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Quantification of DEHP into PVC components of intravenous infusion containers and peritoneal dialysis set before and after UV-A treatment

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The presence of di-(2-ethylhexyl) phthalate (DEHP) in 8 different parts of plastic medical devices that are used in two important medical procedures was determined and influence of UV radiation on DEHP leaching was investigated. DEHP determination was carried out by gas chromatography – mass spectrometry (GC-MS). The results showed that set for peritoneal dialysis contains DEHP approx. 35% by weight of bag and approx. 37% by weight of tubing. Results obtained for samples from transfusion set showed that Quadruple blood bag and transfer bag contain almost the same amount of DEHP (25.63% and 26.92% by weight, respectively) while SAG-M transfer bag contains lower amount of DEHP (16.07%). All samples of tubing material showed the higher concentration level of DEHP than coupled bags. Very low amount was leached by Peritoneal Dialysis Solution from PVC dialysis bag (3.72 μg L^{-1}), despite the fact that dialysis bag contains DEHP in high concentration level. Obtained concentration of DEHP in CPD solution from Quadruple blood bag is higher than concentration in Peritoneal Dialysis Solution about 10 times (37.04 μg L^{-1}). DEHP was not detected in solution from SAG-M transfer bag. Obtained values are under estimated upper-bound dose of DEHP received by adult patients undergoing procedures of peritoneal dialysis 0.01 mg/kg/day (for adult with average body weight 70 kg) and transfusion as part of surgical procedures 8.5 mg/kg/day. Results obtained after UV treatment showed that UV radiation has a certain influence on leaching of DEHP from samples of PVC medical devices. All investigated samples contained smaller amount of DEHP after UV-A treatment than samples which were not treated by UV radiation.

Keywords: di-(2-ethylhexyl) phthalate (DEHP); medical devices; polyvinyl chloride (PVC); UV radiation

INTRODUCTION

Phthalate diesters (phthalates) may be found in a broad range of industrial products because they are widely used as plasticizers. They are added to plastic polymers (e.g. polyvinyl chloride – PVC) to increase flexibility and softness [1-3]. PVC is used in the production of toys, floors tiles, building materials, clothing, automobiles, cleaning materials, cosmetics and food packaging, industrial tubing, medical devices, *etc.* [4-5]. Due to their widespread use, relatively large amounts of these compounds are released into the environment [6].

In general, PVC medical devices contain up to 40% of plasticizers by weight [7-9]. Di-(2-ethylhexyl) phthalate (DEHP) is the most abundant plasticizer, but other phthalates such as diethyl phthalate (DEP), di-*n*-butyl phthalate (DnBP) and benzylbutyl phthalate (BzBP) can be found in PVC materials [10]. Patients undergoing medical procedures, such as parenteral nutrition support, blood transfusion, hemodialysis, peritoneal dialysis, cardiopulmonary bypass (CPB), are in contact with PVC medical devices which contain DEHP [11]. A various types of medical devices are made from PVC, such as enteral and parenteral nutritional tubing, infusion and transfusion tubings, blood bags and tube systems for blood cell separation, bags and tubing for peritoneal dialysis [12]. In humans, phthalates are rapidly hydrolyzed to the monoesters and then further metabolized and they can be detected in urine, breast milk, faeces, *etc*. [13]. DBP, BzBP and DEHP are introduced in the list of potentially endocrine disruptors [14]. Some of these health outcomes may be the result of phthalate-induced increases in oxidative stress or inflammation, which have been demonstrated in animal studies [15].

Phthalates are not bound to plastic material therefore phthalates can migrate to the medium that is in contact [14, 16]. Various conditions may enhance the migration of phthalates from PVC medical devices into the surrounding media. It is possible that the content and transfer properties of phthalates may be influenced by optical radiation and temperature change during storage [7, 17].

Estimated upper-bound dose of DEHP received by adult patients can be various for different medical procedures. Relatively high doses of DEHP can be received by patients who are transfused with large volumes of blood and blood products over a short period. A patient undergoing a routine, elective surgical procedure typically receives about

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two units of packed red blood cells. Transfusion of this volume of blood will result in a DEHP dose equivalent to the TI value, approximately 0.5 mg/kg/day. The highest estimated daily exposure levels for blood transfusion is 8.5 mg/kg for adult trauma patients. Long-term use of some procedures can result in significant total DEHP exposure [18]. Assuming a patient undergoing continuous ambulatory peritoneal dialysis (CAPD) is dialyzed with 8 L of fluid/day, the upper-bound estimate of the daily dose of DEHP infused into the peritoneum would be on the order of 1 mg/day (0.13 g/ml× 8,000 ml/day × 0.001 mg/g) [19].

Various analytical methods can be used for quantitative determination of DEHP, including HPLC, LC-MS, GC-MS, *etc*. [20] Also, numerous preconcentration methods can be applied, such as solid-phase extraction (SPE), solid-phase microextraction (SPME), headspace solid-phase microextraction (HS-SPME), liquid-phase microextraction (LPME) and dispersive liquid-liquid microextraction (DLLME) [6, 21, 22]. The most conventional liquid-liquid extraction method (LLE) performed with hexane, dichloromethane, ethyl acetate or acetone has recovery values in the range between 70 and 100% and is relatively short and easily performed. Because of that, LLE method for extraction followed with GC-MS seems to be the best choice for sample preparation and detection of phthalates. In this study, a GC-MS method was used for determination of DEHP [12, 23-25].

