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Phthalate residue in goat milk-based infant formulas manufactured in China

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ABSTRACT

Phthalates adversely affect the male reproductive system in humans. Through gas chromatography-mass spectrometry analysis, we investigated the residual profile and levels of 15 phthalates in 90 goat milk-based infant formulas from 15 commercial brands of 10 dairy enterprises located in Shaanxi Province, China. In general, dibutyl phthalate was the most detected phthalate, followed by bis(2-ethylhexyl) phthalate, diisobutyl phthalate, and dimethyl phthalate; their geometric mean concentrations in the formulas were 38.1, 24.2, 16.6, and 8.7 μg/kg, respectively. Other phthalates were not detected in the investigated samples. No significant differences were found in the phthalate levels among different stages of infant formulas, even though the samples were packaged in different types of containers. These findings demonstrate that goat milk-based infant formulas may represent the main source of exposure to phthalates in infants.
Key words: phthalate, infant formula, goat milk powder

INTRODUCTION

Diesters of phthalic acid, commonly referred to as phthalates, have been widely used in plastic manufacturing since the 1930s and can be found as a common additive in paints, lubricants, adhesives, insecticides, packaging, and cosmetics (Gao and Wen, 2016). The most important application of phthalates is as plasticizers to improve the flexibility and workability of polymeric materials (Swan, 2008). Phthalates, as plasticizers, are only physically, not chemically, bound to polymer chains, and therefore tend to leach out from polymeric materials and be released into food when polymeric materials containing these compounds are used as packaging (Zhang et al., 2011).

Phthalates have become ubiquitous food contaminants (Cao, 2010). Human exposure to phthalates has become an important issue because of their alarming effects in newborns, infants, and toddlers, discovered in epidemiological studies. Four studies from Taiwan (Hsu et al., 2012), Sweden (Bornehag et al., 2004), Bulgaria (Kolarik et al., 2008), and the United States (Just et al., 2012) suggest that childhood exposure to bis(2-ethylhexyl) phthalate (**DEHP**) and butyl benzyl phthalate (**BBP**) may increase the risk of allergic diseases, including asthma and eczema.

Four prospective cohorts reported that gestational BBP, DEHP, dibutyl phthalate (**DBP**), and diethyl phthalate (**DEP**) exposures were associated with alterations in infant or toddler physical development (Engel et al., 2010; Kim et al., 2011; Miodovnik et al., 2011; Whyatt et al., 2012), as well as parent-reported externalizing and internalizing problems (Whyatt et al., 2012) and child behavior similar to autism (Miodovnik et al., 2011). The latest studies reported that fetal and newborn exposure to phthalates causes a decrease in anogenital distance in male newborns (Bornehag et al., 2015; Swan et al., 2015).

Foodstuffs are the major source of phthalate exposure, particularly for the long-chain phthalates (Wittassek et al., 2011). Infants take milk as their main source of nutrition; thus, monitoring the levels of phthalates in formulas is important to provide data for exposure assessment of phthalates in infants. Some studies have reported that cow milk-based infant formulas contain phthalate residue (Mortensen et al., 2005; Yano et al., 2005), but little or no information has been reported on phthalate residue in goat milk-based infant formulas.

China is one of the largest producers of dairy goats and goat milk-based infant formulas in world (Zhao et al., 2011). The objective of the present study was to evaluate the profile and level of phthalate residue in goat milk-based infant formulas manufactured in China.

MATERIALS AND METHODS

Reagents and Solvents

Acetonitrile and hexane were HPLC-grade and were obtained from Fisher Scientific (Pittsburgh, PA). The 15

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phthalate standards were dimethyl phthalate (**DMP**), DEP, diisobutyl phthalate (**DIBP**), DBP, bis(4-methyl-2-pentyl) phthalate, bis(2-methoxyethyl) phthalate, dipentyl phthalate, bis(2-ethoxyethyl) phthalate, dihexyl phthalate, BBP, bis(2-n-butoxyethyl) phthalate, DEHP, dicyclohexyl phthalate, diphenyl phthalate, and dinonyl phthalate. The phthalate standards were purchased from o2si Smart Solutions (Charleston, SC) in a mixed standard solution with a purity of >98.0% for each.

