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Evaluation of childhood exposure to di(2-ethylhexyl) phthalate from perfusion kits during long-term parenteral nutrition

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Abstract

Leachability of the plasticizer di(2-ethylhexyl) phthalate (DEHP) from administration sets into intravenous parenteral emulsions containing fat was investigated. DEHP is added to polyvinyl chloride (PVC) to impart flexibility. However, DEHP is a lipid-soluble suspected carcinogen that is hepatotoxic and teratogenic in rodents, and has been shown to leach from PVC products containing lipophilic mixtures. Consequently, total parenteral nutrition (TPN) mixtures containing fat emulsions should be stored in ethylvinyl acetate (EVA) bags rather than PVC packs. However, while TPN bags are made of EVA, they contain PVC-DEHP residues and the lines used between TPN bags and venous catheters are made of PVC-DEHP.

The present study quantified the amount of DEHP leached from bags and tubing that could potentially contaminate patients during home TPN. Four types of emulsions containing fat were studied. Levels of DEHP in the bag and at the outlet tubing were measured by high-performance liquid chromatography (HPLC). This was measured during simulated TPN at different times after starting perfusion, 1 day after reconstitution of solutions in the bags, and 1 week later after storage at 4 °C.

Detectable and stable amounts of DEHP were found to leach from bags (0.2 ± 0.008 mg to 0.7 ± 0.02 mg) and DEHP content increased in the outlet tubing (0.8 ± 0.09 mg to 2 ± 0.07 mg) during simulated infusions. The same phenomenon was observed after 1 week of storage at 4 °C. DEHP extraction by TPN depends on the lipid content of each TPN preparation and the flow rate. These results suggest that children treated with prolonged TPN are regularly exposed to significant amounts of DEHP.

Keywords: Parenteral nutrition; Infants; Children; Phthalate; HPLC

1. Introduction

Long-term total parenteral nutrition (TPN) is now routinely used in pediatrics, particularly in infants or children with severe gastrointestinal diseases. As soon as clinical stability is reached, children on long-term TPN can be discharged home under outpatient care (Vanderhoof and Matya, 1999). Although all-in-one parenteral nutrition administered from one compartment is widely used in adults (Beau et al., 1995), experience in children remains limited. Recently, all-in-one TPN systems were developed for pediatric care, including amino acids, carbohydrate, lipids, electrolytes, vitamins and trace elements in the same bag (Cuntz et al., 1997). The results of this

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study and the practical advantages offered supports the use of all-in-one parenteral nutrition in infants and children.

It has been recommended that non-polyvinyl chloride (PVC) containers should be used for administration of these preparations, because it is likely that fat emulsions may extract plasticizers added to impart flexibility to PVC (Allwood, 1986). Di(2-ethylhexyl) phthalate (DEHP) is the predominant plasticizer used to make PVC plastics more flexible and pliable, and, like other lipid-soluble PVC plasticizers, comprises 30–40% of the final polymer weight, and is not covalently bound within the PVC matrix (Turner et al., 1995). Consequently, TPN mixtures containing fat emulsions should be stored in ethylvinyl acetate (EVA) bags rather than PVC containers. However, although TPN bags are made of EVA, they contain PVC-plasticized DEHP sites, and the lines used between TPN bags and venous catheters are made of PVC-DEHP. Previous studies have found detectable amounts of DEHP in blood products (Dine et al., 1991; Turner et al., 1995; Racz and Baroti, 1995), intravenous solutions (Allwood, 1986; Waugh et al., 1991; Faouzi et al., 1995) and intravenous fat emulsions (Mazur et al., 1989) stored in PVC bags. DEHP was shown to confer stability on erythrocyte membranes, reducing hemolysis and increasing in vivo cell survival, whereas it is implicated in reduced platelet function as defined by hypotonic shock recovery and aggregation (Turner et al., 1995). Several studies have reported the exposure of patients to DEHP while undergoing cardiopulmonary bypass surgery (Barry et al., 1989), in infants receiving exchange transfusion (Plonait et al., 1993), and in patients undergoing regular continuous ambulatory peritoneal dialysis (Mettang et al., 1996). Other studies have reported the leaching of DEHP in significant amounts from PVC tubing used in dialysis equipment (Christensson et al., 1991; Turner et al., 1995; Doull et al., 1999; Faouzi et al., 1999). Potential human exposure to DEHP may also be occupational (mainly by inhalation), or may occur in the general population through ingestion of residues in food or water (Steiner et al., 1998; Doull et al., 1999). Infants and children undergoing long-term parenteral nutrition could be particularly at risk of potential toxicity from DEHP due to regular exposure. The purpose of this study was to quantify the amount of DEHP that leaches from bag sites and from outlet PVC tubing containing fat emulsions under various conditions during simulated infusions.

