ELSEVIER

International Journal of Pharmaceutics 229 (2001) 139–146

international journal of pharmaceutics

www.elsevier.com/locate/ijpharm

Comparative study of the leachability of di(2-ethylhexyl) phthalate and tri(2-ethylhexyl) trimellitate from haemodialysis tubing

K. Kambia [a], T. Dine [a,*], R. Azar [b], B. Gressier [a], M. Luyckx [a], C. Brunet [a]

[a] *Laboratoire de Pharmacologie, Pharmacocinétique et Pharmacie Clinique, Faculté des Sciences Pharmaceutiques et Biologiques, 3 rue du Professeur Laguesse, BP 83, 59006 Lille Cedex, France*

[b] *Service de Néphrologie et d'Hémodialyse, Département de Médecine Interne B, Centre Hospitalier Général de Dunkerque, Av. L. Herbaux, BP 6-367, 59385 Dunkerque, France*

Received 26 January 2001; received in revised form 23 July 2001; accepted 31 July 2001

Abstract

The leachability of both Di(2-ethylhexyl) phthalate (DEHP) and Tri(2-ethylhexyl) trimellitate (TEHTM) or Trioctyl trimellitate (TOTM) from haemodialysis tubing was investigated in 20 patients with chronic renal failure undergoing maintenance haemodialysis. The blood tubing made of common polyvinyl chloride (PVC) plasticized with DEHP (group 1 patients) were replaced with tubing plasticized with TOTM-DEHP (group 2 patients). The patient blood obtained from the inlet and the outlet of the dialyzer was analyzed during a 4 h-dialysis session. Thus, the circulating concentrations of both DEHP and TOTM resulting from the release from dialyzer tubes were estimated using High-performance Liquid chromatograph (HPLC). With the common PVC-DEHP blood tubing, a DEHP quantity of 122.95 ± 33.94 mg was extracted from tubing during a single dialysis session (ranging from 55 to 166.21 mg). During the same period, the total amounts of DEHP retained by the patients were 27.30 ± 9.22 mg (ranging from 12.50 to 42.72 mg). As for blood tubing plasticized with TOTM-DEHP, 41.80 ± 4.47 mg of DEHP and 75.11 ± 25.72 mg of TOTM were extracted. During the same period, the amounts of DEHP and TOTM retained by the patients were 3.42 ± 1.37 mg and 4.87 ± 2.60 mg, respectively. The extraction rate both plasticizers was correlated with serum lipid content (cholesterol + triglyceride) ($r^2 = 0.75$ for DEHP and $r^2 = 0.64$ for TOTM). In the present investigation, less TOTM and DEHP were apparently released from haemodialysis tubing plasticized with TOTM-DEHP than DEHP released from haemodialysis tubing plasticized with DEHP only. TOTM seems to be a superior alternative to DEHP for use in medical devices because of its potential lower leachability. To recommend it as an alternative plasticizer, its possible toxicity towards human body should be investigated before it can be used routinely. However, patients undergoing haemodialysis using tubing plasticized with DEHP only are regularly exposed to non negligible amounts of DEHP. In view of several biological effects previously reported, it is time to reconsider the use of DEHP only as a plasticizer. © 2001 Published by Elsevier Science B.V.

Keywords: TOTM; DEHP; PVC; Release; Haemodialysis patients

* Corresponding author. Tel.: +33-320-964040; fax: +33-320-969752.
E-mail address: tdine@phare.univ-lille2.fr (T. Dine).

0378-5173/01/$ - see front matter © 2001 Published by Elsevier Science B.V.
PII: S0378-5173(01)00840-7

1. Introduction

Polyvinyl chloride (PVC) plasticized with DEHP found wide use in medical and paramedical appliances as well as in food storage packaging (Martis et al. 1987; Rathinam, 1988). For this reason, a number of phthalic acid esters, including di(2-ethylhexyl) phthalate (DEHP), have been subjected to fairly extensive safety testing. Since the toxicity of DEHP towards animals was suspected (Parmar et al., 1995; Peters et al., 1997; Doull et al., 1999), an intensive research on new, biologically inert plasticizers to be used in blood bags, haemodialysis material has been initiated. Among the alternative plasticizer studied, tri(2-ethylhexyl) trimellitate (TEHTM or TOTM) has been increasingly attractive (Flaminio et al., 1988).

