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Cumulative risk assessment for plasticizer-contaminated food using the hazard index approach

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ABSTRACT

Phthalates strongly and adversely affect reproduction, development and liver function. We did a cumulative risk assessment for simultaneous exposure to nine phthalates using the hazard index (HI) and the levels of nine phthalates in 1200 foodstuff samples. DEHP (di-2-ethylhexyl phthalate) present the highest level (mean: 0.443 mg/kg) in 1200 samples, and the highest average daily dose (ADD) was found in DEHP, $\Sigma DBP_{(i+n)}$ (the sum of dibutyl phthalate [DBP] isomers [DnBP + DiBP]) posed the highest risk potential of all the phthalates. In seven phthalates, the 95th percentiles of the ADDs for $\Sigma DBP_{(i+n)}$ in 0–6-yr-old children accounted for 91% (79–107%) of the tolerable daily intake, and the 95th percentiles of the HIs for the anti-androgenic effects of five phthalates in 0–3-yr-old children and 4–6-yr-old girls were >1. We conclude that the health of younger Taiwanese may be adversely affected by overexposure of phthalate-contaminated foods.

1. Introduction

Phthalate esters (PAEs) are one of world's most used groups of plasticizers. Phthalates are ubiquitous; thus, human exposure to phthalates occurs via food, medicine, cosmetics, and the environment in general (Wittassek et al., 2011). Phthalate concentrations have been measured in diverse edibles (MAFF, 1996; Tsumura et al., 2002; Yano et al., 2002) and in infant formula and mother's milk (Calafat et al., 2004; Casajuana and Lacorte, 2004; MAFF, 1996, 1998; Mortensen et al., 2005; Petersen and Breindahl, 2000; Tsumura et al., 2002; Yano et al., 2002, 2005). Ubiquitous exposure to phthalates might be critical because toxicological studies have demonstrated that phthalates and their metabolites have considerable adverse effects on human health. Butyl benzyl phthalate (BBzP), di-2-ethylhexyl phthalate (DEHP), diisobutyl phthalate (DiBP), diisodecyl phthalate (DiDP), diisononyl phthalate (DiNP), and di-n-butyl phthalate (DnBP) are reproductive toxicants affecting mainly the male reproductive system in mammals (Foster et al., 2000; Gray et al., 2000; Lee et al., 2004; Mylchreest et al., 1999; Parks et al., 2000). DEHP shortens the duration of human pregnancy (Latini et al., 2003) and disrupts or modulates the human endocrine system (Akingbemi et al., 2004; Boekelheide, 2004; Sharpe, 2005; Sharpe and Irvine, 2004). Increased exposure to DEHP is correlated with a decrease in human sperm quality (Duty et al., 2003; Sharpe, 2005).

Moreover, several phthalates have been identified as anti-androgens, and a dose-additive reduction in testosterone levels has been reported (Hannas et al., 2011; Howdeshell et al., 2008) in rats when they were exposed to nine different phthalates. An assessment of the human risk from the combined exposure to several phthalates is therefore important.

The acceptable level of exposure to chemicals in Europe is regulated by different laws. The regulations on registration, evaluation, authorization, and restriction of chemical substances, which is implemented by the European Chemicals Agency, deals with chemicals in general, whereas the European Food Safety Authorities (EFSA) specifically assesses all risks associated with chemicals in the food chain. In the United States, the Environmental Protection Agency (USEPA) and the Food and Drug Administration (USFDA) perform similar tasks. Because exposure to phthalates comes from many different sources, phthalates are included in several sets of regulations. Due to their possible harmful anti-androgenic effects and the importance of dietary intake as an exposure route, TDIs have been specified by EFSA for several

