Di(2-ethylhexyl)phthalate (DEHP) and di-n-butylphthalate (DBP) exposure through diet in hospital patients

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A B S T R A C T

Ready-to-eat packed meals intended to hospital patients were studied over a two-weeks period to measure the daily intake by total diet. The packaging consisted of polyethylene terephthalate (PET) dishes sealed with polypropylene (PP) foil. The DEHP mean concentrations in total meals varied from 0.061 ± 0.028 to 0.307 ± 0.138 µg/g wet weight (wet wt.); the DBP mean levels varied from 0.025 ± 0.018 to 0.174 ± 0.091 µg/g wet wt. Highest levels of concentration for DEHP and DBP were found in bread with mean values of 0.307 ± 0.138 µg/g wet wt. and 0.174 ± 0.091 µg/g wet wt. for DEHP and DBP, respectively. The daily intake for DEHP was 3.1 ± 0.9 µg/kg bw and 1.5 ± 0.5 µg/kg bw for DBP.

The mean ± sd incidence of DEHP and DBP intake via hospital meals on the respective EFSA TDI was 6 ± 2% (range 4–11%), and 15 ± 5% (range 8–24%), respectively. Even if for hospital patients the major route of exposure may be represented by medical devices, the influence of the diet could have a significant value on TDI.

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1. Introduction

It was recently highlighted that hospital meals, if not complying with safety standards, may represent a high risk to health because they serve hazardous foods to vulnerable people, as children, elderly people, pregnant women, immuno-compromised people, etc., that are more susceptible to food-borne illness than the general population, with potentially severe consequences (SA Department of Health, 2008; UK Department of Health, 2009; IT Ministero della Salute, 2011). Nevertheless, many toxic chemicals may be present in the food, whether they occur naturally, as contaminants, or as deliberate additives or introduced through processing and packaging practices. Among them, particular attention has been recently paid on phthalates (PAEs), mainly on di-(2-ethylhexyl) phthalate (DEHP) and di-n-butylphthalate (DBP), used as plasticizers in polymers such as polyvinyl chloride (PVC), for their adverse effects on human health (Latini et al., 2004; Fromme et al., 2007).

Biomonitoring studies performed on the general population have shown the ubiquitous exposure to phthalates in all age groups (Koch and Calafat, 2009) by main routes of exposure represented by ingestion, inhalation and dermal contact (Schettler, 2006). The ingestion of commonly used drugs and medicines may also be an important PAE’s source (Hauser et al., 2004; Hernández-Díaz et al., 2009) as is the case for many antibiotics, antihistamines, laxatives, herbal preparations and nutritional supplements coated with films made of synthetic polymers containing phthalates. Hospital patients undergoing medical procedures such as intravenous (IV) therapy, enteral and parenteral nutrition support, blood transfusion, hemodialysis and peritoneal dialysis, cardiopulmonary bypass (CPB) and extracorporeal membrane oxygenation (ECMO) can be exposed to phthalates leached from PVC medical devices (Food and Drug Administration, FDA, 2001). Empirical data, although limited, have also demonstrated a positive association between the magnitude of exposure and the use of PVC containing...
paring them with DEHP and DBP TDIs established by EFSA.

of DEHP and DBP in ready-to-eat packed meals served to hospital

Composition of the typical diet served to patients in the studied hospital.

Table 1

<table>
<thead>
<tr>
<th>Meals</th>
<th>Meal component</th>
<th>Composition</th>
<th>Mean serving weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breakfast</td>
<td>Milk, tea or barley coffee</td>
<td>Rusks, fruit jams</td>
<td>Beverages (180), Rusks (30), Jams (25)</td>
</tr>
<tr>
<td></td>
<td>rusk, fruit jams</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lunch</td>
<td>First course</td>
<td>Pasta or rice with tomato sauce, pasta with</td>
<td>Menu (492)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>legumes, rice in vegetable soup</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Second course</td>
<td>Meat and meat products, fish and fish products,</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>eggs, mozzarella</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Accompaniment</td>
<td>Boiled potatoes, carrots, runner beans, spinach</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>and chards dressed with olive oil</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fresh fruit</td>
<td>Apple, peer, orange</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bread</td>
<td>Roll</td>
<td></td>
</tr>
<tr>
<td>Dinner</td>
<td>First course</td>
<td>Pasta or rice with tomato sauce, pasta</td>
<td>Menu (381)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>with legumes, rice in vegetable soup</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Second course</td>
<td>Meat and meat products, fish and fish products,</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>eggs, ham, cheese, mozzarella, processed cheese</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Accompaniment</td>
<td>Boiled potatoes, carrots, runner beans, spinach</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>and chards dressed with olive oil</td>
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<tr>
<td></td>
<td>Fresh fruit</td>
<td>Apple, peer, orange</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bread</td>
<td>Roll</td>
<td></td>
</tr>
</tbody>
</table>