2. Materials and methods

2.1. Parenteral admixture preparation

All preparations (infusion bags) studied were prepared by Fasonut Laboratory (Montpellier, France) and were provided by the Lille University hospital pharmacy (France). The infusion bags were made of EVA but they contained PVC sites. The administration kits were also made of PVC. The infusates were magistral preparations containing amino acids, glucose, electrolytes, vitamins, trace elements in a higher compartment of the bag and lipids in a separate lower compartment of the bag. The all-in-one mixture was prepared just before simulated infusion, by opening a connection between the two compartments of the bag. Four formulae, used in two infants and two children treated in the home parenteral nutrition program of Lille Children's University Hospital, were chosen arbitrarily according to their lipid content and flow rate of infusion dependent on infusion volumes as follows:

- Formula 1: 2200 ml containing 3.85% lipid;
- Formula 2: 2200 ml containing 2.50% lipid;
- Formula 3: 650 ml containing 1.85% lipid;
- Formula 4: 800 ml containing 1.00% lipid.

The four formulae were duplicated in two study groups:

- Group 1: One set of each formula (2 bags) was stored for 24 h at 4 °C immediately after preparation, and then used to simulate infusion.
- Group 2: The other set (2 bags) was stored for 1 week at 4 °C immediately after preparation. This allowed us to simulate the actual clinical conditions in which children receive perfusion at home: all the preparations are used for a week.

Bags stored for 24 h at 4 °C and bags stored for 1 week at 4 °C were used for the same patients. Patients were infants or children 2 years old or younger. Just before simulated administration, each bag containing the total nutrient solution was shaken to mix

the lipid source with the rest of the preparation. Bags were then attached to administration kits connected to infusion pumps (IMED Gemini PC-1 or AVI 270 infusion pumps: VitalAire Nord, France) that allowed the emulsion to flow through at a constant rate. The rate was then increased progressively until the desired values by clinician were reached. Samples were drawn aseptically from the bags and outlet tubing at 0, 0.25, 1, 2, 4, 8, 10 and 11 h, and analyzed for DEHP content. All the material used and procedures of infusion were similar to the clinical practice of home parenteral program.

2.2. Chemicals

DEHP and di-*n*-heptyl phthalate (DNHP), used as internal standards, were obtained from Sigma-Aldrich (Saint-Quentin-Fallavier, France) and used as analytical standards without further purification. HPLC-grade acetonitrile and hexane were purchased, respectively, from SDS (ZI de Valdome Peypin, France) and from Sigma-Aldrich and were each assayed for the presence of DEHP. Analytical grade sodium hydroxide, phosphoric acid and triethylamine were obtained from Prolabo (Paris, France). The water used to prepare aqueous buffers was de-ionized and purified by distillation (Milli-Q, Millipore, Saint-Quentin Yvelines, France). To minimize the risk of contamination with DEHP during sample handling and analysis, all the glassware used in the study was washed with a tetrahydrofuran–methanol mixture then rinsed with hexane. All the other reagents used were of analytical grade or better.