TOTM (an ester of trimellitic acid) like DEHP (an ester of phthalic acid) was used in such medical devices, had applications in pool liners, furniture, outwear and weather-stripping (Martis et al., 1987). However, very little information was available on its biological effects. Before using TOTM as an alternative plasticizer to DEHP, some studies including toxicity, disposition and metabolism, are required.

It is clear that a non negligible amounts of DEHP leaches from PVC into blood products (Dine et al., 1991; Turner et al., 1995), into intravenous solution (Faouzi et al., 1995) and into patients undergoing maintenance haemodialysis (Pollack et al. 1985; Faouzi et al., 1999). On the other hand, the exposure of laboratory animals to high DEHP doses resulted in various biological effects, including testicular atrophy in rats (Flaminio et al., 1988; Parmar et al., 1995), proliferation of peroxisomes in rodents (Bojes and Thurman, 1994; Doull et al., 1999) and liver tumors in rats and mice (Kluwe et al. 1982; Reddy and Lalwani 1984). In addition, studies in rodents have shown that DEHP can induce several changes in hepatic morphology and biochemical functions (Van Den Munckhof et al., 1998). In view of these findings in animals, intensive researches on alternative non toxic plasticizers has been carried out including the current investigation on TOTM as a plasticizer having negligible leachability (Flaminio et al., 1988; Rathinam, 1988; Christensson et al., 1991). With regard to haemodialysis patients using dialysis tubing plasticized with DEHP only, the estimated values previously reported for DEHP exposure range widely (Pollack et al., 1985; Nässberger et al., 1987; Faouzi et al., 1999). For the new dialysis tubing plasticized with TOTM-DEHP, little is known about the real exposure of both plasticizers.

The aim of this study was to quantify the amounts of both DEHP and TOTM released into the blood of haemodialyzed patients using the new dialysis tubing plasticized with TOTM-DEHP and to compare them with the amounts of DEHP released when the plasticizer was DEHP only.

2. Materials and methods

2.1. Subjects

Before entering the study, each patient had been on haemodialysis treatment for periods ranging from 1 to 7 years. Consent was obtained from all subjects. Patients, with congestive heart failure, pulmonary oedema, hepatic or acute renal failure were not included in the study. Plasma levels of TOTM or DEHP were determined for two groups of patients:

Group 1: 10 patients (seven men and three women) aged 61–86 years old with chronic renal failure on maintenance haemodialysis in the dialysis Unit of Dunkerque Hospital, (France) participated in the study using classic tubing plasticized with DEHP only.

Group 2: 10 patients (eight men and two women) aged 37–75 years old with chronic renal failure on maintenance haemodialysis in the dialysis Unit of Dunkerque Hospital, (France) participated in the study using the new tubing plasticized with TOTM-DEHP.

Each patient underwent dialysis for a 4 h-period three times a week, with a double needle access in arterio-venous fistulas. The dialysate flow rate was maintained at 500 ml/min and the blood flow rate in the haemodialysis circuit measured by pump revolutions and bubble transit time was maintained approximately 300 ml/min.

2.2. Chemicals

DEHP and its internal standard, Di-*n*-heptyl phthalate (DNHP), TOTM and its internal standard Di-*n*-decyl phthalate (DNDP), were obtained from commercial sources purchased from Sigma-Aldrich (Saint-Quentin-Fallavier, France) and were used as analytical standards without further purification. HPLC-grade acetonitrile and hexane were purchased from SDS (ZI de Valdome Peypin, France) and from Sigma-Aldrich respectively and both were assayed for the presence of DEHP and TOTM. 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 and TOTM during samples handling and analysis, all the glasswares used in the study were previously washed using tetrahydrofuran-methanol mixture then rinsed with hexane. All the other reagents used were analytical grade or better.