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phthalates, namely BBzP, DnBP, and DEHP, but not for DiNP or DiDP (Lhuguenot, 2009). This is because DiNP and DiDP primarily affect the liver, whereas BBzP, DnBP, and DEHP affect the testes. Therefore, the TDIs which we used for anti-androgenic effects were 10 μg/kg bw/d for DnBP (EFSA, 2005a), 50 μg/kg bw/d for DEHP (EFSA, 2005d), 500 μg/kg bw/d for BBzP (EFSA, 2005e), 500 μg/kg bw/d for diethyl phthalate (DEP) (WHO, 2003) and 10 μg/kg bw/d for DiBP (Hannas et al., 2011; Howdeshell et al., 2008) (Supplemental table). For the adverse hepatic effect, EFSA defined a group-TDI value of 150 μg/kg bw/d for DiNP and DiDP (EFSA, 2005b,c), a specification based on their related chemical structure and common mode of action (i.e., peroxisome proliferation in the rodent liver). The reference doses (RfDs) of 200 μg/kg bw/d for BBzP and of 20 μg/kg bw/d for DEHP have also been specified by the USEPA based on significantly increased liver-to-body weight to liver-to-brain weight ratios and relative liver-weight in adults (USEPA, 1993, 1991). Risk characterization for these phthalates can be done by evaluating the assessed dietary intake against these TDI and RfD values. Therefore, more phthalate intake data are needed, in particular for young children, because they may be a potentially vulnerable subgroup. Children consume more food and drinks compared with adults when expressed as "per kg bw"; this results in relatively higher exposure to compounds with adverse health effects (Kroes et al., 2002).

On May 23, 2011, a severe plasticizer-contaminated food episode occurred in Taiwan because of the improper use of one of plasticizer, DEHP, to replace palm oil in food and drinks as a clouding agent. The affected foods included fruit juices and other beverages, bread, sports drinks, tea, and jam (Uy, 2011). A few days later, the Taiwan government began seizing the contaminated products and announced a ban on exporting them (Whats On Xiamen, 2011). Later, the list for government safety checks was extended to syrups, tablets, pastries, powdered probiotic products, and emulsifiers (Central News Agency, 2011). By May 27, 2011, "up to 465,638 bottles of DEHP-tainted beverages have been pulled out from store shelves. Also, up to 270,822 boxes and 68,924 packs of powdered probiotics and 28,539 kilos of fruit juices, fruit jam, powder and syrup, and yoghurt powder have been removed from shelves" (Galarpe, 2011), according to the EcoWaste Coalition and a report from Taiwan's Food and Drug Administration (TFDA).

By mid-June 2011, roughly 900 products had been recalled from nearly 40,000 Taiwanese retailers (Economist, 2011). Ninety-five Taiwanese food-product manufacturers had used the DEHP ingredients, as had 244 ingredient-manufacturing firms (Kastner, 2011).

An assessment of the dietary risk from the combined exposure to several phthalates is therefore urgently needed. The objectives of this study were: (1) to assess the Taiwan population's intake of nine phthalates—BBzP, DEP, DEHP, DiBP, DiDP, DiNP, dimethyl phthalate (DMP), DnBP, and di-n-octyl phthalate (DnOP)—in contaminated food items (baby food, milk and other beverages, cooking and salad oils, and dietary supplements); (2) to evaluate ion the intake of seven phthalates—BBzP, DEP, DEHP, DiBP, DiDP, DiNP, and DnBP—against the TDI values. We used the EFSA TDI value for DnBP in the risk assessment of both DnBP and DiBP; (3) to assess the cumulative risk for simultaneous exposure to the seven phthalates in objective (2) using the HI; and (4) to assess the contribution of the different food groups to the intake of the nine phthalates in objective (1).

We report the 50th (P50), 95th (P95), and 99th percentiles (P99) of the plasticizer-contaminated food intake distributions of the seven phthalates (separately for different age groups), because the higher end of the intake distribution is more important. Moreover, for each phthalate, we report the average daily dose (ADD) contribution (as a percentage) of nine phthalates in different foodstuffs.

2. Materials and methods

The preliminary survey was focused on commercially available brands and people's buying habits. The principle of sampling was the probability proportional to size, and the number of samples was decided upon based on each product's real market share of sales. After reaching an agreement with the TFDA, we bought infant formula ($n = 136$), non-staple foods for infants ($n = 158$), supplementary foods for infants ($n = 46$), beverages (e.g., soft drinks, juices) ($n = 211$), other beverages (containing no tea leaves) ($n = 19$), and milk and other dairy products ($n = 126$), animal fat ($n = 4$), vegetable oils ($n = 129$), and health supplements (capsules, tablets, and powders) ($n = 371$) at drug stores, convenience stores, and hypermarkets (retail discount department stores that combine a supermarket on one floor with a department store on another) to investigate their levels of phthalates. High-performance liquid chromatography/electrospray ionization tandem mass spectrometry (HPLC-ESI-MS/MS) was used to determine the levels of the nine phthalates in 1200 foodstuff samples. Suitable extraction methods were developed for the three food matrices (low-fat products, high-fat products, and dairy products). Representative samples of four groups of foods were purchased using a systematic sampling strategy.