2.3. Analytical methods

Chromatographic analysis was performed using an HP 1090 high-performance liquid chromatograph (Hewlett-Packard, Orsay, France) equipped with a variable-volume injector, an automatic sampling system and a Hewlett-Packard Model 79994A diode-array UV detector operating at 202 nm wavelength. The output from the detector was connected to a Hewlett-Packard 9000 Model 300 integrator and the data were recorded on a Hewlett-Packard Thinkjet printer. Separation was achieved using a 5 μm Waters Spherisorb^{Æ} C_{18} column (4.6 mm × 150 mm) (Waters, Milford, MA). During assay development, DEHP and the internal standard were eluted isocratically using a mobile phase consisting of acetonitrile and aqueous buffer (triethylamine 0.08% adjusted to pH 2.8 with 1 M phosphoric acid) mixture (88:12, v/v) at a flow rate of 1.0 ml/min. The mobile phase was filtered through a 0.45 μm membrane and degassed under helium before use.

2.4. TPN sampling and assays

Each sample aliquot collected was placed in glass tubes and stored at 4 °C until HPLC analysis. Samples (1 ml aliquots) were spiked with 50 μl of DNHP (250 ng) as an internal standard, followed by sodium hydroxide 1 M (1 ml) and hexane (2 ml). The mixture was vortexed (5 min), centrifuged (1620 × *g* for 5 min) and the separated organic layer (Fraction 1) was transferred to clean conical glass tubes. The aqueous phase was extracted again with 2 ml hexane and the mixture was treated as above. The separated organic phase (Fraction 2) was combined with Fraction 1 and the total organic phase was evaporated to dryness in a water-bath at 40 °C under nitrogen. The residue was dissolved in 100 μl acetonitrile and, after centrifugation, 20 μl aliquots of the supernatant were injected into the column.

For DEHP quantification, the peak area ratio (DEHP/DNHP) (*y*) was calculated for each sample. The amount of DEHP (*x*) was determined using a calibration curve ranging from 125 to 5000 ng/ml obtained during the validation of the method. The mean linear regression equation obtained from five replicates samples was $y = 0.00156x + 0.4266$ with a correlation coefficient $r = 0.9998$. The mean coefficient of variation was 3.65% and the recovery of DEHP was nearly 97%. The detection limit (LOQ) was less than 25 ng/ml.

2.5. Data analysis

The amount of DEHP (*Q*) extracted from bags was determined and the amount of DEHP extracted from infusion tubing during 10–11 h parenteral administration was obtained by calculating the area under the curve (AUC), multiplied by the infusion flow rate (*D*): $Q = D \times \text{AUC}$.

The area under the emulsion concentration time curve (AUC) was calculated by the trapezoidal rule.