2.3. Analytical method

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 and at 215 nm for DEHP and for TOTM respectively. The output from the detector was connected to a Hewlett-Packard 9000 Model 300 integrator and the data were recorded on a HP Thinkjet printer. Separation was achieved using a 5 μm Waters Spherisorb® C_{18} column (4.6 × 150 mm) (Waters, Milford, MA) for DEHP and a 5 μm Waters Symmetry® C_{18} column (4.6 × 150 mm) (Waters, Milford, MA) for TOTM operating each at 20 ± 2 °C. During assay development, DEHP and TOTM were eluted isocratically with a mobile phase consisting of acetonitrile-aqueous buffer (triethylamine 0.08% adjusted to pH 2.8 with phosphoric acid 1 M) mixture (88:12,v/v) at a flow-rate of 1.0 ml/mn and acetonitrile 100% at a flow-rate of 1.2 ml/mn, respectively with a system back-pressure averaging ~230 kPa. The mobile phase was filtered through a 0.45 μm membrane and degassed under a helium stream before use. The run time were 10 min and 15 min for DEHP and TOTM, respectively.

2.4. Blood samples and extraction

Samples were directly collected on the haemodialysis line by sample site into heparinized glass tubes. Venous blood samples (5 ml) were drawn immediately prior to dialysis, and then arterial and venous blood samples entering and leaving the dialyzer were simultaneously obtained from the inlet and outlet tubing of the diayzer in the two groups study at 5, 15, 30, 60, 120, 180, 240 min during the dialysis session and after centrifugation, plasma was frozen (−20 °C) into glass tubes until HPLC analysis.

2.5. DEHP plasma sample extraction

After defrosting, each plasma sample (1 ml aliquot) was spiked with 50 μl of DNHP (250 ng) as an internal standard in the glass tubes, followed by sodium hydroxide 1 M (1 ml), acetonitrile (2 ml) and hexane (2 ml). The mixture was stirred (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 of 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 of acetonitrile and after centrifugation, 20 μl of the supernatant were finally injected into the chromatograph.

2.6. TOTM plasma sample extraction

As for DEHP, TOTM quantification needs an extraction procedure before chromatographic analysis.

Each plasma sample (1 ml aliquot) was spiked with DNDP (2 μg) as an internal standard in the

glass tubes. Extraction was carried out in one step by addition of sodium hydroxide 1 M (500 μl) followed by acetonitrile (1 ml) and hexane (3 ml). The mixture was stirred (5 min), centrifuged (1620 g for 5 min) and the separated organic phase was evaporated to dryness in a water-bath at 40 °C under nitrogen. The residue was dissolved in 100 μl of acetonitrile and after centrifugation, 20 μl of the supernatant were finally injected into the chromatograph.

2.7. Quantitative determination

For DEHP or TOTM quantification, the peak area ratio (DEHP/DNHP), or (TOTM/DNDP) (y) was calculated for each sample and the amount of DEHP or TOTM (x) was determined using the calibration curve ranged from 62.5 to 4000 ng/ml and from 0.1 to 5 μg/ml respectively, obtained during the validation of methods. Mean linear regression equations obtained were $y = 0.0046x + 0.426$ ($r = 0.999$) for DEHP (five replicates) and $y = 0.61x - 0.015$ ($r = 0.999$) for TOTM (seven replicates) with y, peak-ratio$x$, analyte concentration ng/ml and μg/ml respectively. DEHP and DNHP or TOTM and DNDP were well separated, identified and quantified by this HPLC procedure and no co-extracted endogenous compound exists at the retention times of both DEHP and TOTM. The retention times were 6.60 and 8.60 min for DNHP and DEHP, respectively; 11.80 and 14.60 min for DNDP and TOTM, respectively. These methods had acceptable accuracy and precision with intra-assay and inter-assay coefficients of variation all below 5.2%, and the recoveries for DEHP and TOTM were all better than 97%. The limit of quantification of both compounds was 25 ng/ml.

2.8. Serum biochemistry

Approximately 70% of the DEHP present in plasma stored in PVC bags is associated with low-density and very low-density lipoproteins (Albro and Corbett, 1978). Thus, cholesterol and triglycerides concentrations in serum were measured to determine the influence of the aforementioned serum constituents on the extraction of DEHP or TOTM from dialysis tubing into blood.

2.9. Data analysis

The amounts of both DEHP and TOTM extracted from haemodialysis tubing over a 4 h-dialysis session were estimated following transit of blood through the dialyzer.

