, and five phthalates [dimethyl phthalate, diethyl phthalate, di-*n*-butyl phthalate, butylbenzyl phthalate, and bis(2-ethylhexyl) phthalate] in milk in order to use it for routine quality analysis. The method was optimized by testing extraction and different cleanup sorbents and desorption solvents. Finally, five milk samples of different brands packed either in Tetra Brik or in high-density polyethylene (HDPE) or infant formula have been analyzed as a preliminary work to determine the levels of some legislated EDCs or mutagens in commercial whole milk and to prove the validity of the method.

EXPERIMENTAL PROCEDURES

Chemicals and Reagents. HPLC grade water, methanol, hexane, ethyl acetate dichloromethane, sodium hydroxide, and lauric acid for organic trace analysis were supplied from Merck (Darmstadt, Germany). The standards were nonylphenol,(NP) kindly supplied by AGBAR (Aigües de Barcelona, Spain); di-*n*-butyl phthalate (DnBP) and butylbenzyl phthalate (BBP) from Supelco (Bellefonte, PA); Bisphenol A (BPA), bisphenol A diglycidyl ether (BADGE), bis(2-ethylhexyl) phthalate (BEHP), diethyl phthalate (DEP), and dimethyl phthalate (DMP) from Aldrich (Milwaukee, IW). 4-*n*-Nonylphenol (4-n-NP) was from Dr. Ehrenstorfer (Augsburg, Germany) and deuterated bis(2-ethylhexyl) phthalate (BEHP d4) from Cambridge Isotope Laboratories (U.K.). Cartridges of C-18 (0.5 and 1 g) and Extrelut were a gift from Merck. Cartridges of Florisil (2 and 5 g) and alumina (2 g) were purchased from Waters (Milford, MA).

Samples Analyzed. Five milk samples processed in different ways were analyzed.

Ultrahigh-Temperature (UHT)-Treated Milk. Whole milk, after separation, standardization, and pasteurization, is heated to 137−140 °C for 2−10 s to obtain commercial sterility. Sterilization is performed before packaging, and filling takes place into presterilized Tetra Brik packages. Tetra Brik is a carton-based package composed of a laminate of paper, polyethylene, and aluminum foil, which has a rectangular shape and is specifically designed to be stacked on pallets. The only material to touch the contents of the package is foodgrade polyethylene. Two samples packed in Tetra Brik containers of different brands were analyzed, designated Tetra Brik brands 1 and 2.

In-Bottle-Sterilized Milk. Whole milk is filtered, homogenized, heated, and maintained at a temperature of no less than 100 °C for a length of time sufficient to render it commercially sterile. Sterilized milk is packed in high-hensity polyethylene (HDPE) bottles, which are suitable for long-term storage of milk, can be blown aseptically, and present light barriers to increase the shelf life of the product. Two different brands packed in hermetically (HDPE) sealed bottles were analyzed, designated HDPE brands 3 and 4.

Powdered Milk Infant Formula Packed in a Can. In this case, milk is pasteurized and homogenized and is dehydrated at 150 °C. After

cooling, it is sifted and packed in preferably tin cans. Only one sample was analyzed, designated infant formula.

All milk samples were purchased from supermarkets.

Sample Preparation. Powdered milk infant formula was previously reconstituted with HPLC grade water at the ratio prescribed (3 g of powdered milk in 30 mL of water). Ten milliliters of milk was introduced in a prerinsed volumetric glass flask, and 10 mL of methanol was added. The solution was spiked with the surrogate 4-n-NP at a concentration of 1.7 ng/mL and sonicated for 10 min in the flask. Afterward, 80 mL of HPLC water was added. HPLC grade water blanks were prepared according to the same method as described for milk.