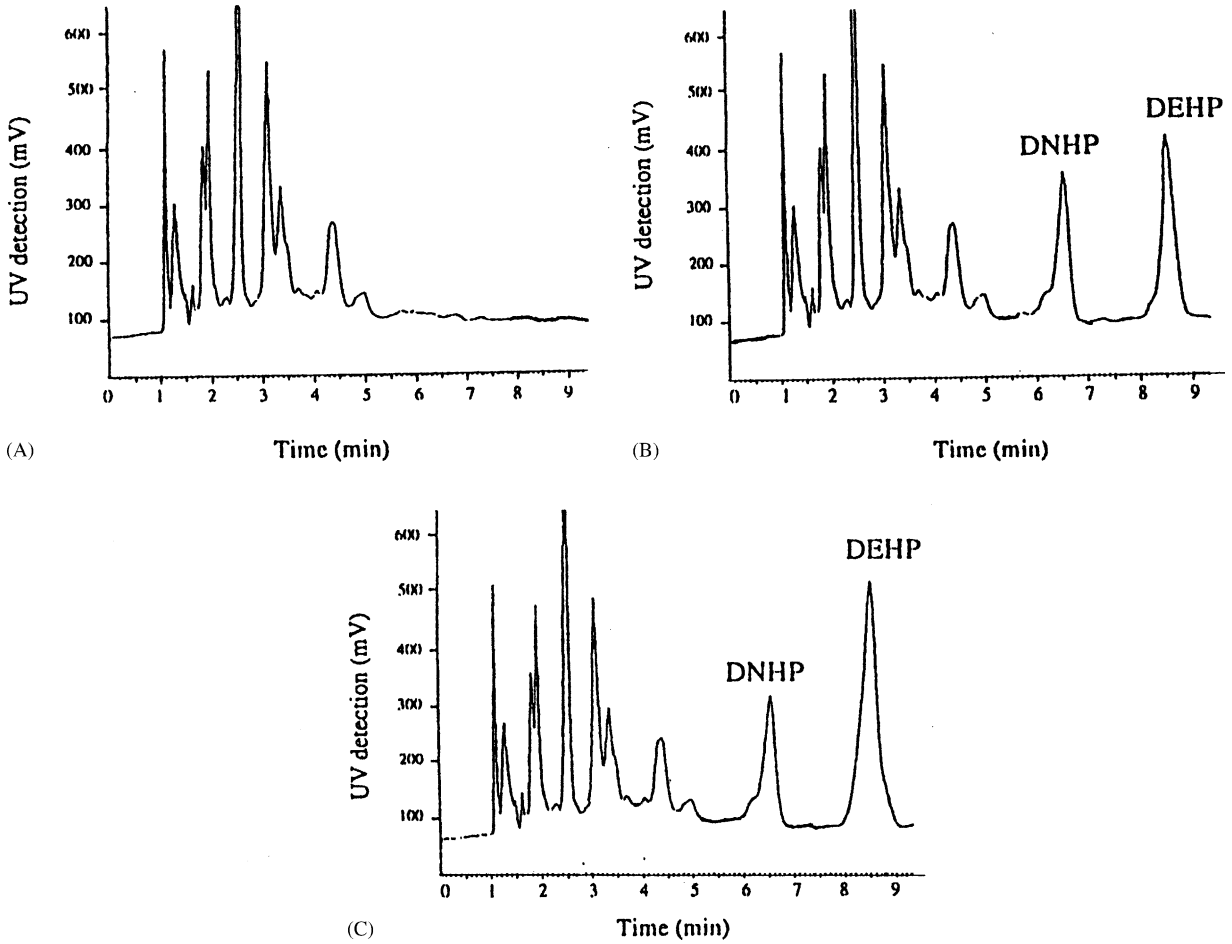

Fig. 1. Chromatograms obtained from extracted TPN samples. (A) Blank TPN; (B) blank TPN spiked with 400 ng DEHP and 250 ng of internal standard (DNHP); (C) extracted TPN sample at the outlet tubing (DEHP concentration was estimated to be 600 ng/ml).

3. Results

Fig. 1 illustrates chromatograms obtained from extracted emulsion samples, using a double extraction procedure with hexane Figs. 2–5 show the DEHP concentration time-course obtained from bags and outlet tubing during 10–11 h simulated infusion of TPN 24 h after storage at 4 °C, and 1 week later. As shown in the study, TPN emulsion extracts DEHP from bags and tubing. Extraction depends on the lipid content of each preparation and the flow rate used. It remains stable after 1 week at 4 °C. In the bag samples, DEHP was detectable immediately after mixing and remained stable during infusion. The outlet samples also demonstrated detectable levels of DEHP during infusion, and the concentrations of DEHP in the sample preparations increased throughout the 10–11 h study period. This finding is consistent with earlier reports that significant amounts of DEHP are leached in TPN regimens containing lipid (Allwood, 1986; Mazur et al., 1989). In the present study, quantities of DEHP released into TPN were estimated to be 0.2 ± 0.008 mg to 0.7 ± 0.02 mg from the bags and 0.8 ± 0.09 mg to 2 ± 0.07 mg from the outlet tubing.