The amounts of DEHP or TOTM contaminating the patient during dialysis session, Q were obtained by calculating the AUC_{out} (area under the output dialyzer) concentration-time curve (venous line) and multiplied by the plasma flow rate D.

$$Q = D \times AUC_{out}.$$

The amounts of DEHP or TOTM retained by the patient during the same period Q' were estimated by calculating the difference between AUC_{out} and AUC_{in} for the area under the input dialyzer concentration-time curve (arterial line) and multiplied by the plasma flow rate D.

$$Q' = D(AUC_{out} - AUC_{in}).$$

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

3. Results and discussion

Fig. 1 shows DEHP and TOTM concentrations time-course obtained from one haemodialysis patient at the inlet and outlet of the dialyzer during a 4 h-dialysis session. At the beginning of the treatment, detectable concentrations of TOTM (< 0.7 μg/ml) and DEHP (< 0.1 μg/ml) were found in all the blood samples. This observation is consistent with the fact that both plasticizers might accumulate in patients undergoing regular treatment resulting from the plasticizer redistribution in the body due to lipophilic characteristics. The present study provides quantitatives data on the concentrations of DEHP and TOTM in the arterial and venous blood tubes of 20 patients. In Fig. 2, plasma DEHP concentrations of the group 1 patients undergoing 4 h-dialysis sessions increased while for the group 2 patients, slight increase was observed. The tubes plasticized with TOTM-DEHP show a low and constant leachability of TOTM. So, with the blood lines used in

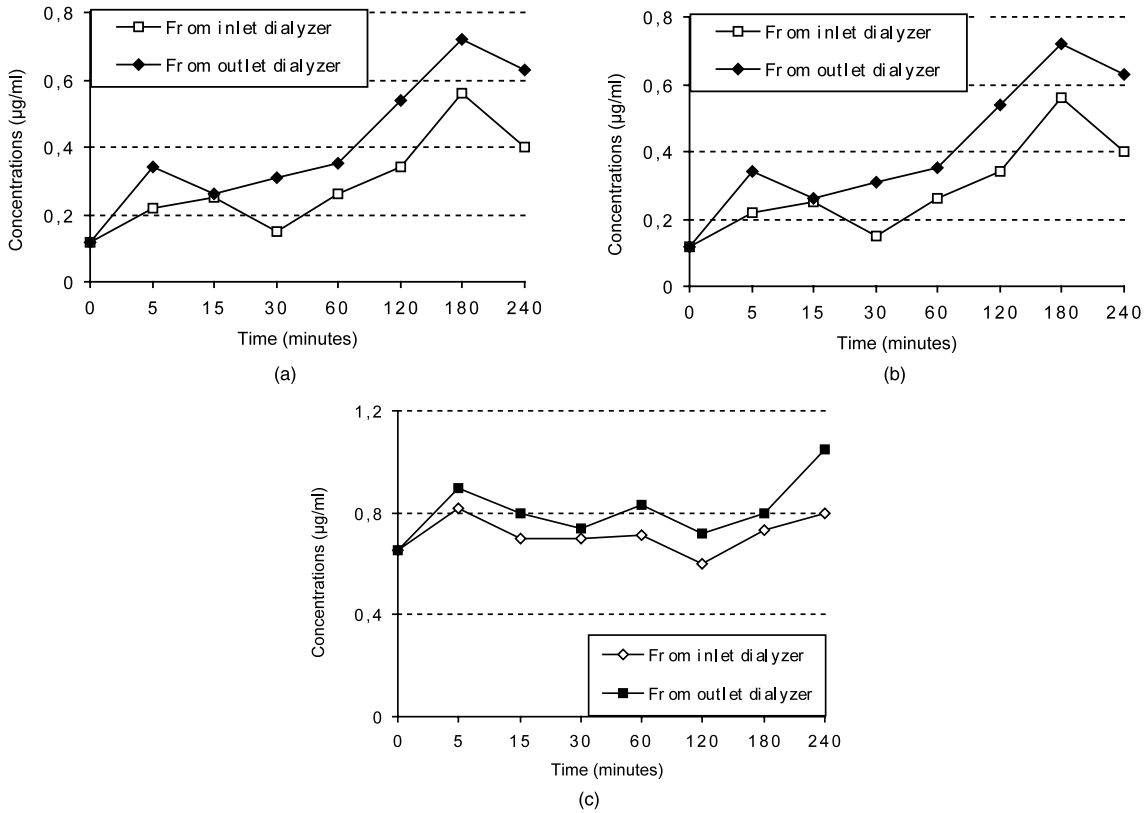

Fig. 1. Time-course of DEHP and TOTM plasma concentrations at the inlet and outlet of the dialyzer during dialysis session for one patient: (A) Kinetics of DEHP leachability when classic lines plasticized PVC-DEHP were used (group 1 patients). (B) Kinetics of DEHP leachability when lines plasticized PVC-TOTM/DEHP were used (group 2 patients). (C) Kinetics of TOTM leachability when lines plasticized PVC-TOTM/DEHP were used (group 2 patients).

