JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Exposure to Di-2-Ethylhexyl Phthalate, Di-*N*-Butyl Phthalate and Bisphenol A through Infant Formulas

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ABSTRACT: Phthalates and bisphenol A (BPA) are ubiquitous contaminants identified as endocrine disruptors. Phthalates are worldwide used as plasticizers, in particular to improve the mechanical properties of polymers such as polyvinyl chloride. Because they are not chemically bound to the polymer, they tend to leach out with time and use. Di-2-ethylhexyl phthalate (DEHP) and di-n-butyl phthalate (DnBP) are the two most common phthalates. BPA is an estrogenic compound used to manufacture polycarbonate containers for food and drink, including baby bottles. It can migrate from container into foods, especially at elevated temperatures. Diet is a predominant source of exposure for phthalates and BPA, especially for infants. The aim of this study was to test the presence of DEHP, DnBP, and BPA in infant formulas. DEHP, DnBP, and BPA concentrations were measured in 22 liquid and 28 powder milks by gas chromatography with flame ionization detection and high performance liquid chromatography with fluorimetric detection, respectively. DEHP concentrations in our samples were between 0.005 and 5.088 μg/g (median 0.906 μg/g), DnBP concentrations were between 0.008 and 1.297 μg/g (median 0.053 μg/g), and BPA concentrations were between 0.003 and 0.375 μg/g (median 0.015 μg/g). Concentrations of the investigated contaminants in liquid and powder milks were not significantly different, even though samples were packed in different types of containers. These data point out potential hazards for infants fed with baby formulas. Contamination seems more related to the production of formulas than to a release from containers.

KEYWORDS: *di-2-ethylhexyl phthalate, di-n-butyl phthalate, bisphenol A, infant formula*

INTRODUCTION

Endocrine disruptors (EDs) are chemicals known to mimic steroid hormones' action and to interfere with the synthesis, secretion, transport, activity, or elimination of natural hormones.[1−5] In particular, they modify the programming of the normal endocrine-signaling pathways during pre- and early postnatal life, thus determining adverse health effects such as neurological and immune effects, reproductive disorders, cancers, lowered fertility, and increased incidence of endometriosis.[1−5] Recent papers show that EDs pose the greatest risk during prenatal and early postnatal development, when organ and neural systems are forming.[2,3] The possible relationships between combined exposures to environmental contaminants and diseases are now attracting attention, especially if they occur early in life.[6,7] Recently, some studies correlated the combined exposure to phthalates and BPA with human health.[6,7] Phthalates are widely used in many products to impart softness, flexibility, transparency, and longevity to an otherwise rigid polyvinyl chloride (PVC). Because there is not a chemical bond with the polymer, they leach out with time and use, thus becoming ubiquitous environmental contaminants.[8] Di-2-ethylhexyl phthalate (DEHP) and di-*n*-butyl phthalate (DnBP) are two of the most common phthalates.[8] Human exposure occurs through ingestion, inhalation, and dermal contact during the whole lifetime, including intrauterine life, but exposure in children exceeds that in adults. Phthalates determine toxic effects in laboratory animals, especially on the developmental and reproductive systems.[9] Human studies correlated phthalate exposure with adverse health effects such as liver, kidney, and lung damage as well as sexual developmental abnormalities.[1,4,10−12] Moreover, phtalates may alter the methylation status of DNA and consequently the DNA sequence itself, thus transmitting these effects to future generations.[13] Bisphenol A (BPA), 2,2-bis(27 4-hydroxyphenyl) propane, is at the same time an estrogenic compound and a main monomer for the synthesis of polycarbonate and epoxy resins. Polycarbonate is used for many products like water and baby bottles, children's toys, sport equipment, medical and dental devices, and so forth, whereas coatings of many food and beverage containers consist of epoxy resins.[2,8] BPA tends to migrate from can containers into foods, especially at elevated temperatures.[2,14] As a consequence, potential risks of exposure to BPA raised concern over the years because of suspicion of affecting reproduction, development, and metabolism. There is a consensus that infants are at the greatest risk of harm, even

Received: December 3, 2014
Revised: February 23, 2015
Accepted: March 2, 2015
Published: March 2, 2015