The sample was solid-phase extracted with a 0.5 g C-18 cartridge, which was conditioned by passing 12 mL of dichloromethane/hexane (4:1 v/v), 12 mL of methanol, and 12 mL of water. The diluted milk sample was loaded at a flow rate of 5 mL/min. After preconcentration, the sorbent was rinsed with 15 mL of water and dried with a vacuum system for 45 min. To improve the method sensitivity, the same procedure was applied to a larger sample size. In this case, 20 mL of milk was combined with 20 mL of methanol, and after sonication, the solution was diluted with HPLC water to a final volume of 200 mL and the sample extracted with a 1 g C-18 cartridge. Trapped compounds were desorbed using a two-step elution procedure with solvent mixtures of different polarities: fraction A, 12 mL of dichloromethane/hexane (4:1 v/v); fraction B, 12 mL of methanol/dichloromethane (9:1) or 12 mL of ethyl acetate. From the two solvent mixtures tested in fraction B, the solution of ethyl acetate was more selective, and it was used in all analyses.

The cleanup process was optimized using three sorbents: Florisil, alumina, and Extrelut. Of the three sorbents used, only Florisil gave clean extracts and, therefore, was the one selected for further studies. With this sorbent, we evaluated its adsorption capacity using the lauric acid assay on SPE Florisil cartridges of 1 g, following the method described in the FDA *Pesticide Analytical Manual* (*25*). Florisil SPE cartridges of 5 g were thoroughly conditioned by passing 60 mL of methanol and 60 mL of dichloromethane/hexane (4:1 v/v) in order to avoid possible contaminations. The extracts resulting from fractions A and B (ethyl acetate) were placed on top of the cartridge and eluted with two cartridge volumes (cartridge = 20 mL) of dichloromethane/hexane (4:1 v/v) and ethyl acetate, respectively. The final extract was collected in a single fraction. After rotavaporization to near dryness, the extract was reconstituted with ethyl acetate to a final volume of 0.3 mL. The internal standard BEHPd4 was added to the final extract to yield a concentration of 2.1 mg/L. Finally, 2 μL was injected into the GC-MS system.

GC-MS Analysis. Purified extracts were analyzed on a GC-MS Trace 2000 GC coupled to a Voyager quadrupolar MS (Thermo Quest Instruments), following the same protocol detailed elsewhere (*31*). An HP 5MS fused-silica capillary column containing 5% phenyl and 95% methyl polysiloxane (30 m × 0.25 mm i.d., 0.25 μm film thickness) was used. He was the carrier gas at a flow rate of 1 mL/min. Two microliters was injected in splitless mode (time splitless delay was of 1 min) with the injector temperature set at 250 °C. The oven was programmed from 60 °C (1 min) to 175 °C at 6 °C/min, letting it stay for 1 min, and further programming to 280 °C at 3 °C/min and to 300 °C at 7 °C/min. Electron ionization was performed at 70 eV, with an interface temperature set at 270 °C. The emission current was of 100 μV, and the detector voltage was set at 380 V. Acquisition was performed in scan mode from 80 to 400 amu for compound confirmation and in time-scheduled selected ion monitoring (SIM) for quantification using retention windows from 5 to 19.5 min (DMP and DEP), from 19.5 to 25.5 min (NP), from 25.5 to 49 min (DBP, BPA, BBP, and BEHP), and from 49 to 55 min (BADGE), which included three or four monitored ions per compound, as indicated in **Table 1**.

Quantification and Quality Parameters. Quantification of target compound was done from the SIM chromatogram using 4-n-NP as surrogate and BEHPd as internal standard. Calibration was performed from 0.002 to 4 μg/mL. The recoveries and intraday variation of the method were determined by spiking 10 mL of milk with target compounds to a concentration of 8 μg/kg. The solution was equilibrated for 30 min, and afterward it was extracted by applying the method described above, together with a milk blank. In the same way, HPLC blanks were analyzed. All units are reported as micrograms per kilogram, assuming 1 L of milk equal to 1 kg (the density of whole milk calculated at 20 °C is 1.029 kg/L).

