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Simulated neonatal exposure to DEHP and MEHP from PVC enteral nutrition products

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Abstract

The leaching of di(2-ethylhexyl)phthalate (DEHP) and mono(2-ethylhexyl)phthalate (MEHP) from medical products made of polyvinyl-chloride (PVC) to enteral nutrition (EN) for neonatal patients was determined in a simulated study. The study simulated a typical case of EN administration to a neonatal patient (body weight, 3 kg) in a neonatal care unit (temperature, 25 °C); the medical products used were an irrigator and catheter containing DEHP (9.1–31.8%, w/w) as a plasticizer. The worst-case daily exposures of the neonatal patient to DEHP and MEHP by the administration of EN were estimated to be 148 and 3.72 μg/(kg day), respectively, as assessed from the levels of these compounds leaching from the medical products to the EN. The use of DEHP-free medical products reduced the exposure of DEHP and MEHP to the minimum levels contained in the EN at preparation. A transition to DEHP-free medical products for neonatal patients would be effective in reducing the exposure of neonatal patients to DEHP via EN administration.
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1. Introduction

Di(2-ethylhexyl)phthalate (DEHP) is a common plasticizer used to impart flexibility to polyvinyl-chloride (PVC). It readily leaches from PVC into the environment and transfers to other materials attached to the PVC or via the atmosphere. PVC is used in a variety of medical products for its excellent physical characteristics such as flexibility, strength, and transparency. Patients undergoing medical procedures such as intravenous therapy, nutritional support, blood transfusion, haemodialysis, cardiopulmonary bypass, or extracorporeal membrane oxygenation (EMO) are potentially exposed to DEHP released from medical products. Previous studies have shown detectable amounts of DEHP in blood products, intravenous solutions, and intravenous fat emulsions stored in PVC bags (Mazur et al., 1989; Dine et al., 1991; Waugh et al., 1991; Faouzi et al., 1999; Hanawa et al., 2000). DEHP is hydrolyzed to mono(2-ethylhexyl)phthalate (MEHP) *in vivo* and in blood products by esterase activities (Albro and Thomas, 1973; Lake et al., 1977). DEHP and MEHP have also been detected in the blood of haemodialysed patients (Flaminio et al., 1988a). Orally administrated DEHP in enteral nutrition (EN) can be hydrolyzed to MEHP by lipase, which is then absorbed via the intestine as well as within food and water (Albro and Thomas, 1973).

In animal studies, DEHP and/or MEHP are toxicants to the reproductive and developmental systems (Gray and Gangolli, 1986; Sjöberg et al., 1986; Moss et al., 1988; Davis et al., 1994). The toxicities of DEHP and/or MEHP are dose-, time-, and age-dependent for the target organ and tissue deposition (Latini, 2000). Recent epidemiologic studies report that certain phthalates exposure levels of pregnant women were significantly associated with duration of pregnancy and reproductive health of male infants (Latini et al., 2003; Swan et al., 2005; Marsee et al., 2006), and the possible effects of phthalates on the reproductive systems of male infants are well documented

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(Lottrup et al., 2006). Male neonates and infants are considered a sensitive population to toxicity because their reproductive systems are still developing.

Calafat et al. (2004) reported that the geometric mean of MEHP and its glucuronide concentrations in the urine of ill neonates who spent time in neonatal intensive care units was 30 times higher than that in healthy children; the neonatal patients were exposed to DEHP via medical procedures. The Center for Devices and Radiological Health in U.S. Food and Drug Administration (FDA/CDRH) has reviewed the potential health risks of DEHP leaching from medical devices (FDA/CDRH, 2001, 2002) and recommended considering alternatives when high-risk procedures such as transfusion, haemodialysis, total parenteral nutrition (TPN), EMO, or EN are to be performed on male neonatal patients, pregnant women carrying a male fetus, or peripubertal males (FDA/CDRH, 2002). The FDA report estimated the daily exposure of neonatal patients to DEHP from EN administration to be 140 μg/(kg day) (FDA/CDRH, 2001), while the exposure of infants to DEHP from infant formula and breast milk was estimated to be 8–21 μg/(kg day) (Latini et al., 2004). The difference in exposure level between EN and infant formula or breast milk is deriving from the DEHP leached from medical products; however, information regarding the exposure of neonatal patients to DEHP and MEHP via EN administration is limited. We previously developed a method for determining DEHP and MEHP levels in human sera without severe contamination of DEHP, using a liquid chromatography with tandem mass spectrometry (LC/MS/MS) (Takatori et al., 2004). In the present study, we use this method to investigate the exposure of neonatal patients to DEHP and MEHP via EN administration.