DOI: 10.1021/jf505563k
J. Agric. Food Chem. 2015, 63, 3303−3310

with a low level exposure to BPA.[2] Recent studies of the National Toxicology Program (NTP) and the U.S. Food and Drug Administration (FDA) pointed out the potential BPA effects on brain, behavior, and prostate gland in fetuses, infants, and young children.[15] Indeed, BPA can affect the hormone-mediated neurologic and behavioral development in early life.[15−19] In addition, high BPA exposure has been associated with heart disease, diabetes, abnormally high levels of liver enzymes, and alterations of the thyroid function.[20−22] For these reasons, BPA containing baby bottles have been banned in Europe since March 2011.[23] Diet remains the predominant source of exposure for both phthalates and BPA especially for infants, since these compounds have been found in breast milk and in baby formulas.[24−27] The present paper analyzes the presence of DEHP, DnBP, and BPA in infant formulas to assess possible neonatal exposure and to reduce the gap of knowledge in this field.

MATERIALS AND METHODS

Sampling. Fifty infant formula samples were collected at different neonatal nurseries in Naples 53 hospital during three months (May−July 2013). Liquid ready to use (n = 22) and powder (n = 28) milk samples were collected. Among them, there were seven special milk samples, that is, milks for infant with gastrointestinal problems (n = 3), rice milk formulas (n = 3), and a premature formula. Liquid samples were packed in polyethylene terephthalate (PET) and Tetrapak, whereas milk powders were contained in aluminum (Al) containers. The infant formula samples were collected in glass vials and were rapidly transferred to the laboratory of the Department of Agriculture, where analytical samples were obtained for the different procedures. All samples were labeled. For DEHP and DnBP analysis, aliquots (15 mL) of liquid milk were lyophilized and stored a −18 °C until analyses, whereas powder sample aliquots (1 g) were just stored in the dark. For BPA determination, aliquots (5 mL) of liquid milk were stored at −18 °C until analyses, whereas powder sample aliquots (2 g) were reconstituted with high-performance liquid chromatography (HPLC) water (15 mL), were split into 5 mL aliquots, and were stored at −18 °C until analyses. For each reconstituted vial, an additional 5 mL vial with HPLC water was stored at −18 °C as a negative control to avoid possible bias because of a contamination of HPLC water.

DEHP and DnBP. *Chemical Reagents.* Acetonitrile, n-hexane, acetone for organic trace analysis, and anhydrous Na_2SO_4 were supplied by Merck (Darmstadt, Germany). Florisil (60/100 mesh) was furnished by Supelco (Bellefonte, PA, U.S.), and Bondesil (PSA 40UM) was furnished by Varian (Palo Alto, CA, U.S.). Standard solutions of DEHP and DnBP were purchased from Sigma-Aldrich (St. Louis, MO, U.S.).

Instrumental Parameters. The analyses of phthalates (PAEs, phthalic acid esters) were carried out by a Shimadzu GC-17 (Shimadzu, Kyoto, Japan) capillary gas chromatography with a flame ionization detector (GC-FID) and an HP-5 (cross-linked 5% PHME siloxane, 30 m length, 0.32 i.d., 0.25 μm film thickness) glass-capillary column. Helium was used as the carrier, and a hydrogen/air mixture was used to sustain the flame. The volume of injection was 1 μL in splitless mode, and the injector and detector temperatures were 260 and 310 °C, respectively. The temperature program was 100 °C for 1 min, increase of 15 °C/min up to 280 °C, and retention of this temperature for 10 min.