Table 1. Retention Time (t_R), Ions Monitored (*m*/*z*), Recovery, Coefficient of Variation (CV), and Limit of Detection (LOD) Obtained for Target Compounds in Milk Spiked at 8 μg/kg

compd[a]	t_R (min)	*m*/*z* monitored	% recovery	CV ($n = 3$)	LOD (μg/kg)
DMP	16.33	163, 77, 135	89	18	0.06
DEP	19.13	149, 105, 177	100	21	0.06
NP	21.12–22.53	135, 220, 149	73	5	0.18
DBP	27.47	149, 76, 223	111	3	0.09
BPA	32.98	213, 119, 228	81	5	0.15
BBP	37.57	149, 91, 206	88	3	0.12
BEHP	42.66	149, 167, 279	86	27	0.06
BADGE	50.73	325, 340, 269, 213	119	9	0.36

[a] Abbreviations: DMP, dimethyl phthalate; DEP, diethyl phthalate; NP, nonylphenol; DBP, di-*n*-butyl phthalate; BPA, bisphenol A; BBP, butylbenzyl phthalate (BBP); BEHP, bis(2-ethylhexyl) phthalate; BADGE, bisphenol A diglycidyl ether.

RESULTS AND DISCUSSION

Method Performance and Quality Control. To ensure the reliability of the results, instrumental and method performance was evaluated by using standards, spiked milk samples, and method blanks. **Table 1** indicates the retention time of each target compound and the main ions monitored, in order of abundance. Confirmation of each target compound was performed by retention time match and spectral comparison against a standard at least at three *m*/*z* with a maximum deviation of 10% in abundance. Instrumental quality parameters were as follows: Limits of detections (LODs), calculated by a signal-to-noise ratio of 3, were from 0.002 and 0.01 μg/mL. Good linearity (R^2 values >0.990) was obtained from 0.002 to 4 μg/mL. The coefficient of variation (CV) of BEHPd and 4-n-NP was below 5.4%, ensuring the use of these compounds for an accurate measurement of all target compounds. Method intraday variation, calculated on five consecutive days, was between 6.6 and 17.6% for all compounds.

The extraction method follows previous works dealing with the analysis of wastewater (*32*) and drinking water (*31*). The process for analyzing milk is more complex than for wastewater, because it is necessary to perform a destabilization emulsion with methanol, which is an adaptation of the method used to analyze organochlorinated residues in milk (*33*), and a later cleanup step. In this sense, the most critical part in the analysis of milk samples is the cleanup step. Coextracted compounds may inhibit the detection of target compounds through matrix suppression in the GC-MS analysis or produce coelution problems. On the other hand, if milk fat globule membranes are not disrupted, target compounds may not be effectively extracted, thus yielding low recoveries. Among the three sorbent products tested (alumina, Extrelut, and Florisil), Florisil was the only one that gave translucent extracts and chromatograms free of interferences. For alumina and Extrelut extracts, the surrogate presented coeluting peaks and target compounds were immersed in the coextracted matrix, indicating an incomplete cleanup. Thus, these sorbents were not effective enough to remove sample interferences and were rejected for this study.

The cleanup capacity of Florisil was tested by analyzing 10 and 20 mL of milk preconcentrated with C-18 cartridges of 0.5 and 1 g, respectively, and using a Florisil cartridge of 5 g. Although in both cases good resolution was achieved, the chromatogram baseline corresponding to 10 mL of sample was