In the present study, Formula 1 (containing 3.85% lipids) and Formula 2 (containing 2.5% lipids) were perfused at a flow rate of 177 and 215 ml/h, respectively. Formula 3 (containing 1.85% lipids) and Formula 4 (containing 1% lipids) were perfused at flows rate of 46 and 65 ml/h, respectively. These

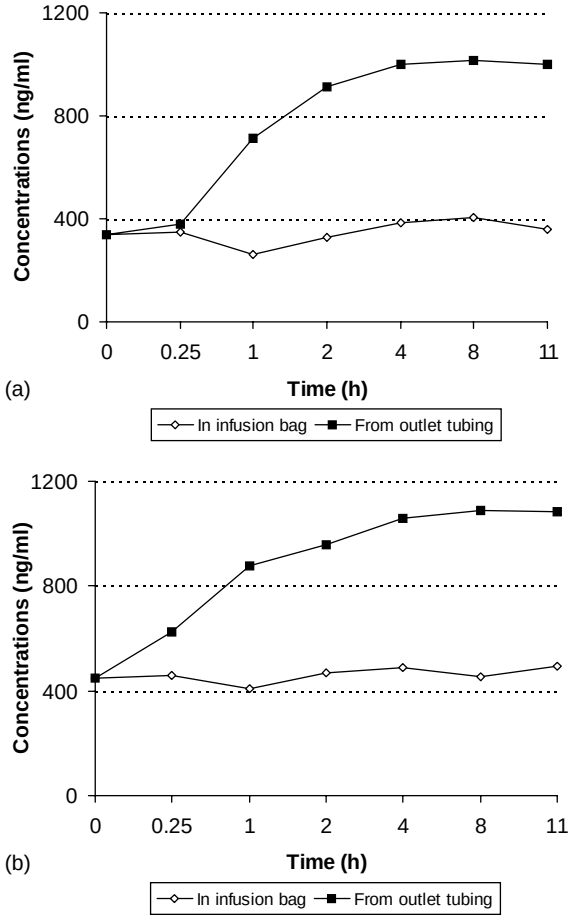

Fig. 2. Formula 1: infusion volume of 2200 ml (flow rate 177 ml/h, lipid concentration 3.85%). (a) Kinetics of DEHP leachability during simulated infusion of TPN 24 h after reconstitution of the preparation ($n = 2$ bags). (b) Kinetics of DEHP leachability during simulated infusion of TPN after storage at 4 °C for 1 week ($n = 2$ bags).

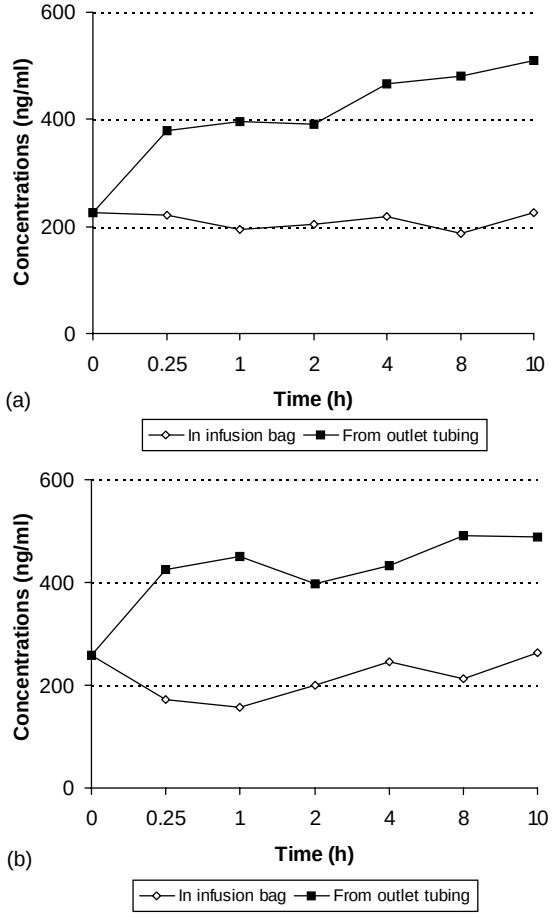

Fig. 3. Formula 2: infusion volume of 2200 ml (flow rate 215 ml/h, lipid concentration 2.50%). (a) Kinetics of DEHP leachability during simulated infusion of TPN 24 h after reconstitution of the preparation ($n = 2$ bags). (b) Kinetics of DEHP leachability during simulated infusion of TPN after storage at 4 °C for 1 week ($n = 2$ bags).