DEHP and DnBP Measurement. Because of PAE ubiquity, any contact with plastic was avoided. All the glassware was thoroughly washed, was rinsed twice with acetone and n-hexane, was heated at 250 °C for 2 h, and finally was stored away from any environmental contamination. In accordance with the method by Cirillo et al.,[25] the lyophilized samples were (1) extracted three times with 15 mL of acetonitrile in an ultrasound bath for 15 min; (2) centrifuged at 2000 rpm for 10 min, and the acetonitrile layer was transferred to a separatory funnel; and (3) added with 10 mL of n-hexane saturated with acetonitrile, and the funnel was vigorously shaken for 5 min. The acetonitrile phase was transferred into a flask and was dried under vacuum at 55 °C. The extracts were reconstituted by 5 mL of n-hexane and were purified through a column containing 2 g of Florisil activated for 2 h at 200 °C, 0.5 g of Bondesil, and 1 g of anhydrous Na_2SO_4. The column was eluted three times with 10 mL of n-hexane/acetone mixture (100:5 v/v). The eluates were collected in a flask, were evaporated under vacuum at 40 °C, and were reconstituted with 1 mL of n-hexane for GC analysis. The calibration curves were obtained using standard solutions at 0.625, 1.250 and 2.500, 5.00 and 10.00 μg/mL for DEHP and at 0.312, 0.625, 1.250, 2.500, and 5.00 μg/mL for DnBP. The regression coefficients (R) were >0.99 for both contaminants. The PAE concentrations in the samples were obtained by comparing the relevant peak areas with calibration curve. Limits of detection (LODs) and quantification (LOQs) were evaluated as the mean blank value plus three blank standard deviations and LOQ was evaluated as 3 times the LOD. LODs and LOQs were 5.0 ng/g and 15.0 ng/g for DEHP and 7.5 ng/g and 22.5 ng/g for DnBP, respectively. A run without sample was carried out every six determinations to reduce the instrumental background due to contamination. Moreover, solvents used to wash the syringe were frequently replaced. The intra- and interday repeatabilities of the method were evaluated by injecting standard solutions at three different concentration levels (2.50, 5.00, and 10.00 μg/mL for DEHP and 1.25, 2.50, and 5.00 μg/mL for DnBP) five times during a day (intraday) and during five consecutive days (interday). The intraday repeatability ranged from 7.0 to 9.5% for DEHP and from 5.5 to 8.5% for DnBP, whereas interday repeatability varied from 6.0 to 8.5% for DEHP and from 4.5 to 6.5% for DnBP. Samples with DEHP and DnBP concentrations lower than LOD were used for recovery tests. Three liquid and three powder milk samples (each in triplicate) were spiked with standard solutions at concentration 2.0, 4.0, and 8.0 μg/mL for DEHP and 1.0, 2.0, and 4.0 μg/mL for DnBP and then were processed as milk samples. Recoveries were 98 ± 10% for DEHP and 98 ± 9% for DnBP. Because of the ubiquity of these compounds, a blank sample (only solvents) for each batch was analyzed, and the average concentration value was subtracted from PAE detected values.

Bisphenol A. *Chemical Reagents.* Acetonitrile, methanol, and water (HPLC grade) were supplied by Merck (Darmstadt, Germany). Solid-phase extraction cartridges (Bond Elut C18 SPE, 1 g/6 mL) were purchased from Agilent Technologies (Palo Alto, CA, U.S.). A BPA standard (purity ≥99%) was purchased from Sigma-Aldrich (St. Louis, MO, U.S.).

Instrumental Parameters. BPA detection was performed through an HPLC (LC-10AT VP Shimadzu, Kyoto, Japan) equipped with a fluorescence detector (Shimadzu RF-10A XL) and a reversed-phase column (Ascentis C18 Length × i.d.: 15 cm × 4.6 mm; particle size: 5 μm, Supelco, Bellefonte, PA). The column was kept at a constant temperature of 40 °C. The

DOI: 10.1021/jf505563k
J. Agric. Food Chem. 2015, 63, 3303−3310

mobile phase consisted of 60% of acidified water (1% of acetic acid), 35% of acetonitrile, and 5% of methanol. The flow rate of mobile phase was set at 0.950 mL/min (isocratic run). The fluorimetric detection was carried out at an excitation wavelength of 275 nm and an emission wavelength of 305 nm.