2.2. Chemical

The standard of di(2-ethylhexyl)phthalate (DEHP) and di-n-butylphthalate (DBP), Gas Chromatography (GC) analytical grade, were obtained from Sigma Aldrich Company Ltd. Dorset, U.K. Acetonitrile, n-hexane and anhydrous sodium sulfate were obtained from Merck KGaA Darmstadt, Germany. Florisil (60/100 mesh) was purchased from Supelco Bellefonte, PA USA and Bondesil (PSA 40/UM) from Varian Palo Alto, CA USA. All the reagents used in the experiment were of the highest grade available and their purity was periodically checked by GC injection.

2.3. Instrumentation

PAE analyses were carried out by a Shimadzu GC-17 (Shimadzu, Kyoto-Japan) capillary gas chromatograph with flame ionization detector (FID), injecting 1 µl of each extract on a HP-5 (Crosslinked 5% PHME Siloxane, 30 m length, 0.32 i.d., 0.25 μm film thickness) glass-capillary column. Helium was used as carrier and hydrogen/air for the flame. The injection mode was splitless, the injector temperature was 260 °C, the detector temperature was 310 °C. The temperature program was 100 °C for 1 min, increasing by 15 °C/min to 280 °C, staying at this temperature for 10 min.

2.4. Calibration curves

The calibration curves were carried out diluting DEHP and DBP standards in n-hexane at concentration of 10 mg/ml. Working standard solutions were prepared by diluting the stock solutions in n-hexane, at concentrations of 2.5, 5.0 and 10.0 µg/ml for DEHP and 1.25, 2.5 and 5.0 µg/ml for DBP. Determinations were carried out in triplicate. The curves were constructed using the areas of the chromatographic peaks measured at the three increasing concentrations. The linearity obtained by regression analysis for both chemicals showed the regression coefficients ($R^2$) >0.99.

2.5. Analytical parameters and quality assurance

Since PAEs are ubiquitous contaminants, during each analytical phase many precautions were followed in order to avoid samples contamination. All the glassware used during sampling and analytical activities were thoroughly washed, rinsed twice with acetone and n-hexane, heated at 250 °C for 2 h and finally stored, quickly portioning in disposable dishes consisting of trays made of PET. The PET containers were then thermally sealed with plastic PP foil. The hot drinks as milk, tea and barley coffee, were packed in plastic cups with lid both in PET. Packed and sealed meals were stored in carts Burloge until hospital patient's delivery. Burloge carts are divided into two parts, in this way, hot meals (pasta, rice, legumes, meat, etc.) are stored at temperatures above 60 °C and cold meals (salads, cheeses, ham, etc.) at temperatures below 10 °C. In the hospital cooking center, the meals were placed in carts as they were packed, starting from cold meals. The time span between the packaging of the meals and the arrival at the bed-side of the patient was about 1 h.

All sampled food was put in glass jars and quickly transported to the laboratory where it was weighed and codified. A total of 225 different food specimens relating to each component of breakfast, lunch and dinner were collected, from which 165 analytical samples were constituted. In fact, the first course, second course and side dishes, that daily constituted the menus of lunch and dinner, were mixed together, homogenized, divided into aliquots of 15 g and lyophilized. Fresh fruit were peeled before being homogenized, aliquoted in 5 g, lyophilized and analyzed individually. About breakfast beverage, milk, tea, or barley coffee were ali- quoted in samples of 15 g and lyophilized. The freeze-dried analytical samples were stored at −20 °C until analysis.

Bread and rusks were ground and subdivided into aliquots of 5 g and 10 g, respectively, and analyzed individually. Fruit jams were analyzed as they were.
keeping them away from any environmental contamination. Besides a blank sample containing only the reagents was submitted to the analytical procedure, as described below, with every series of three sample analysis and the mean value was subtracted from PAE detected values.