data demonstrated that extraction of DEHP by TPN depends on the lipid content of each magistral preparation and the flow rate used: DEHP leachability is higher when lipid content is increased and reduced when the flow rate is low. The total contact time between preparations and tubing increased the leachability of DEHP. The present study provides quantitative data on the amounts of DEHP that can contaminate the TPN during simulated infusion.

The concentration of DEHP measured at the outlet tubing in four magistral formulae during a single nutrition session showed a linear increase followed by a plateau, whereas it remained stable in the bag. The same phenomenon was observed with the emulsion infused after 1 week of storage at 4 °C (Figs. 2–5). In the present study, the quantity of DEHP that could potentially contaminate infants and children during a single nutrition session ranged from 0.8 to 2 mg per day.

4. Discussion

Plastic materials require the addition of a certain amount of plasticizer to obtain the specific

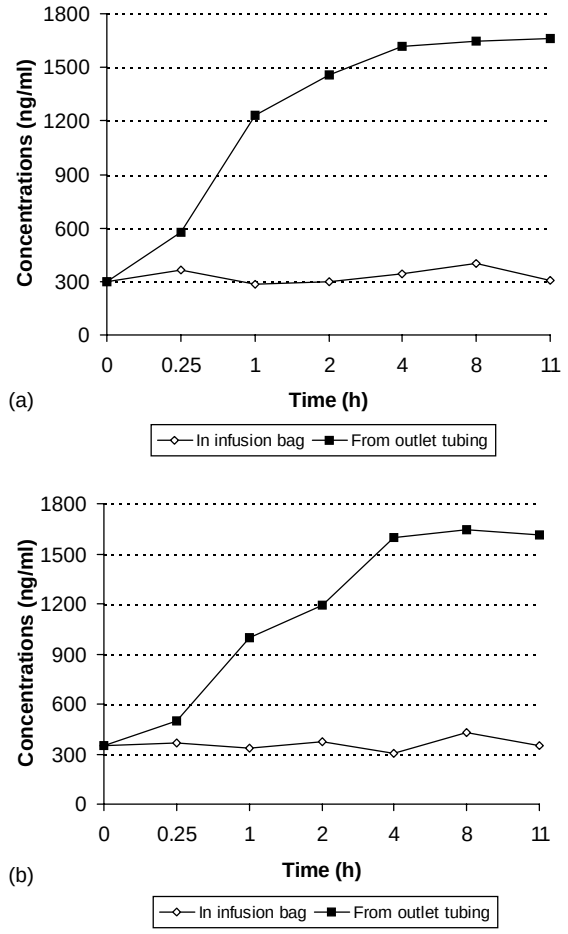

Fig. 4. Formula 3: infusion volume of 650 ml (flow rate 46 ml/h, lipid concentration 1.85%). (a) Kinetics of DEHP leachability during simulated infusion of TPN 24 h after reconstitution of the preparation ($n = 2$ bags). (b) Kinetics of DEHP leachability during simulated infusion of TPN after storage at 4 °C for 1 week ($n = 2$ bags).