2. Materials and methods

2.1. Materials

DEHP (99.6%), MEHP (99.3%), DEHP-d_4 (99.0%), and MEHP-d_4 (99.8%) were purchased from Hayashi Pure Chemical Industries Ltd. (Osaka, Japan), while analytical-grade acetone, hexane, acetonitrile, ethanol, and tetrahydrofuran were purchased from Wako Pure Chemical Co. Ltd. (Osaka, Japan). Water for HPLC was purified using the Milli-Q system (Milli-Q, Millipore, Bedford, MA). The water used in this study (except for eluent of LC/MS/MS) was prepared by washing the Milli-Q water with hexane. To eliminate contamination by DEHP and MEHP, glassware was washed twice with acetone and hexane and then baked at 200 °C for 2 h in a clean oven.

In the present study, we used two types of irrigators and catheters that are commercially available in Japan and are commonly used for neonatal or infant patients: PVC (standard-type) containing DEHP as a plasticizer, and poly-butadiene (DEHP-free type) that contains no DEHP. Both types of irrigators consist of four components (bag, tube, drip tube, and adaptor) and a clamp for flow control. The catheters consist of a connector and a tube. The sizes of the two types of irrigators and catheters are shown in Table 1.

Table 1
Sizes of irrigators and catheters

Component	Size	
	Standard	DEHP-free
Irrigator		
Bag[a]	500	500
Tube[b]	890, 3.5	1010, 3.5
Drip tube[b]	55, 16	50, 18
Adaptor[b]	35, 3.5	40, 3.5
Catheter		
Tube[b]	420, 1.7	420, 1.7
Adaptor[b]	25, 3.5	25, 3.5

[a] Volume (ml).
[b] Total length and internal diameter (mm).

2.2. LC/MS/MS conditions

LC/MS/MS was used to determine DEHP and MEHP. LC/MS/MS analysis was performed using an API3000 mass spectrometer (Applied Biosystems, Foster City, CA, USA) equipped with an electro-spray ionization (ESI) interface and an Agilent 1100 series high performance liquid chromatography (HPLC, Agilent Technologies, Waldbronn, Germany). The HPLC system consisted of a G1312A HPLC binary pump, a G1367A autosampler, and a G1379A degasser. We used a reverse-phase HPLC column (Wakosil3C18, 2.0 mm × 150 mm, 3 μm; Wako Pure Chemical Co. Ltd.). The mobile phases consisted of 100% acetonitrile (A) and 5×10^{-4}% aqueous acetic acid (B). Elution was performed using an isocratic mode (A/B: 80/20, v/v) at 0.2 ml/min, and the ESI interface was controlled by Analyst Software (v.1.3.2). The MS/MS was operated in negative or positive ion mode. The heated capillary and voltage were maintained at 500 °C and ±4.0 kV (negative/positive mode), respectively. MEHP and MEHP-d_4 were detected in the negative mode. The declustering potential (DP), focussing potential (FP), and collision energy (CE) for MEHP and MEHP-d_4 measurements were −31, −110 V, and −22 eV, respectively. DEHP and DEHP-d_4 were detected in the positive mode. The DP, FP, and CE for DEHP and DEHP-d_4 measurements were 26, 100 V, and 27 eV, respectively. The respective combinations of precursor ions and product ions were as follows: MEHP (−277, −134), MEHP-d_4 (−281, −138), DEHP (391, 149), and DEHP-d_4 (395, 153).

2.3. DEHP and MEHP content in irrigators and catheters

To determine the DEHP content in the components of the standard-type irrigator and catheter, sections of each were cut into 2 mm × 2 mm squares. To dissolve the pieces, 5.0 ml tetrahydrofuran was added to a 50-ml volumetric flask containing 0.2 g of the cut squares. PVC polymer was precipitated by adding ethanol up to the volumetric line of the flask. After removing the precipitant by centrifugation, the aliquot of supernatant was diluted with acetonitrile before being analyzed using LC/MS/MS.