BPA Measurement. BPA measurement was performed by adapting the procedure by Sun et al.[9] An aliquot of each sample (5 mL) was inserted into a 250 mL glass round-bottom flask and was added with acetonitrile (20 mL). Flasks were placed onto a Heidolph Promax 2020 shaker for 25 min. The content of each flask was then filtered through a filter paper and was transferred into a separatory funnel. The flask was rinsed with 5 mL of acetonitrile, which was added to the funnel. Afterward, 35 mL of *n*-hexane was also added to the separatory funnel, and the resulting mixture was shaken for 25 min. The acetonitrile layer was removed from the funnel and was stored in a round-bottom flask, whereas the hexane layer was washed twice with acetonitrile (first with 15 mL, then with 10 mL) which was collected and added in the same round-bottom flask. The solvent was removed from the extract through a rotavapor, and then the flask was washed with 3 mL of a methanol:water (5:95 v/v) solution to be processed by solid-phase extraction (SPE). SPE cartridges were first conditioned with 5 mL of methanol and then with 5 mL of water. Later, the sample was loaded, and the elution was carried out at a flow rate of 3−4 mL/min using a Supelco Visiprep SPE vacuum manifold. The cartridges were then washed with 2 mL of a methanol:water solution (30:70 v/v) and were dried under 152 vacuum pump for 1 min. Finally, the BPA retained in the cartridge was eluted with 3 mL of a methanol:water (80:20 v/v) solution. The eluate was dried by a rotavapor, was dissolved with 1 mL of methanol, and finally was collected in an amber vial before the HPLC analysis. A calibration curve with a correlation coefficient of 0.998 was obtained by injecting standard solutions of BPA at concentrations of 10.0, 20.0, 30.0, 40.0, and 50.0 μg/L. An instrumental LOD equal to 0.003 μg/g dry weight (dw) was calculated using the standard deviation of the response (σ) and the slope of the calibration curve (S) according to the formula 3.3 σ/S. Similarly, an LOQ equal to 0.009 μg/g dw was calculated as 10 σ/S. Recovery percentages at three concentration levels were assessed on six samples (three liquid and three powder milk samples with BPA level below the LOD) by spiking each sample with BPA solutions in methanol at concentrations of 50.0, 100.0, and 1000.0 μg/L. The recoveries were 87 ± 3%. BPA quantification was performed comparing the peak areas obtained in the samples with the BPA standard calibration curve.

For each batch of samples, a blank sample was processed according to the procedures mentioned previously. A total of 16 blanks were analyzed, and all of them showed BPA concentrations well below the LOD value.

BPA Confirmation by LC MS/MS. Because BPA measurements could be affected by matrix-related interferences, a confirmation by Liquid Chromatography Tandem Mass Spectrometry (LC MS/MS) was carried out according to the Shao et al. method.[28]

Instrumental Parameters. Identification was carried out using an alliance 2695 (Waters, U.S.) liquid chromatography equipped with a Quattro Ultima Pt (Micromass, U.K.) tandem mass spectrometer and a symmetry C-18 column (150 mm × 2.1 mm i.d., 3.5 m). The temperature of the column oven was set at 40 °C, the flow rate was 0.2 mL/min, and the injection volume was 10 μL. Mobile phases consisted of methanol and water with 0.1% ammonia. The methanol was linearly increased from 10 to 55% in 10 min, then was increased to 85% in 10 min, was held for 7.5 min, and finally was brought back to 10% and was held for 15 min before the following injection. The mass spectrometer was operated in negative mode electrospray ionization in multiple-reaction monitoring (MRM) mode. The capillary voltage was 3.5 kV, the cone voltage was 70 V, and the multiplier voltage was 650 V. Nitrogen was used as nebulizing, desolvation, and cone gas. In particular, the nebulizing gas was adjusted to the maximum, whereas the flow of the desolvation gas and cone gas was set to 550 L/h and 80 L/h, respectively. The source temperature and the desolvation gas temperature were held at 100 and 300 °C, respectively. The RF lenses 1 and 2 were set at 50 and 0.5, the ion energy 1 and the ion energy 2 were both 0.5, the entrance and exit were zero, and the collision gradient was 3.2 eV. Ultra High Purity (UHP) argon was used as the collision gas for the tandem mass spectrometric analysis, and the pressure in the collision chamber was kept at 2.8×10^{-3} mbar. A calibration curve in the concentration range 1−100 ng/g was obtained by linear regression of the normalized (to the internal standard area) standard solution areas against BPA concentrations. The correlation coefficient was ≥0.999. The intra- and interday repeatabilities of the method were evaluated by injecting standard solutions at three different concentration levels (10, 50, and 100 ng/g) five times during a day (intraday) and during five consecutive days (interday). The intraday reproducibility ranged from 4.0 to 6.5%, while interday reproducibility varied from 4.5 to 6.2%.