For determinations of Detection Limits (LODs) and Quantification Limits (LOQs), 20 blanks were used. These blanks were obtained submitting to the analytical procedure only the reagents and they were GC injected in triplicate. LODs were set as mean blank value plus three times standard deviation and LOQs were set as three times of LODs. For DEHP LOD was 5.0 ng/g and LOQ was 15.0 ng/g; while for DBP mean blank value plus three times standard deviation and LOQs were set as three times of LODs. Final recovery results were obtained as the difference between the PAE detected values.

## 2.6. DEHP and DBP detection

In accordance with the method described by Tsumura et al. (2001a), with minor modifications (Cirillo et al., 2011), DEHP and DBP were extracted three times from food samples by 15 ml of acetonitrile in an ultrasound bath for 15 min; the samples were centrifuged at 2000 rpm for 10 min, collecting the acetonitrile layers in a separatory funnel. 10 ml of n-hexane saturated with acetonitrile were added and the funnel was vigorously mechanically shaken for 5 min. The acetonitrile phase containing the PAEs was transferred to a flask and dried under vacuum. The dried extracts were reconstituted by 5 ml of n-hexane and cleaned up on a column of anhydrous Sodium Sulfate (Na2SO4). The column was eluted three times with 1 ml of each of the three standard working solutions containing DEHP and DBP mean values of 0.270 ± 0.138 and 142.8 ± 78.0 ng/g wet wt., respectively. As PAE certified matrixes are not available on sale, for recovery tests 10 lunch and 10 breakfast samples were randomly analyzed through PAE detection method described below. Among these, were chosen to recovery tests the samples that showed DEHP and DBP levels quite similar to LOQ. In particular, 3 analytical samples, chosen as described above from one lunch menu, one dinner menu and three components of breakfast were spiked respectively with 1 ml of each of the three standard working solutions containing DEHP at 10.0, 20.0 and 40.0 g/ml and DBP at 5.0, 10.0 and 20.0 g/ml, stored overnight and then processed as food samples. Each spiked extract was GC injected three times. Final recovery results were obtained as the difference between the PAE amounts detected and those detected before spiking. Mean recoveries were: 80.3 ± 3.5% for DEHP and 102.8 ± 4.4% for DBP.

## 3. Results and discussion

The DEHP and DBP concentrations in the foods and meals analyzed and the mean contents by serving are shown in Table 2. The highest levels of concentrations were found in bread with DEHP and DBP mean values of 0.307 ± 0.138 µg/g wet wt. (range 0.110–0.420 µg/g wet wt.) and 0.174 ± 0.091 µg/g wet wt. (range 0.093–0.273 µg/g wet wt.), respectively. Bread rolls are industrially packed in plastic bags quickly after baking, at a temperature that can favor the plasticizer release. These values are quite similar to levels found by Cirillo et al., 2011 in bread served in school meals (DEHP and DBP mean values of 0.270 ± 0.143 and 142.8 ± 78.0 µg/g wet wt. respectively).

The dinners showed the highest mean concentrations of DEHP and DBP (0.158 ± 0.098 and 0.067 ± 0.052 µg/g wet wt., respectively). Even if the composition of the lunch menus was quite similar to that of the dinners, sliced ham and cheese industrially produced and packed under vacuum in PET were served exclusively at dinner. This may explain the higher values of contamination found in dinner menus considering also that ham and cheese have a fat amount higher than other foods. DEHP and DBP contamination in lunches and dinners may be attributable to a contamination due to the foodstuffs and to the preparation and packaging of meals. As described in the literature, the main sources of such contamination result from the products containing PAEs that are in contact with food during food processing and packaging (Sharman et al., 1994; Tsumura et al. 2001a; Casajuana and Lacorte, 2004; Mortensen et al., 2005; Cirillo et al., 2011).