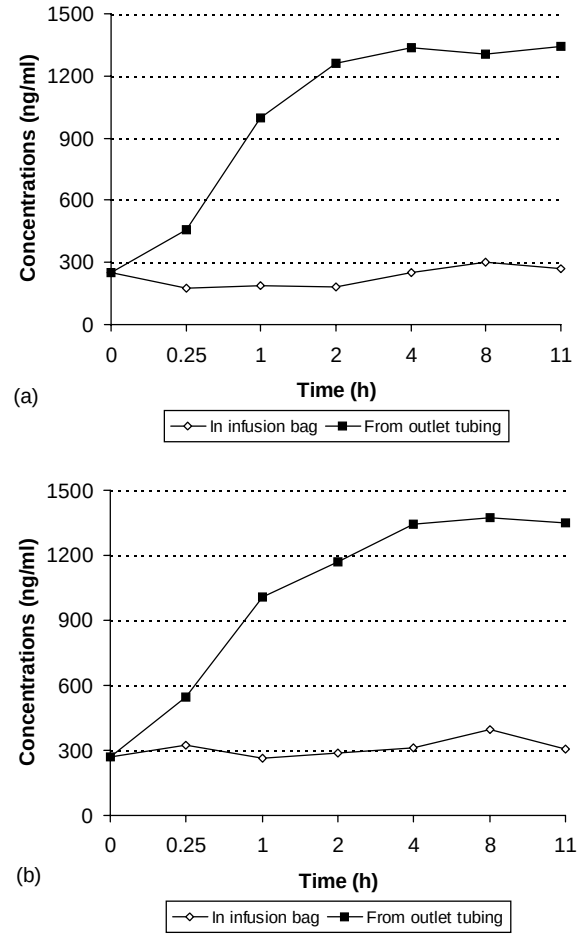

Fig. 5. Formula 4: infusion volume of 800 ml (flow rate 65 ml/h, lipid concentration 1.00%. (a) Kinetics of DEHP leachability during simulted infusion of TPN 24 h after reconstitution of the preparation ($n = 2$ bags). (b) Kinetics of DEHP leachability during simulated infusion of TPN after storage at 4 °C for 1 week ($n = 2$ bags).

physicochemical and mechanical properties required for practical applications. A high degree of safety is required, and leaching of potentially toxic or noxious compounds from plastic materials into patients should be avoided. In the present study, using the usual PVC-DEHP tubing and EVA bags with PVC-DEHP connections, we found that a constant amount of DEHP leaches from bags. A linear increase of DEHP from the outlet tubing occurred during simulated infusion of TPN containing fat emulsions. It is clear from this study that the leachability of DEHP depends on the lipid content of each preparation and the flow-rate used. This process is time-dependent. Infants and children receiving intravenous TPN infused through PVC-administration sets will thus potentially receive between 0.8 and 2 mg of DEHP every day.

Exposure of the general human population to DEHP is approximately 30 μg/kg of body weight per day, the major source being from residues in food. Higher daily exposures can occur occupationally mainly by inhalation (up to about 700 μg/kg), and from certain medical devices used for hemodialysis patients (up to

457 μg/kg) (Doull et al., 1999). Thus, prolonged exposure to DEHP by particular risk groups, such as expectant mothers or young children including neonates, should be considered unacceptable (Allwood, 1986). Information from toxicological studies on plasticizers has been used by the European Commission Expert Committee, the Scientific Committee for Food, to set tolerable daily intakes (TDI) for plasticizers. For example, the TDI for DEHP is 0.05 mg/kg body weight per day (Steiner et al., 1998). From our data, infants and children treated with long-term parenteral nutrition are regularly exposed to non-negligible amounts of DEHP ranging from 0.8 to 2 mg per day, depending on the lipid content used for TPN.

Species differences have been reported in the absorption, distribution, metabolism, and excretion of DEHP (Huber et al., 1996). It is rapidly metabolized and excreted from the body (plasma half-life for DEHP is 10–18 h, while that for mono-ethylhexyl phthalate (MEHP), the principal metabolite, is 3–6 h), and there is no evidence of persistence or tissue accumulation following repeated daily exposures (Doull et al., 1999). A previous study with rats indicated that phthalates and other peroxisome proliferators increased the growth-promoting effects of fat and increased total hepatic lipid content. This suggests that phthalates interfere with lipid metabolism and with mitochondrial energetics, including fatty acid oxidation (Winberg and Badr, 1995). However, the exact mechanism and site of action of these chemicals on mitochondrial lipid metabolism remain undefined. Data from that study suggest that MEHP inhibits mitochondrial fatty acid metabolism by inhibiting the respiratory chain at the level of cytochrome *c* reductase.