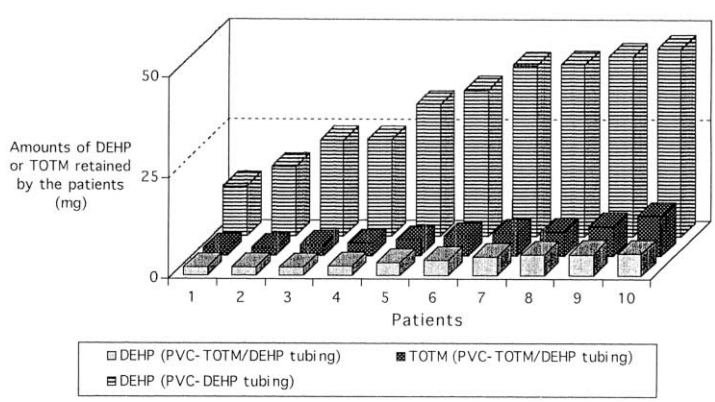

Fig. 2. Comparative amounts of TOTM or DEHP retained by patients according plasticized tubing used (Significant difference of total DEHP input between PVC-DEHP tubing and PVC-TOTM/DEHP ($P < 0.01$).

Table 1
Total DEHP exposure, total DEHP input and plasma concentrations of cholesterol plus triglycerides in 10 dialyzed patients

Patients	DEHP exposure (mg)	DEHP retained (mg)	Plasma cholesterol + triglycerides (g/l)
Group 1: Patients on maintenance haemodialysis with PVC-DEHP tubing			
1	130.27	33.00	3.40
2	166.21	42.72	3.98
3	95.54	12.50	2.70
4	147.02	36.56	5.21
5	110.32	17.19	ND
6	55.00	24.13	1.98
7	145.76	23.58	3.80
8	121.48	31.75	ND
9	158.42	30.28	4.45
10	99.43	21.35	3.20
	122.95 ± 33.94	27.30 ± 9.22	

ND, not determined.

the group 2 patients, less TOTM and DEHP were apparently leached than with the blood lines used in the group 1 patients. These findings are consistent with earlier reports suggesting that significant amounts of DEHP are introduced into the systemic circulation of patients undergoing haemodialysis with blood lines plasticized with DEHP (Pollack et al., 1985; Faouzi et al., 1999). Our data indicated that, there was a decrease of DEHP released when using new lines plasicized with TOTM-DEHP. However, the estimated values previously reported for DEHP exposure ranged widely. Gibson et al. (1976) found a larger range (from 9 to 150 mg per dialysis). Pollack et al. (1985) estimated that patients received 23.8–360 mg of DEHP during 4 h-dialysis session. Therefore, a high degree of interindividual variability in total DEHP exposure was noted. The same observation was found in our study with a range of 55–166.21 mg and 37.55–49.20 mg of DEHP with PVC-DEHP tubes and PVC-TOTM/DEHP tubes respectively, while TOTM leachability during the same session ranged from 47.60 to 125.70 mg. The reason for this discrepancy between these studies is not found, but it may be due to variations in DEHP or TOTM content of the dialyzer tubing or to differences in the dialysis protocol. Other factors such as lipid plasma content could influence the extraction of both plasticizers and could explain these differences.

No significant correlation was statistically found between the amount of DEHP or TOTM extracted during a dialysis session and the number of years of the prior dialysis treatment. In contrast, the released of both DEHP or TOTM was linked to the sum of the serum cholesterol and triglycerides concentrations ($r^2 = 0.75$ for DEHP; $r^2 = 0.64$ for TOTM).

In the present study, results obtained after HPLC data analysis are summarized in Tables 1 and 2. The comparative amounts of TOTM or DEHP retained by patients are shown in Fig. 2.