Statistical Analysis. A power calculation was undertaken to determine an appropriate sample size for this study. On the basis of literature data,[29] considering DEHP as the most abundant phthalate in infant formula, a two-sided test power calculation was performed. Double of the range value was used as the sigma (0.780 μg/g dry weight). This power calculation indicated that 11 samples in each group would be necessary to detect a 15% difference in the DEHP concentration with a power of 80% at a 5% level of significance. Data distribution was assessed with the Shapiro Wilk's test. A two-sample t test was performed with SPSS 20.0 software (IBM) to assess the differences between DEHP, DnBP, and BPA concentrations in liquid and powder milks. Significance was set at $p < 0.05$. The concentrations below LOD were assumed to be equal to LOD.

Dietary Intake Assessment for Italian Infants (Age 0−4 Months). Daily intake was estimated as

$$\text{Intake} = (C \times V)/\text{BW}$$

where C is concentration, V is volume of milk per day, and BW is body weight. This equation was used to evaluate DEHP, DnBP, and BPA exposure of young children through artificial milk. Dietary exposure was calculated using the blueprint to the budget method (BM) model[30] in accordance with Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO) and with the help of weight growth charts by WHO[31] and pediatric nutrition suggestions for our range of age. We considered two possible scenarios: (1) median concentrations of contaminants, infants with average weight to development at the 50th percentile or at the 97th percentile (according to the growth curve by WHO[31]) who introduce daily a medium quantity of milk (medium case); (2) maximum concentrations of contaminants, children who have grown at the 50th percentile or at the 97th percentile and introduce daily a higher quantity of milk (worst case).

DOI: 10.1021/jf505563k
J. Agric. Food Chem. 2015, 63, 3303−3310

RESULTS

Most milk samples showed detectable levels of DEHP (80%, 86% of liquid and 96% of powder milks), DnBP (90%, 82% of liquid milks and 96% of powder milks), and BPA (60%, 43% of liquids milks and 67% of powder milks) (Table 1). The average concentration of DEHP in all milk samples was 1.327 ± 0.724 μg/g dw, and in particular, it was 1.112 ± 0.716 μg/g dw in liquid milks and 1.496 ± 0.729 μg/g dw in powder milks. For DnBP, the average concentration in all milk samples was 0.354 ± 0.305 μg/g dw, namely, 0.384 ± 0.385 μg/g dw in liquid milks and 0.330 ± 0.229 μg/g dw in powder milks. The average concentration of BPA in all milk samples was 0.021 ± 0.022 μg/g dw; it was 0.019 ± 0.037 μg/g dw in liquid milks and 0.023 ± 0.028 μg/g dw in powder milks (Table 1). DEHP concentrations varied from 0.092 to 3.552 μg/g (median 1.136 μg/g), DnBP concentrations varied from 0.008 to 1.624 μg/g (median 0.244 μg/g), and BPA concentrations varied from 0.003 to 0.169 μg/g (median = 0.008 μg/g) (Table 1). Similar concentrations of the three analytes were found in liquid and powder milks, even though containers were of different types. DEHP, DnBP, and BPA concentrations in the HLPC water samples stored as negative controls for reconstituted powder milk were below the LODs.

The concentrations of DEHP, DnBP, and BPA in liquid and powder milks together with the type of packaging are reported in Tables 2 and 3, respectively. Estimates of dietary exposure to DEHP, DnBP, and BPA in the medium and worst cases are shown in Tables 4 and 5. The daily intake of DEHP in the medium case ranged from 19.34 to 24.85 μg/kg body weight (bw) day at the 50th percentile and from 17.54 to 21.14 μg/kg bw day at the 97th percentile. In the worst case, DEHP intake varied between 42.57 at 54.68 μg/kg bw day at the 50th percentile and 38.61 at 46.52 μg/kg bw day at the 97th percentile (Tables 4 and 5). Estimates of dietary exposure to DnBP in the medium case ranged from 4.15 μg/kg bw day to 5.34 μg/kg bw day at the 50th percentile and from 3.77 μg/kg bw day to 4.54 μg/kg bw day at the 97th percentile. In the worst case, the DnBP intake varied between 13.62 and 17.50 μg/kg bw day at the 50th percentile and between 12.35 and 14.89 μg/kg bw day at the 97th percentile (Tables 4 and 5). BPA intakes in the medium and worst cases are shown in Tables 4 and 5. In the medium case, values ranged from 0.14 to 0.17 μg/kg bw day at the 50th percentile and from 0.12 to 0.15 μg/kg bw day at the 97th percentile. In the worst case, the BPA intake varied between 0.99 and 1.27 μg/kg bw day at the 50th percentile and between 0.90 and 1.08 μg/kg bw day at the 97th percentile. Both in the medium and worst cases, the highest intake occurred at the 30th day of life because the amount of consumed milk starts increasing while the baby's weight is still pretty low. As for BPA, for both DnBP and DEHP, the higher values of intake occurred in children at 30 days of age (Tables 4 and 5).