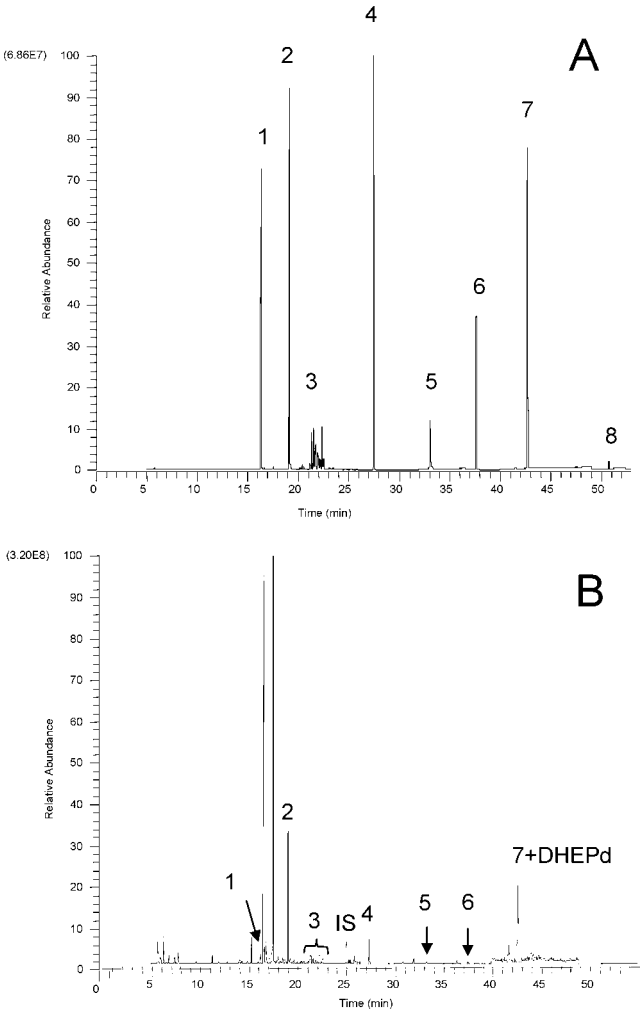

Figure 1. GC-MS chromatogram in SIM mode of (**A**) a standard mixture and (**B**) a milk extract preconcentrated with C-18 and Florisil cleanup. Peaks: 1, DMP; 2, DEP; 3, NP; 4, DBP; 5, BPA; 6, BBP; 7, BEHP and BEHPd.

cleaner than the 20 mL baseline, due to the fact that 5 g of Florisil was not enough to remove all fat contained in the 20 mL milk extract. These results were corroborated by the lauric acid assay, which gave a result of 89 mg, indicating that 1 g of Florisil can adsorb 89 mg of fat. According to this result, and taking into consideration that commercial whole milk analyzed had a maximum fat content of 3%, SPE cartridges of at least 3.4 g of Florisil were needed to remove the 300 mg of fat in the extract from 10 mL of milk. We chose the commercially available 5 g SPE cartridges.

Figure 1 shows GC-MS chromatograms in selected ion monitoring (SIM) mode corresponding to a mixture of standards (**A**) and to a milk sample with Florisil cleanup (**B**). Using 10 mL of milk it was possible to detect target compounds at LODs between 0.06 and 0.36 μg/kg (**Table 1**). Recoveries obtained by spiking milk at a concentration of 8 μg/kg were from 73 to 119%, as shown in **Table 1**. The CV calculated in triplicate analyses was below 10% for all analytes except DMP, DEP, and BEHP, which ranged from 18 to 27%. This higher variation was attributed to environmental contamination during sample preparation due to the ubiquity of some phthalate esters, especially BEHP, or due to phthalate contamination from the SPE cartridges. However, thorough washing of cartridges and sorbents minimized contamination by phthalates. HPLC water blanks contained traces of all compounds except BPA and BADGE. The HPLC blank contribution was subtracted from all samples. Milk blank samples always contained traces of target compounds except for BADGE, and therefore, the standard addition method was used to calculate the recoveries and to calculate target compounds in milk samples. As for elution solvents, ethyl acetate gave better recoveries for BEHP ($R = 86\%$) than the solvent mixture methanol/dichloromethane (9:1, v/v) used in a previous work (*31*).

Table 2. Average Concentration (Micrograms per Kilogram ± Standard Deviation, $n = 2$) of Target Compounds in Five Milk Samples[a]

compd	Tetra Brik brand 1	Tetra Brik brand 2	HDPE brand 3	HDPE brand 4	infant formula
DMP	1.75 ± 0.1	1.30 ± 0.2	0.97 ± 0.1	1.19 ± 0.2	1.38 ± 0.01
DEP	36.5 ± 4.6	72.0 ± 17.8	85.3 ± 2.9	70.9 ± 6.7	76.4 ± 12.5
NP	16.5 ± 1.5	34.8 ± 5.2	23.6 ± 2.2	27.7 ± 4.6	27.1 ± 3.7
DBP	7.30 ± 0.9	9.49 ± 1.0	50.3 ± 1.4	40.6 ± 5.4	18.4 ± 0.2
BPA	0.99 ± 0.1	2.64 ± 0.6	1.17 ± 0.1	1.29 ± 0.1	0.28 ± 0.02
BBP	1.11 ± 0.3	2.93 ± 0.1	2.88 ± 0.5	1.23 ± 0.8	1.18 ± 0.3
BEHP	15.1 ± 3.4	24.7 ± 0.6	23.2 ± 4.1	27.2 ± 9.4	20.5 ± 4.5
BADGE	bld	bld	bld	bld	bld
sum	79.3	147.8	187.4	170.2	145.2

[a] Compound abbreviations as in **Table 1**. Samples analyzed correspond to processed UHT whole milk samples packed in Tetra Brik (brands 1 and 2), sterilized milk in HDPE bottles (brands 3 and 4), and powdered infant formula packed in a can. bld: below limit of detection.