Assuming a three times a week-treatment schedule, the average patient in the group 1 would be yearly exposed to ~ 19.20 g of DEHP (ranging from 8.6 to 25.93 g). In the same period, an average patient of the group 2 will be yearly exposed to 6.5 g of DEHP and 11.70 g of TOTM (ranging from 5.86 to 7.70 g and 7.40 to 19.61 g) respectively. On the other hand, quantities of both plasticizers retained by an average patient during a single dialysis session was evaluated. Tables 1 and 2 show a high degree of interpatient variability in both DEHP and TOTM retained. The mean quantity of DEHP retained by the group 1 patients during a single dialysis session would be $\sim 27.30 \pm 9.22$ mg, while in the group 2, 3.42 ± 1.34 mg of DEHP and 4.87 ± 2.60 mg of TOTM would be retained by an average patient. The quantities of DEHP retained by the group 2 patients were < 8 fold than for the group 1 patients. This data shows that in the group 2 patients, less

Table 2
Total TOTM and DEHP exposure, total TOTM and DEHP input and plasma concentrations of cholesterol plus triglycerides in 10 dialyzed patients

Group 2 Patients on maintenance haemodialysis with PVC-TOTM/DEHP tubing					
Patients	DEHP exposure	TOTM exposure (mg)	DEHP retained	TOTM retained (mg)	Plasma cholesterol + triglycerides (g/l)
1	39.68	105.72	5.33	5.80	5.80
2	37.74	47.60	2.05	5.00	3.10
3	39.54	65.16	3.33	7.60	4.21
4	49.20	125.70	6.67	3.30	5.60
5	37.55	74.50	2.17	10.00	5.21
6	37.73	54.77	2.49	2.80	3.58
7	45.92	50.82	3.72	1.90	2.20
8	38.56	55.91	2.00	4.13	1.99
9	47.00	88.22	5.50	2.05	3.81
10	45.01	82.73	2.90	6.10	3.68
	41.80 ± 4.47	75.11 ± 25.72	3.42 ± 1.34	4.87 ± 2.60	

TOTM is leached from dialysis tubing, moreover it seems to reduce the leachability of DEHP. TOTM or DEHP retained by the patients might be certainly responsible for biological effects. Toxicity studies in animals have demonstrated an association between exposure to DEHP and changes in hepatocellular structure and liver functions, testicular atrophy in rats and proliferation of peroxisomes in rodents (Isseman and Green, 1990; Parmar et al., 1995; Doull et al., 1999).

In contrary, the investigations of Rathinam et al. (1990) on the toxicity of TOTM in rats after intraperitoneal did not show any change in the activities of hepatic enzymes. These studies are indicative of the safer toxicokinetic properties of TOTM compared with DEHP. In the other hand, the amount of peroxisome induction in TOTM-treated rats is less than those treated with DEHP. In addition, the monoester effects attributed to mono-ethylhexyl phthalate (MEHP) as the main metabolite of DEHP, was not seen with TOTM (Hodgsson, 1987). The results of the present study indicate that patients undergoing dialysis with the new lines are less exposed to both DEHP and TOTM than with the classics lines. These findings are consistent with earlier reports (Flaminio et al., 1988; Christensson et al., 1991). Therefore, TOTM can be recommended as an alternative plasticizer to DEHP, but its possible toxicity towards the humans body should be investigated before it can be routinely used. As very little information is available on the biological effects of TOTM, a number of studies including toxicity, disposition and metabolism are needed before replacing DEHP with TOTM.

References

Albro, P.W., Corbett, J.T., 1978. Distribution of di- and mono(ethylhexyl) phthalate in human plasma. Transfusion 18, 750–755.

Bojes, H.K., Thurman, R.G., 1994. Peroxisome proliferators inhibit acyl CoA synthetase and stimulate protein kinase C in vivo. Toxicol. Appl. Pharmacol. 126, 233–239.

Christensson, A., Ljunggren, L., Nilsson-Thorell, C., Arge, B., Diehl, U., Hagstam, K.E., Lundberg, M., 1991. In vivo comparative evaluation of hemodialysis tubing plasticized with DEHP and TEHTM. Int. J. Artif. Org. 14, 407–410.

Dine, T., Luyckx, M., Cazin, M., Brunet, C., Goudaliez, F., Cazin, J.C., 1991. Rapid determination by high performance liquid chromatography of di(2-ethylhexyl) phthalate in plasma stored in plastic bags. Biomed. Chromatogr. 5, 94–97.