DISCUSSION

Our data indicate the presence of DEPH, DnBP, and BPA in infant formulas. Data relevant to all contaminants showed a wide variability, but no significant differences between liquid and powder milks were found, even though samples were packed in different types of containers. This finding would suggest that DEHP, DnBP, and BPA contamination could arise from raw materials or from manufacturing processes rather than from different packaging. Phthalates, in particular, may

Table 1. Di(2-ethylhexyl)phthalate (DEHP), Di-*n*-butylphthalate (DnBP) and Bisphenol A (BPA) Concentrations in μg/g Dry Weight[a]

	DEHP				DnBP				BPA			
sample	POS (%)	mean ± sd	median	min−max	POS (%)	mean ± sd	median	min−max	POS (%)	mean ± sd	median	min−max
liquid milk (n = 22)	86	1.112 ± 0.716	0.926	0.092−2.919	82	0.384 ± 0.385	0.280	0.008−1.624	43	0.019 ± 0.037	0.003	0.003−0.169
powder milk (n = 28)	96	1.496 ± 0.729	1.159	0.702−3.552	96	0.330 ± 0.229	0.212	0.101−0.812	67	0.023 ± 0.028	0.011	0.003−0.108
total (n = 50)	80	1.327 ± 0.724	1.136	0.092−3.552	90	0.354 ± 0.305	0.244	0.008−1.624	60	0.021 ± 0.022	0.008	0.003−0.169

[a]Mean ± sd, median, and range. There were no significant differences between liquid and powder milk (coupled t test, $p < 0.05$).

DOI: 10.1021/jf505563k
J. Agric. Food Chem. 2015, 63, 3303−3310

Table 2. Concentrations of Di(2-ethylhexyl)phthalate (DEHP), di-*n*-butylphthalate (DnBP) and Bisphenol A (BPA) in Liquid Milk Samples and Type of Packaging

type	packaging	DEHP (μg/g dry weight)	DnBP (μg/g dry weight)	BPA (μg/g dry weight)
infant formula	Tetrapak	0.696	0.075	0.003
		1.831	0.067	0.003
infant formula	PET	0.092	0.082	0.030
		0.219	0.084	0.020
		0.633	0.142	0.009
		2.067	0.0075	0.003
		1.456	0.287	0.003
		0.301	0.067	0.003
		2.099	0.624	0.003
		0.784	0.482	0.058
		0.606	0.216	0.014
		1.877	0.787	0.003
		1.202	0.351	0.003
		0.923	0.899	0.017
		0.256	0.14	0.018
		0.929	0.088	0.003
		2.919	1.624	0.169
		0.852	0.423	0.030
		1.428	0.384	0.003
		0.796	0.807	0.003
		1.137	0.272	0.003
		1.371	0.548	0.003

Table 3. Concentrations of Di(2-ethylhexyl)phthalate (DEHP), Di-*n*-butylphthalate (DnBP) and Bisphenol A (BPA) in Powder Milk Samples in Aluminium Packaging

type	DEHP (μg/g dry weight)	DnBP (μg/g dry weight)	BPA (μg/g dry weight)
infant formula	1.408	0.321	0.003
	1.134	0.199	0.003
	0.702	0.155	0.003
	0.871	0.212	0.028
	1.274	0.161	0.008
	0.883	0.137	0.003
	3.552	0.809	0.011
	2.909	0.765	0.100
	1.023	0.101	0.009
	1.142	0.356	0.022
	0.981	0.392	0.003
	1.024	0.161	0.003
	0.922	0.337	0.043
	1.052	0.709	0.054
	1.018	0.575	0.026
	2.341	0.187	0.012
	0.982	0.123	0.003
	1.723	0.118	0.016
	1.899	0.704	0.003
	1.175	0.148	0.035
	2.409	0.349	0.041
infant formula for gastrointestinal problems	1.213	0.301	0.003
	1.897	0.812	0.108
	1.821	0.201	0.018
rice milk formula	1.723	0.211	0.003
	2.871	0.321	0.003
	0.951	0.184	0.046
premature formula	0.997	0.201	0.003

contaminate milk during the production or preparation of formulas. A main source of contamination results from migration of phthalates from products in contact with food during processing. Several studies concerned the migration of DEHP from the PVC tubing of the milking machine used in dairy farms.[32−34] PVC tubing contains up to 40% DEHP by weight. A Norwegian study showed a clear difference in DEHP levels between raw milk collected by hand milking (about 5 μg/