Table 2
DEHP content of the standard-type irrigator and catheter ($n = 3$)

Component	Content (%, w/w)
Irrigator	
Bag	26.1 ± 0.8
Tube	22.0 ± 1.4
Drip tube	15.2 ± 1.6
Adaptor	9.1 ± 1.3
Catheter	
Tube	31.8 ± 0.6
Adaptor	31.3 ± 0.6

2.4. Preparation of EN

Four commercially available brands of EN (EN-A, -B, -C, and -D) were used in the present study. Both EN-A and -B are used for neonatal and infant patients, while EN-C and -D are used for adult patients. EN-A, -B, and -C are supplied as powders that were prepared immediately prior to the experiments with warmed hexane-washed Milli-Q water (40 °C) to minimize contamination with DEHP and MEHP. EN-A, -B, and -C were prepared at standard concentrations (EN-A: 20%, w/v; EN-B: 17%, w/v; EN-C: 27%, w/v) according to the manufacturer's directions. EN-D is a canned liquid that was opened just before the experiments and used without further preparation.

2.5. Leaching of DEHP and MEHP from medical products to EN

Simulated studies were performed assuming that EN-A is administrated to a neonatal patient. Details of the case are as follows. A neonatal patient (body weight, 3 kg) was cared for in a neonatal patient care unit at 25 °C. Daily nutrition (312 kcal/day) was supplied by EN-A (20%; 392 ml) with an irrigator and catheter that contained DEHP. The daily EN-A was divided into seven portions (56 ml/portion), administered seven times a day. Each portion was administered over 15 min. The simulated studies were performed in our laboratory in an incubator regulated at 25 °C. The glass flask of prepared EN-A was equilibrated to 25 °C in the incubator for 30 min. The EN-A was then poured into the irrigator before flowing into clean glass tubes via the catheter. The flow time of 56 ml of EN-A from the irrigator to the glass tubes was regulated using a clamp to 15 min. The flow rate was approximately 3.5–4 ml/min. To compare leaching of DEHP and MEHP between the ENs, the same volume (56 ml) of EN-A (10–15%), -B, -C, -D, and water was run through the irrigator and catheter set as described above. The collected EN solutions were frozen at −40 °C until analysis.

2.6. Determination of DEHP and MEHP in EN

DEHP and MEHP in the EN were determined using the previously described procedure for determining these compounds in human serum (Takatori et al., 2004). Briefly, 50 ng DEHP-d_4 and MEHP-d_4 were added to a glass tube containing 0.5 ml EN. DEHP and MEHP in the EN were initially extracted with acetone. The acetone extraction was dried under an N_2 stream and then dissolved with 0.5 ml hexane-washed Milli-Q water containing 4 μl acetic acid. Next, DEHP and MEHP in the acetic acid aqueous solution were extracted three times with hexane. The extract was dried as described above and then dissolved in 0.5 ml acetonitrile before being analyzed using LC/MS/MS.

2.7. Raman microspectroscopic analysis of the medical products

A Raman microspectrometer, NRS-3100 (JASCO, Tokyo, Japan), was used to measure the Raman spectra of the internal surface of irrigator and catheter. The Raman microspectrometer was coupled with thermoelectrically cooled charge-coupled device detector. The holographic grating was 1800 g/mm. The exciting wavelength was 532 nm from a green laser with a power of 9.2 mW. The laser was focused on the surface of the sample by using a 5× microscope objective. The spatial resolution was 120 μm in the X–Y plane. The spectral resolution was 1 cm^{-1}. Raman spectrum was collected over the range from 1879 to 541 cm^{-1} and the integration time was 30 s. The measurements were repeated five times to normalize data.