Doull, J., Cattley, R., Elcombe, C., Lake, B.G., Swenberg, J., Wilkinson, C., Williams, G., Van Gemert, M., 1999. A cancer risk assessment of di(2-ethylhexyl) phthalate: Application of the New US EPA risk assessment guidelines. Regul. Toxicol. Pharmacol. 29, 327–357.

Faouzi, M.A., Dine, T., Luyckx, M., Brunet, C., Mallevais, M.L., Goudaliez, F., Gressier, B., Cazin, M., Kablan, J., Cazin, J.C., 1995. Stability and plasticizer extraction of

miconazole injection added to infusion solutions and stored in polyvinyl chloride containers. J. Pharm. Biomed. Anal. 13, 1363–1372.

Faouzi, M.A., Dine, T., Gressier, B., Kambia, K., Luyckx, M., Pagniez, D., Brunet, C., Cazin, M., Belabed, A., Cazin, J.C., 1999. Exposure of hemodialysis patient to di(2-ethylhexyl) phthalate. Int. J. Pharm. 180, 113–121.

Flaminio, L.M., De Angelis, L., Ferazza, M., Marinovich, M., Galli, G., Galli, C.L., 1988. Leachability of a new plasticizer TEHTM from haemodialysis tubing. Int. J. Art. Org. 11, 435–439.

Gibson, T.P., Briggs, W.A., Boone, B.J., 1976. Delivery of di(2-ethylhexyl) phthalate to patients during hemodialysis. J. Lab. Clin. Med. 87, 519–524.

Hodgsson, J.R., 1987. Results of peroxisome induction studies on TOTM and 2-ethylhexanol. Toxicol. Indust. Health 3, 49–61.

Isseman, I., Green, S., 1990. Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators. Nature 347, 645–650.

Kluwe, W.M., Haseman, J.K., Douglas, J.F., Huff, J.E., 1982. The carcinogenicity of dietary di(2-ethylhexyl) phthalate in Fischer 344 rats and B6C3F mice. J. Toxicol. Environ. Health 10, 797–815.

Martis, L., Freid, E., Woods, E., 1987. Tissue distribution and excretion of TEHTM in rats. J. Toxicol. Environ. Health 20, 357–366.

Nässberger, L., Arbin, A., Östelius, J., 1987. Exposure of patients to phthaltes from polyvinyl chloride tubes and bags during dialysis. Nephron 45, 286–290.

Parmar, D., Srivastava, S.P., Singh, G., Seth, P.K., 1995. Testicular toxicity of di(2-ethylhexyl) phthalate in developing rats. Vet. Human. Toxicol. 34, 310–313.

Peters, J.M., Taubeneck, M.W., Keen, C.L., Gonzalez, F.J., 1997. DEHP induces a functional zinc deficiency during pregnancy and teratogenesis that is independant of PPARα. Teratol 56, 311–316.

Pollack, G.M., Buchanan, J.F., Slaughter, R.L., Kohli, R.K., Shen, D.D., 1985. Circulating concentrations of di(2-ethylhexyl) phthalate and its de-esterified phthalic products following plasticizer exposure in patients receiving hemodialysis. Toxicol. Appl. Pharmacol. 79, 257–267.

Rathinam, K., 1988. Biotransformation of intravenously administered TEHTM in rabbits. Ind. J. Pharmacol. 19, 149–150.

Rathinam, K., Srivastava, S.P., Seth, P.K., 1990. hepatic studies of intraperitoneally administered TOTM and DEHP in rats. J. Appl. Toxicol. 10, 39–41.

Reddy, J.K., Lalwani, N.D., 1984. Carcinogenesis by hepatic peroxisome proliferators: evaluation of the risk of hypolipidemic drugs and industrial plasticizers to human. Crit. Rev. Toxicol. 12, 1–58.

Turner, V.S., Mitchell, S.G., Kang, S.K., Hawker, R.J., 1995. A comparative study of platelets stored in polyvinyl chloride containers plasticized with butyryl trihexyl citrate or triethylhexyl trimellilate. Vox. Sang. 69, 195–200.

Van Den Munckhof, R.J.M., Bosch, K.S., Frederiks, W.M., 1998. The different effects of the peroxisome proliferators clfibric acid and DEHP on the activities of peroxisomal oxidases in rat liver. Histochem. J. 30, 339–349.