Lower levels of contamination of both DEHP and DBP were found in breakfast (DEHP mean 0.061 ± 0.028 µg/g wet wt. and range 0.012–0.116 µg/g wet wt.; DBP mean concentration 0.025 ± 0.018 µg/g wet wt. and range 0.008–0.072 µg/g wet wt.). Regarding breakfast food components, the highest DEHP concentrations were found in fruit jams with mean 0.080 ± 0.030 µg/g wet wt., while for DBP, the highest concentration levels were found in rusks with mean 0.045 ± 0.012 µg/g wet wt., products both industrially packed. Among the hot drinks, milk showed the highest levels of concentration of both DEHP (mean 0.048 ± 0.024 µg/g wet wt.) and DBP (mean 0.021 ± 0.008 µg/g wet wt.) (data not shown), probably due to lipid content of milk.
The highest DEHP and DBP mean contributions by serving were from dinners (79 ± 48 μg and 34 ± 27 μg, respectively). The daily intakes of DEHP and DBP by total diet in hospital patients, in the studied conditions, and the contribution to daily total intakes (TDI), established by EFSA for adult people of 70 kg bw (EFSA, 2005a,b), 50 μg/kg bw for DEHP and 10 μg/kg bw for DBP, are reported in Table 3. The estimate of the daily DEHP intakes, obtained for all total diets ingested by patients during the fifteen days of this study, showed a mean value of 3.1 ± 0.9 μg/kg bw (range 1.9–5.4 μg/kg bw); the daily DBP intakes showed mean value of 1.5 ± 0.5 μg/kg bw (range 0.8–2.4 μg/kg bw). These values are lower than those established by EFSA both for the DEHP and DBP. On the basis of the mean and the maximum values obtained in the study, DEHP and DBP intakes by hospital diet can represent on average the 6 ± 2% (max. 11%) and the 15 ± 5% (max. 24%) respectively of the EFSA TDIs. The daily DEHP total diet intake is also lower than tolerable intake (TI) value of 0.6 mg/kg b.w./day established by the FDA (FDA, 2001).

### 3.1. Literature data comparison

Although phthalate contamination of foods has been widely demonstrated, up to now few studies are available about dietary phthalate intake under real-life conditions. These studies, carried out by different methods, evidenced on adult populations DEHP and DBP daily total diet intakes varying meanly from 2.1 to 7.4 μg/kg bw and from 0.2 to 4.1 μg/kg bw, respectively. Therefore, even if Italian diet scheme may be quite different from those of other countries, our data (3.1 μg/kg bw for DEHP and 1.5 μg/kg bw for DBP) fit data from other studies, as shown in Table 4.

### 4. Conclusion

This study regarded the evaluation of DEHP and DBP levels in hospital packed meals and the consequent evaluation of intakes of hospital patients through daily diet. The DEHP and DBP levels found in this study ranged from 0.012 to 0.420 μg/g wet wt. (DEHP) and from 0.008 to 0.273 μg/g wet wt. (DBP); while daily DEHP intake range was 1.9–5.4 μg/kg bw and daily DBP intake range 0.8–2.4 μg/kg bw. The evaluated daily total intakes by diet may cover up to 11% for DEHP and 24% for the DBP of the TDI established by EFSA. Considering that in hospital patients the medical practices and devices may be relevant routes of exposure the diet can be considered not negligible in overcoming of DEHP and DBP TDIs. These compounds were found mainly in the processed and packed foodstuffs employed in supplying hospital meals, suggesting that manufacturing and contact with plastic wrapping can play a major role in the phthalate contamination on foods; as shown also by the high levels of DEHP and DBP found in bread rolls, in rusks, in marmalade fruit jams and in foods, as ham and cheese.

In this study foods have not been studied before packaging and therefore we cannot say with certainty that contamination by DEHP and DBP can be relative to the process and to packaging of meals or was endogenous to the food itself. In addition, the influence of the temperature and the time of contact of the food with the container inside the Burlodge cart have not been evaluated, because the aim of this study was to carry out the evaluation of hospital patients exposure under real conditions, but it is plausible that these parameters could influence the migration of the plasticizers from the packages into the foods as demonstrated by the same authors in a previous study (Cirillo et al., 2011).

A close examination is needed in order to assess the whole exposure of hospital patients by all pathways. Therefore, to sum up, next studies may be intended to assess the levels of dermal and inhalatory exposure (indoor air quality in hospital areas) and the total exposure by DEHP and DBP urinary metabolite detection. About the food processing, the evaluation and control of influencing parameters, mainly the storage time/temperature and the lipiddic food content to be in contact with package, and the reduction of the contamination during food handling, cooking and portioning, etc., by the use of plants, machinery, equipments and packaging materials not containing PAEs or obtained by new production techniques avoiding any PAE migration (Navarro et al., 2010), could be strategies of mitigation of exposure to PAEs through diet.

### Conflict of Interest

The authors declare that there are no conflicts of interest.

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