Sample Collection

Ninety powdered infant formulas were collected from stores of 10 randomly selected dairy enterprises in Shaanxi Province, China. These samples covered 15 commercial brands. For each brand, 2 samples, with plastic bag and metal can packaging, respectively, were collected from stage I (for infants 0–6 mo old), stage II (for infants 6–12 mo old), and stage III (for infants 12–36 mo old) infant formulas.

Sample Preparation

Sample preparation was performed according to the method described by Schecter et al. (2013) with minor modification. The samples of powered infant formula (1 g) were directly extracted with 10 mL of acetone:hexane (1:1, vol/vol) 3 times and then centrifuged (8,000 × g, 10 min, room temperature) after shaking for 30 min. The upper organic layers were combined and concentrated to near dryness. The sample residue was redissolved in 2 mL of hexane and subjected to a glass column packed with 7 g of Florisil (60–100 mesh, Sigma-Aldrich, St. Louis, MO) conditioned with 20 mL of acetone:hexane (2:8, vol/vol) and 20 mL of hexane. Phthalates were eluted with 70 mL of acetone:hexane (2:8, vol/vol). The final eluate was concentrated to 5 mL under a gentle stream of nitrogen for instrumental analysis.

Instrumental Analysis

The 15 phthalates were measured using a GC (7890A; Agilent Technologies, Santa Clara, CA) coupled with an MS (5975C; Agilent Technologies) equipped with an electron impact ion source in the selective ion monitoring mode. The GC-MS operating conditions were set at 70 eV ionization potential with the source at 230°C and electron multiplier voltage at 2,000 eV. The injection port was maintained at 250°C and 1 μL of the sample was injected in splitless mode, followed by a 1-min purge after the injection. Helium was used as the carrier gas at a flow rate of 1.2 mL/min. A fused-silica capillary column (Rxi-5Sil MS; 30 m × 0.25 mm i.d. and 0.25 μm film thickness; Restek, Bellefonte, PA) was used for separation. Oven temperature was programmed at 100°C (held for 1.0 min) and raised to 260°C at 8°C/min (held for 9 min). The retention times on the Rxi-5Sil MS column and the fragment ions monitored for the 15 phthalates are listed in Table 1.

Quality Control and Quality Assurance

Glassware was baked at 450°C overnight. The detailed quality-control operations were performed according to the method described by Fierens et al. (2012). The efficiency of the analytical method was evaluated before sample analysis. The limits of detection (**LOD**) for the 15 phthalates were estimated as $3 \times S_0$, where S_0 is the value of the standard deviation as the concentration approaches zero (Blount et al., 2000). The S_0 was determined by analyzing quintuplicate sets of the lowest 5 standards and plotting the standard deviation versus the known standard concentration. The y-intercept of the best-fit line of this plot was used as S_0. Recovery was determined based on the results of 6 replicate analyses of a sample spiked at a 0.10-mg/kg level of each phthalate standard. The recovery of each phthalate was calculated as the ratio between the experimentally observed concentration and the theoretical concentration as a percentage. Precision was determined from these data by calculating the relative standard deviation of each phthalate.

Data Treatments and Statistics

Both DBP and DEHP were present in the procedural blank, which was carried through the same processing procedure as the samples, but no samples were used. Their values were subtracted from sample values. Statistical analyses were performed using the statistical software package SPSS (version 15.0, SPSS Inc., Chicago, IL). For concentrations below the LOD, a value equal to the LOD divided by the square root of 2 was used (Hornung and Reed, 1990). The analyses were considered statistically significant when $P < 0.05$.

RESULTS AND DISCUSSION

Method Performance

To ensure the reliability of the results, instrumental and method performance was evaluated before sample analysis. Table 1 indicates the retention time and the main ions monitored for each phthalate. Confirmation of each phthalate was performed by comparison of the retention time and fragments (abundance ratios between quantitative and qualitative ions) with those