Treatment of laboratory animals with high doses of DEHP has resulted in a variety of biological effects, including testicular toxicity in rats and mice (Li et al., 1998; Akingbemi et al., 2001; Moore et al., 2001; Foster et al., 2001; Park et al., 2002; Tanaka, 2002), proliferation of peroxisomes in rodents, and liver tumors in rats and mice (Kluwe et al., 1982; Doull et al., 1999). Several types of chemicals have been shown to produce liver enlargement and enhanced peroxisomes proliferation in the rat and mouse (Reddy and Lalwani, 1983; Lake, 1995). Classes of chemicals that can produce these effects include hypolipidemic drugs, herbicides, plasticizers, industrial solvents and food flavors. Toxicity studies on peroxisome proliferators in animals and humans have demonstrated a clear evidence for species differences in hepatic peroxisome proliferation and liver tumor formation (Cattley et al., 1998; Doull et al., 1999). However, the exact mechanism of hepatocarcinogenesis of these compounds is poorly understood. It has been postulated that DEHP and several structurally dissimilar compounds increase hepatic oxidative stress by lipid peroxidation and lipofuscin deposition (Lake, 1995; Doull et al., 1999). Other studies have revealed that peroxisome proliferation in rodent hepatocytes is mediated through peroxisome proliferator-activated receptors (PPARs) (Isseman and Green, 1990; Gebel et al., 1992; Cattley et al., 1998; Doull et al., 1999). PPARs have been identified in several species, including rats, mice and humans, and are members of the steroid hormone receptor superfamily. Four different PPAR subtypes have been identified (Auboeuf et al., 1997; Cattley et al., 1998; Doull et al., 1999) but only the alpha-form (PPARα) is directly involved in the induction of peroxisome proliferation in the rodent liver.

Rats and mice are uniquely responsive to the morphological, biochemical and chronic carcinogenic effects of peroxisome proliferators, while non-human primates and humans are essentially non-responsive or refractory (Cattley et al., 1998; Doull et al., 1999). These differences are explained, in part, by marked interspecies variations in the expression of PPARα, with levels of expression in humans being only 1–10% of expression levels in rodents (Tugwood et al., 1996; Cattley et al., 1998; Palmer et al., 1998).

In rats, DEHP is both a male and female reproductive toxicant. Data from few studies in rodents reported that phthalates effects on reproductive cells are influenced by the stage of development at exposure (Moore et al., 2001; Akingbemi et al., 2001; Park et al., 2002). Effects of DEHP are more pronounced early in the development than at later stage.

Accordingly, results obtained in animal models cannot be applied to humans. However, disturbances in lipid metabolism and hepatic function observed in chronic hemodialysis patients, as in children treated by long-term parenteral nutrition, could be explained by the inducer effects of DEHP on peroxisomal proliferation and β-oxidation of fatty acids (Bell et al., 1978). Such effects have not been identified clinically from humans exposed to phthalates or by in vitro studies

with human hepatocytes, although PPARα is expressed at a very low level in the human liver. Further investigations are therefore required to better define the sensitivity of humans to phthalates in comparison to rodents that have been more extensively studied.

However, in the light of the present study, it would appear advisable to use non-phthalate-containing sets or tubing to administer TPN-containing lipid sources to children, especially infants and neonates. Although DEHP is not an acutely toxic compound and prolonged exposure to DEHP leached into the blood of patients undergoing hemodialysis was not associated with specific toxicity, its use in TPN tubing, when it could be avoided through careful selection of material, is both unprofessional and undesirable.

In infants and children on regular intravenous parenteral nutrition containing fat emulsions, hepatic toxicity with lipid disturbances is a frequent complication and its pathology is still poorly understood. The role of DEHP in TPN-related liver disease needs to be investigated further.

Further studies should be performed to determine the plasma concentrations of phthalates in infants and children under long-term parenteral nutrition and to confirm the risk of exposure.

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