3. Results

The content of DEHP and MEHP was determined for each part of the irrigator and catheter made of PVC (standard-type) without surrogates. DEHP and MEHP were recovered from the extraction solution to satisfactory levels (95–105%). All parts

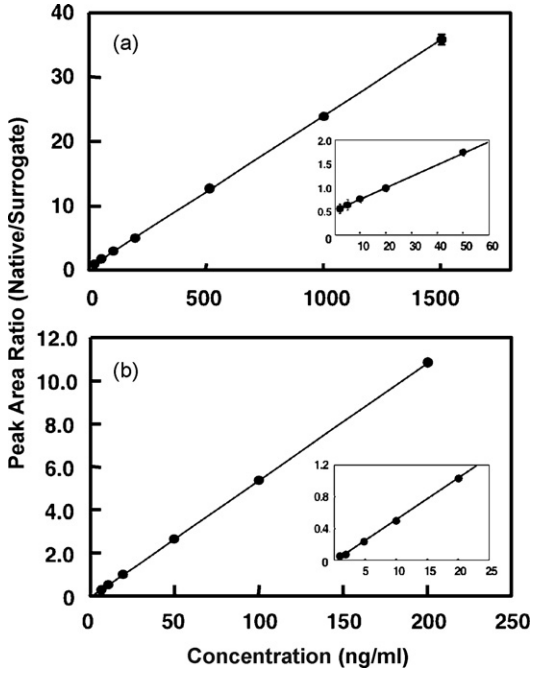

Fig. 1. Calibration curves for DEHP and MEHP. Points and bars represent the averages and standard deviations of five independent experiments. (a) Vertical axis corresponds to the peak-area ratio (DEHP/DEHP-d_4). Inset indicates the low range (2–60 ng/ml) of the curve. (b) Vertical axis corresponds to the peak-area ratio (MEHP/MEHP-d_4). Inset indicates the low range (1–25 ng/ml) of the curve.

Table 3
DEHP and MEHP concentrations in the EN at preparation and after flowing through the irrigator and catheter ($n = 6$)

EN	Concentration (%, w/v; kcal/ml)	Fat (mg/ml)	DEHP (ng/ml)		MEHP (ng/ml)	
			Before	After	Before	After
A	20; 0.8	7.2	49.6 ± 8.4	1130 ± 190	<LOQ[b]	28.5 ± 4.8
A	15; 0.6	5.4	32.8 ± 4.1	1080 ± 40	<LOQ[b]	30.5 ± 1.5
A	10; 0.4	3.6	21.6 ± 4.1	1000 ± 65	<LOQ[b]	28.6 ± 1.6
B	17; 0.6	4.3	58.6 ± 3.0	203 ± 43	6.0 ± 0.3	23.5 ± 1.4
C	27; 1.0	1.7	64.3 ± 2.4	513 ± 60	6.9 ± 0.7	26.1 ± 0.1
D	–; 1.0	33.2	158 ± 7.7	721 ± 93	<LOQ[b]	24.9 ± 1.9
Water	–; 0	0	<LOQ[a]	54 ± 17	<LOQ[b]	24.4 ± 0.9

[a] 15 ng/ml.
[b] 5 ng/ml.

contained 9.1–31.8% (w/w) of DEHP (Table 2), while the content of MEHP in the same parts was less than 0.1%.

For DEHP measurement in EN, the calibration curve was obtained for the peak-area ratio (DEHP/DEHP-d_4) versus DEHP concentration (Fig. 1(a)). The curve was linear over the range of 2.0–1500 ng/ml. The mean linear regression equations obtained from five replicates were $y = 0.0237x + 0.566$ ($r = 0.999$), with mean values for the slope and intercept of 0.0237 ± 0.0003 and 0.566 ± 0.129, respectively (y, peak-area ratio; x, DEHP concentration, ng/ml). For MEHP measurements, the calibration curve was obtained for the peak-area ratio (MEHP/MEHP-d_4) versus the MEHP concentration (Fig. 1(b)). The curve was linear over the range of 1.0–200 ng/ml. The mean linear regression equations obtained from five replicates were $y = 0.0541x - 0.0530$ ($r = 0.999$) with mean values for the slope and intercept of 0.0541 ± 0.0012 (mean ± S.D. (standard deviation)) and −0.0530 ± 0.0186, respectively (y, peak-area ratio; x, MEHP concentration, ng/ml). The recoveries of 20 ng/ml DEHP and MEHP fortified into EN-A (20%) were 91 ± 3.0 and 99 ± 3.0%, respectively ($n = 6$). The average and S.D. of background levels of DEHP and MEHP of the preparation of test solution were 5.5 ± 1.8 and less than 1.0 ng/ml, respectively ($n = 6$). The limits of quantification (LOQ) of DEHP and MEHP determined by the equation LOQ = background + S.D. × 5 were 15 and 5 ng/ml, respectively.