DOI: 10.1021/jf505563k
J. Agric. Food Chem. 2015, 63, 3303−3310

Table 4. Medium Case, Estimated Daily Dietary Intake of Di(2-ethylhexyl)phthalate (DEHP), Di-*n*-butylphthalate (DnBP) and Bisphenol A (BPA) in Newborns Fed with Liquid or Powder Formulae According to the 50th and 97th of Infant Weight Growth Curve by WHO (2006)

	infant's average weight (kg)		milk assumption (g dry weight/day)		DEHP intake (μg/kg bw day)		DnBP intake (μg/kg bw day)		BPA intake (μg/kg bw[b] day)	
age (days)	50th pctl[a]	97th pctl	50th pctl	97th pctl	50th pctl	97th pctl	50th pctl	97th pctl	50th pctl	97th pctl
15	3.70	4.75	67.61	76.06	20.78	18.21	4.46	3.91	0.15	0.13
30	4.25	5.45	92.96	101.41	24.85	21.14	5.34	4.54	0.17	0.15
45	4.76	6.20	101.41	109.86	24.23	20.15	5.20	4.33	0.17	0.14
60	5.41	6.84	98.59	105.63	20.72	17.54	4.45	3.77	0.15	0.12
75	5.76	7.26	105.63	112.68	20.83	17.64	4.47	3.79	0.15	0.12
90	6.10	7.65	112.68	126.76	20.98	18.82	4.51	4.04	0.15	0.13
120	6.70	8.35	114.08	129.58	19.34	17.63	4.15	3.79	0.14	0.12

[a]pctl, percentile. [b]kg bw, kg body weight.

Table 5. Worst Case, Estimated Daily Dietary Intake of Di(2-ethylhexyl)phthalate (DEHP), di-*n*-butylphthalate (DnBP) and Bisphenol A (BPA) in Newborns Fed with Liquid or Powder Formulae, According to the 50th and 97th of Infant Weight Growth Curve by WHO (2006)

	infant's average weight (kg)		milk assumption (g dry weight/day)		DEHP intake (μg/kg bw day)		DnBP intake (μg/kg bw day)		BPA intake (μg/kg bw[b] day)	
age (days)	50th pctl[a]	97th pctl	50th pctl	97th pctl	50th pctl	97th pctl	50th pctl	97th pctl	50th pctl	97th pctl
15	3.70	4.75	67.61	76.06	45.74	40.07	14.64	12.82	1.06	0.93
30	4.25	5.45	92.96	101.41	54.68	46.52	17.50	14.89	1.27	1.08
45	4.76	6.20	101.41	109.86	53.32	44.33	17.06	14.19	1.24	1.03
60	5.41	6.84	98.59	105.63	45.60	38.61	14.59	12.35	1.06	0.90
75	5.76	7.26	105.63	112.68	45.85	38.83	14.67	12.42	1.06	0.90
90	6.10	7.65	112.68	126.76	46.18	41.43	14.78	13.26	1.07	0.96
120	6.70	8.35	114.08	129.58	42.57	38.80	13.62	12.41	0.99	0.90

[a]pctl, percentile. [b]kg bw, kg body weight.

kg) and machine milking involving PVC tubing (30 μg/kg in milking chamber and 50 μg/kg in collection tank).[33]

Dietary Intake Assessment for Italian Infants (Age 0−4 Months). To assess postnatal exposure to phthalates and BPA, the estimation of daily dietary intake of these contaminants was carried out in 0−4 month old children, as milk is the only food introduced in this age group. Four possible nutrition scenarios were possible, namely, nutrition with infant powder, liquid formula, breast milk, or a combination of these, but we only considered artificially fed babies assuming liquid or powder formulas (or both). The European Food Safety Authority (EFSA) established a tolerable daily intake (TDI) of 50 μg/kg bw for DEHP and 10 μg/kg bw for DnBP.[35,36] As expected, the highest intakes of DEHP and DnBP were estimated among infants with growth at the 50th percentile, who have a lower body weight than those at the 97th percentile.