Levels in Milk. Target compounds were detected in all five samples analyzed, independent of the type of the sterilizing process and packing material. **Table 2** shows the mean concentration in the five samples and the standard deviation of duplicate analyses. The concentration of individual compounds in commercial whole milk ranged from 0.28 and 85.3 μg/kg, which is higher in comparison with those of dioxins, furans, or PCBs (*34*). The most abundant compounds identified were DEP, DBP, BEHP, and NP with levels from 7.3 to 85.3 μg/kg. DMP, BBP, and BPA were found at levels from 0.28 and 2.93 μg/kg. BADGE was not detected in any of the samples. The levels of NP found in our work are somewhat higher than those published by Guenther et al. for several dairy products (*10*). It is very difficult to determine why the levels differ between the studies, although it is probable that the milk source, processing, packing, and storage conditions differ between the samples analyzed, thus affecting the final concentration of NP in milk. For BPA, the concentration detected in all studied milks was much lower than the levels published by Kuo et al. in infant formula, which varied between 45 and 113 μg/kg (*19*).

The total concentration of potential endocrine disrupters in the five samples analyzed showed higher values in both sterilized HDPE bottles (187.1 and 170.2 μg/kg), whereas levels varied between UHT Tetra Brik brands (79.3 and 147.6 μg/kg, respectively). Infant formula presented a medium value (145.2 μg/kg). The concentrations of phthalates were similar in all samples, although milk in sterilized HDPE bottles presented higher values of DBP than Tetra Brik UHT milk. It is likely that the container enhances leaching, and this depends on when heat is used, while the milk is in a bottle (in-bottle sterilization) or in a larger vat prior to bottling (UHT).

The sources of potential endocrine disrupters in milk may be diverse. Part could originate from environmental contamination, uptake, and accumulation by animals. However, Fries demonstrated that among many organic contaminants present in sludge, only lipophilic halogenated hydrocarbons accumulated in animal tissues. Compounds such as phthalate esters, PAHs, and aromatic surfactants, among others, were metabolized and

did not accumulate (*35*). Furthermore, Snyder et al. indicated that BPA was metabolized to BPA glucuronide via glucuronidation, which accounted for a detoxication reaction of BPA in rats (*36*). Despite this, Walsh et al. (*37*) reported that xenobiotics (PCBs, phthalates, pharmacologic agents, insecticides, and heavy metals) affect milk secretion and composition by inducing changes in nutrient delivery to the mammary gland. Another source would be the plastic material and coating epoxy resins from which phthalates, NP, BPA, and BADGE could migrate into milk, as demonstrated in previous works (*38*).

None of the compounds analyzed, or the sum of total compounds, achieved the maximum leached level allowed by law (*6*, *7*, *24*). In principle, the encountered levels cannot be considered a health risk. However, the impact these compounds may have on organisms and human beings needs to be further studied, especially with regard to accumulation, degradation, and possible effects within the endocrine system. Also, the findings need to be considered from the perspective that milk represents a continuous low-level exposure to endocrine-active compounds.

ABBREVIATIONS USED

SPE, solid-phase extraction; LODs, limits of detection; GC, gas chromatography; MS, mass spectrometry; NP, nonylphenol; DnBP, di-*n*-butyl phthalate; BBP, butylbenzyl phthalate; BPA, bisphenol A; BADGE, bisphenol A diglycidyl ether; BEHP, Bis-(2-ethylhexyl) phthalate; DEP, diethyl phthalate; DMP, dimethyl phthalate; 4-n-NP, 4-*n*-nonylphenol; BEHP d4, deuterated bis (2-ethylhexyl) phthalate.

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