At preparation, the concentrations of DEHP and MEHP in the EN were 21.6–158 and <5–6.9 ng/ml, respectively (Table 3). After flowing the EN through the irrigator and catheter, we observed that DEHP and MEHP had leached from the medical products. Typical chromatograms of EN-A are shown in Fig. 2. The degree of DEHP leaching was dependant on the brand of EN; in contrast, MEHP leaching was approximately 20 ng/ml for all EN. The leaching of DEHP was not affected by the concentration of EN-A.

We calculated the exposure of a neonatal patient to DEHP from EN administration using the DEHP concentration in EN-A

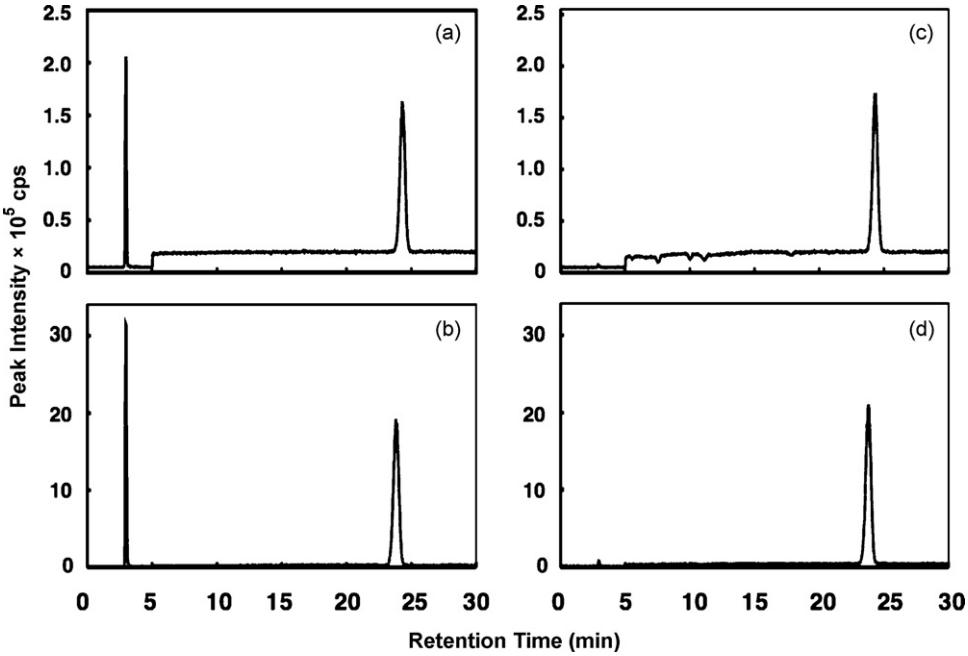

Fig. 2. DEHP and MEHP chromatograms. Retention times of MEHP and DEHP were 3.0 and 23.8 min, respectively. (a) Standard mixture 50 ng/ml; (b) standard mixture 1000 ng/ml; (c) EN-A (20%) at preparation; (d) EN-A (20%) after flowing through the irrigator and catheter.