2.1. Sample extraction

2.1.1. Low-fat sample extraction

One gram of a food sample was transferred to a 15-mL centrifuge tube, and 10 μL of internal standard mixture (DMP-d_4: 0.03 μg/mL; DBP-d_4 and BBzP-d_4: 0.05 μg/mL; and DEHP-d_4: 0.05 μg/mL) was added. Then 8 mL of methanol was added, and the mixture was shaken vigorously for 30 min. Methanol was then added to make up a volume of 10 mL. The mixture was centrifuged at 3000 rpm for 15 min. The 0.5–1 mL supernatant (hexane phase) was used for analysis. The hexane/ether phase was transferred to another tube and the combined extract was evaporated to 0.5 mL using the rotary evaporation method. The residue was redissolved in 10 mL of acetonitrile. The solvent was changed to 10 mL of acetonitrile, and 1 mL of solvent was used for analysis.

2.1.2. Oil sample extraction

After using the double heating method to melt 0.5 g of solid cream, 0.5 g (weight) of oil was transferred to a 15-mL centrifuge tube and 10 μL of internal standard mixture was added. To prevent the mixture from immediately curdling, 3.0 mL of pentane/methanol (1:4) was added, and the mixture was shaken vigorously for 2 min and then centrifuged at 1000 rpm for 1 min. The supernatant (pentane phase) was transferred to another tube, and the extraction was repeated twice with 3.0 mL of pentane each time. The combined extract was evaporated to 0.5 mL using the rotary evaporation method. The solvent was changed to 10 mL of methanol, and 1 mL of solvent was used for analysis.

2.1.3. Dairy sample extraction

One gram of milk was transferred to a 15-mL centrifuge tube, and 10 μL of internal standard mixture was added. The sample was mixed first with 1 mL of ethanol and then with 2.0 mL of pentane/acetone (1:1). The mixture was next shaken vigorously for 1 min and then centrifuged at 1500 *g* for 1 min. The supernatant (pentane phase) was transferred to another tube, and the extraction was repeated with 3.0 mL of pentane. The combined extract was evaporated to 0.5 mL using the rotary evaporation method. The residue was redissolved in 10.0 mL of acetonitrile. The solvent was changed to 10 mL of acetonitrile, and 1 mL of solvent was used for analysis.

2.2. LC/MS/MS

The LC system consisted of a binary pump, isocratic pump, solvent degasser, autosampler, and column oven (1200 series; Agilent Technologies, Waldbronn, Germany). The mass spectrometer was a triple quadrupole instrument equipped with a positive ion electrospray ionization source (Agilent 6410B; Applied Agilent Technologies, Wilmington, DE, USA). A 35 μm, 2.1 × 30 mm trap column (Agilent Zorbax SB-C18) was used between the pump and autosampler to retain any phthalates originating from the HPLC system. Separation was done using a C18 column (3.5 μm, 2.1 mm × 100.0 mm i.d.) (XBridge; Waters, Wexford, Ireland). Sample extracts were kept at ambient temperature until analysis. Ten microliters of the extract was injected into the HPLC system under isocratic conditions. The column flow rate was 0.3 μL/min and the column temperature was kept at 40 °C. The isocratic mobile phase composition was a mixture of 5 mM of ammonium acetate in water and 100% methanol. The total run time between injections was 17 min. Detailed MS conditions were: desolvation temperature: 350 °C; cone gas flow: 10 L/min; nebulizer pressure: 276 kPa; and ion spray voltage: 4 kV. Electrospray ionization was operated in the positive ion mode in the multiple reaction monitoring mode.

The following m/z ion combinations (precursor → product) were monitored: m/z 195 → 163 for DMP; m/z 223 → 149 for DEP; m/z 279 → 149 for DiBP; m/z 313 → 91 for BBzP; m/z 279 → 149 for DBP; m/z 391 → 149 for DEHP; m/z 391 → 149 for DnOP; m/z 419 → 71 for DiNP; and m/z 447 → 149 for DiDP. The mobile phase consisted of 5 mM of ammonium acetate (solvent A) and 100% methanol (solvent B). The gradient elution procedure was as follows: 25% solvent B at 0 min; 50% solvent B