Daily intake of DEHP in the medium case varied between 20 and 25% and between 18 and 21% of TDI at 50th and 97th percentile, respectively. In the worst case, intake was also lower than TDI, except for the 50th percentile infants aged 30 and 45 days (Table 5).

Daily intake of DnBP in the medium case varied between 42 and 53% and between 38 and 45% of TDI at 50th and 97th percentile, respectively. In the worst case, instead, intake always exceeded TDI, up to 175%. Muller et al.[37] estimated for 0−6 month old Danish infants a daily intake via infant formulas of 9.8 μg/kg bw/day for DEHP and 16.4 μg/kg bw/day for DnBP.[37] Our values for DEHP intake were higher than Muller's both in the medium and in the worst cases, whereas DnBP intake levels were lower in the medium case and similar in the worst case. Our estimates of DEHP and DnBP daily intake were higher than those reported by MAFF[29] for infants 0−3 months old, that is, 13 μg/kg bw/day for DEHP and 2.4 μg/kg bw/day for DnBP. Our estimated BPA daily intakes were well below the temporary tolerable daily intake (t-TDI) established by EFSA in 2015 (4.0 μg/kg bw). In the medium case, our intake ranges were 3.5−4.3% and 3.0−3.8% of t-TDI for the 50th and 97th percentile respectively, which increased in the worst case to 25−31% and 23−28% of t-TDI for the 50th and 97th percentile, respectively. The results reported in the worst case are consistent with oral exposure data shown in Draft Scientific Opinion on the risks to public health related to the presence of bisphenol A (BPA) in foodstuff by EFSA.[38] Diet is the main source of exposure to BPA in infants aged 0−4 months.[38] Minor pathways of introduction could be the inhalation or ingestion of dust, dermal contact, and the mouthing of toys. Until a few years ago, babies could introduce BPA from polycarbonate baby bottles, especially when bottles were heated and reused multiple times.[2,7] The EU Regulation No. 321/2011 imposed not to use BPA in the manufacture of baby bottles, thus reducing exposure. In 2008, a report of the U.S. National Toxicology Program (NTP) provided daily exposure estimates for infants, children, and adults on the basis of realistic scenarios.[39] The highest daily exposure to BPA was estimated to occur in infants and children. Formula-fed infants (0−6 months of age) had estimated daily intakes of 1−11 μg/kg bw. In 2010, the FAO and WHO jointly held an Expert Meeting on BPA, whose final report was published in 2011.[40] The report identified 0−6 month infants fed with liquid formulas in polycarbonate bottles as the subpopulation with the highest dietary exposure to BPA, namely, 2.4 μg/kg bw per day

DOI: 10.1021/jf505563k
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(average) and 4.5 μg BPA/kg bw per day (95th percentile). In 2012, a probabilistic exposure assessment using data from recent Canadian surveys suggested that daily exposure to BPA in children ranged from 0.083 μg/kg bw (0−1 month old) to 0.164 μg/kg bw (children 4−7 months of age).[41] Our data resemble those of Health Canada but are lower than those of NTP and FAO/WHO, probably because the problem of BPA migration from baby bottles in Europe has been solved. The different packages (Tetrapak, PET, and aluminum) represent a possible bias of the present study. However, the studied contaminants can be found not only in Tetrapak and PET but also in aluminum packages, as these are often internally coated with plastic derivatives.

Our data show a widespread contamination of infant formulas from the three investigated contaminants, either of environmental or process origin. Our findings demonstrate that infant formulas may represent a main source for the simultaneous exposure to DEHP, DnBP, and BPA in babies. This risk is particularly relevant for DEHP and DnBP because intake from formulated milk could exceed in the worst case the TDI from EFSA. In conclusion, potential hazards exist for infants fed with baby formula, as these endocrine disruptors show the highest toxicity in infant population. TDIs for the three investigated contaminants set by EFSA refers to adult and infant populations, indiscriminately, but since children and infants are developing individuals, providing a specific TDI could be worthwhile as TDIs intended to children would help the protection of the most vulnerable part of the population from a severe public health hazard.

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Notes

The authors declare no competing financial interest.

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