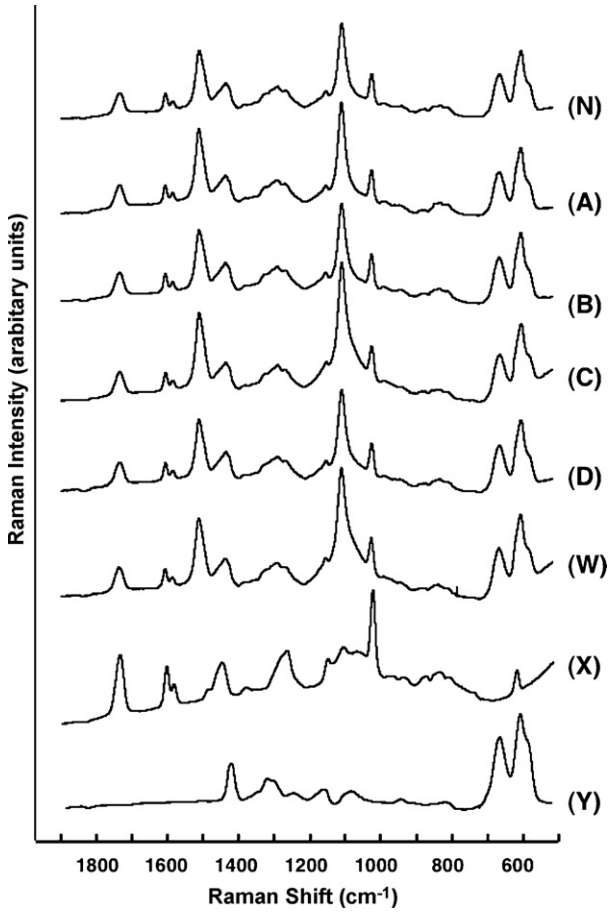

Fig. 3. Raman spectra of the internal surface of irrigator bags used for administration of EN under the conditions as described. (N) unused; (A) used for EN-A administration; (B) used for EN-B administration; (C) used for EN-C administration; (D) used for EN-D administration; (W) used for water administration; (X) DEHP standard; (Y) Pure PVC, which did not contain plasticizers including DEHP.

(20%) and the following equation:

$$Y\,(\mu g/(kg\,day)) = \frac{X\,(ng/ml)\ \times\ 392\,(ml/day)}{3\,(kg)\ \times\ 1000}$$

Where Y is the daily exposure per kg body weight and X is the concentration of DEHP in EN-A (20%).

In this case, the exposures to DEHP and MEHP from EN-A administration were calculated as 148 and 3.72 μg/(kg day), respectively. For the case of EN-B (17%), supplying the same calories (312 kcal/day) via EN administration, exposure to DEHP and MEHP was estimated to be 35.4 and 4.09 μg/(kg day), respectively.

When a standard-type irrigator and DEHP-free type catheter set were used, the leaching of DEHP and MEHP to EN-A (20%) was reduced to 795 ± 44 and 24.0 ± 3.1 ng/ml, respectively ($n = 3$). When a DEHP-free type irrigator and a standard-type catheter set were used, the leaching of DEHP and MEHP was reduced to 439 ± 19 and 17.3 ± 4.2 ng/ml, respectively ($n = 3$). When a DEHP-free type irrigator and DEHP-free type catheter set were used, leaching of DEHP and MEHP was not observed. Thus, in this case the exposures of neonatal patients to DEHP and MEHP can be reduced to a minimum level that can be calculated from the concentration at preparation. The exposure to DEHP and MEHP from EN-A (20%) administration was 6.48 and <0.653 μg/(kg day), respectively.

Raman spectroscopic analysis was conducted to examine the loss of DEHP at the internal surface of medical products. Fig. 3 shows the Raman spectra of the internal surface of the irrigator bags. The spectra consist of peaks derived from PVC (1429, 692 and 632 cm^{-1}), DEHP (1725, 1597, 1585, 1454, 1273 and 1037 cm^{-1}) and unknown components of the part (1505 and 1118 cm^{-1}). Significant differences were not found between the spectra of unused and used irrigators. The Raman intensity ratio (RIR) between 1725 and 692 cm^{-1} ($I_{peak\ 1725}/I_{peak\ 692}$) was calculated as an index of content ratio of DEHP to PVC at the internal surface. RIR of the irrigators were as follows: unused, 0.54; used for EN-A, 0.55; used for EN-B, 0.56; used for EN-C, 0.55; used for water, 0.55. Significant differences were also not found between the spectra of unused and used catheters (data not shown).

4. Discussion

To prevent adverse effects such as diarrhea, EN can be prepared in a range of concentrations appropriate to the physical condition of the patient. We observed that the lipid content of the EN was not a factor in promoting the leaching of DEHP; interestingly, leaching of DEHP from the irrigator and catheter was not affected by the concentration of EN-A. Some EN products incorporate an emulsifier to dissolve lipophilic components uniformly; lecithin and polysorbate 80 (PS80) are added to EN-A and EN-C, while lecithin alone is added to EN-D. The lecithin and PS80 exert emulsification in the ranges of appropriate concentration. Neither lecithin nor PS80 is added to EN-B, which recorded the lowest levels of DEHP leaching. Thus, we consider that the presence of an emulsifier in EN is an important factor that promotes leaching of DEHP from EN medical products. Hanawa et al. (2000) reported that the leaching of DEHP from medical products to saline reached a plateau with the addition of PS80 at around its critical micelle concentration; this finding corresponds with the observations of the present study. The leaching of DEHP from medical products to intravenous solutions is also promoted by the addition of lipophilic components and emulsifiers (Haishima et al., 2005). In these cases, emulsifiers would be potentially one of the most important factors in determining the level of leaching.

We observed that a small amount of MEHP leached from the medical products into all the ENs and Milli-Q water. MEHP is produced from DEHP in medical products by gamma ray irradiation or ethylene oxide gas treatment for sterilizing. MEHP detected in the present study would have been produced from DEHP in the medical products after sterilization with ethylene oxide gas (Ito et al., 2006). MEHP is more soluble in water than DEHP; thus, the components of the EN would not have affected the leaching of MEHP.

The daily exposure of a neonatal patient to DEHP by EN-A administration with the irrigator and catheter set was 148 μg/(kg day), which is close to the amount of 140 μg/(kg day) estimated by the FDA (FDA/CDRH, 2001). This exposure

level corresponds to the upper acceptable daily intake of DEHP (140 μg/(kg day)). Leaching of DEHP and MEHP from DEHP-free medical products was not observed; thus, by using DEHP-free type medical products, exposure to DEHP via EN administration can be reduced to the minimum level that is present in the EN at initial preparation. Recently, DEHP-free medical products have become widely used. It would seem reasonable that such products should be used for the population sensitive to the toxicity of DEHP and MEHP, including pregnant women, male neonatal patients, and infants; alternatively, medical products with substitute plasticizers such as tri(2-ethylhexyl)trimellitate (TOTM) and di(2-ethylhexyl)adipate (DEHA) should be used. The leachability of TOTM from haemodialysis tubes to blood is lower than that of DEHP (Flaminio et al., 1988b). The hepatic toxicity of TOTM is lower than that of DEHP (Hodgson, 1987; Kambia et al., 2004). The testicular toxicity of DEHA has not been observed in animal studies (Kang et al., 2006); however, there is much less information regarding the toxicities of these plasticizers compared to DEHP, and further investigation into these potential plasticizers is required to guarantee pregnant women and neonatal and infant patients against the risk associated with usage.

A Raman spectrometer is useful to determine the content of components in resin (Kikuchi et al., 2004; Norbygaard and Berg, 2004). Norbygaad and Berg successfully determined the content of phthalate diesters in PVC by a Raman spectrometer (Norbygaard and Berg, 2004). We utilized a Raman microspectrometer to examine the loss of DEHP at the internal surface of medical products. No obvious differences between the unused products and used products were found on the Raman spectra. Norbygaad and Berg determined the content of DEHP in PVC by the RIR at 1726 and 696 nm, which are independent and characteristic peaks of DEHP and PVC, respectively. In this study, the corresponding RIR of the internal surface of irrigator bags remained at approximately 0.5, whether they were unused or used. The main parts of the irrigator and catheter have a total content of DEHP corresponding to around 25% of their total weight of 47 g. Their total DEHP content is thus estimated to be 11.8 g. From the results described above, the leached DEHP to EN was estimated to be at most 59 μg, which is only 5.0×10^{-4}% of the total DEHP contained in those medical products. These observations suggest that the loss of DEHP would be too low to detect it by Raman spectroscopic changes even though at the surface, and/or DEHP corresponding to the loss by leaching to EN may be promptly supplied to the surface from the inside of the resin. Our results imply that used medical products have the potential to leach similar amounts of DEHP to EN to new products. Further studies to determine correlations of DEHP leaching from medical products with times of their use may be informative and may provide a basis for avoiding excessive exposure of patients to DEHP.

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