The aim of this work was DEHP determination in medical devices – dialysis set and transfusion set – based on polyvinyl chloride (PVC), and investigation of UV-A (ultraviolet radiation) effects on DEHP leaching. Quantitative determination of DEHP was performed by total dissolution of plastic material. Determination of DEHP was done by GC-EI-MS as the one of the most common methods for phthalate quantification, due to specificity, sensitivity and availability of instrumental technique.

EXPERIMENTAL

Materials and chemicals

Medical devices were taken from the local Clinical Center Niš, Serbia. Samples consisted of filled plastic bag and tubing from peritoneal dialysis set (Baxter, USA) and bags and tubing from transfusion set (TIANHE Pharmaceutical, China).

DEHP, DBA, hexane and tetrahydrofurane (THF) were purchased from Sigma Aldrich, USA. Solvents were HPLC grade and screened to determine the DEHP background. Hexane was used as a solvent for stock solutions and working standards.

Preparation of standards

Amount of DEHP standard was accurately weighted out by analytical balance with precision at ±0.00001 g (Kern, Germany) and diluted with *n*-hexane to 5 ml. This solution was labeled as stock solution and was stored in the fridge. Working standard solutions were obtained by diluting of stock solution, obtaining the series of the concentration range from 0.25 to 10 μg ml^{-1}.

All sample manipulation was done avoiding any contact with plastic equipment. Special care was taken to avoid the contamination of solvents with plastic laboratory materials during standards and sample preparation. All glassware was washed with hot water and sodium lauryl sulfate, rinsed with ultrapure deionized water and subsequently thoroughly rinsed with dichloromethane. After cleaning, glassware was sealed with aluminum foil and stored in a clean environment to avoid adsorption of phthalates from the air.

Instrumental analysis

Analysis was carried out by gas chromatography coupled to mass spectrometer (Hewlett Packard 6890 series GC System with autosampler connected with Agilent 5973 Mass Selective Detector (Electron Ionization MSD-EI, single quadrupole). The separation was achieved with 30 m × 0.25 mm × 0.25 μm a non-polar AGILENT DB-5MS column coated with 5% phenyl, 95% dimethylpolysiloxane. The oven temperature was programmed from 65 °C (holding time 1 min) to 220 °C (1 min) at rate of 20 °C min^{-1}, then to 280 °C at rate of 5 °C min^{-1} (4 min). Volume of 1 μL was injected in the splitless mode. Helium was the carrier gas (1.0 ml min^{-1}) and the inlet temperature was 250 °C. The operating temperature of the MSD was 280°C with the emission energy of 70 eV. The MSD was used in the single ion-monitoring (SIM) mode at *m/z* 149. The identification of target compounds was based on the relative retention time, the presence of target ions and their relative abundance. The most abundant ion *m/z* 149 was chosen for quantification of DEHP, with no qualifier ions, due to the simplicity of the matrix. The dwell time was 100 ms. DEHP fragmentation pathways are given in Fig. 1 [26]. Ion *m/z* 185 was chosen as representative ion of DBA internal standard.

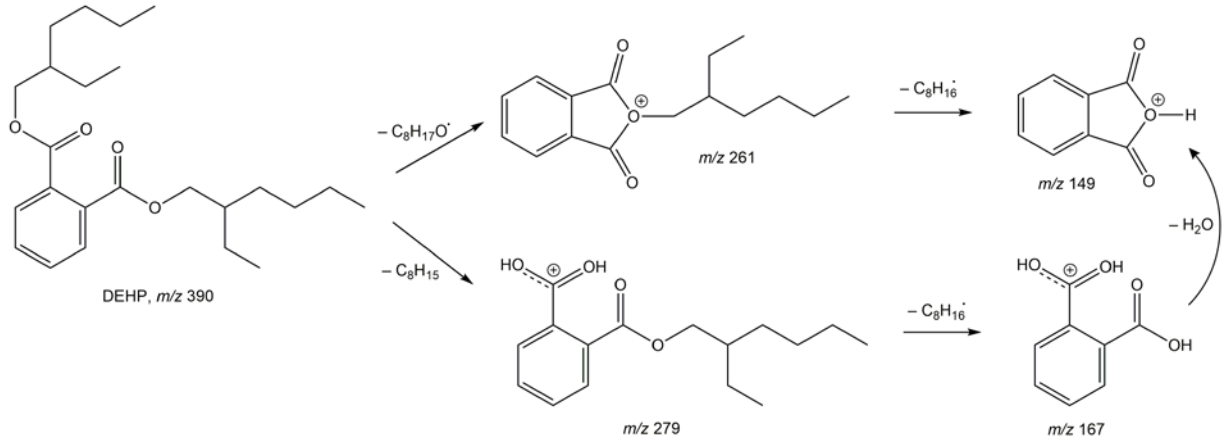

Fig. 1. DEHP fragmentation pathways.

Treatment of solid samples from PVC medical devices

PVC medical devices were kept up in the shade and at room temperature. Some PVC samples were irradiated with UV-A light, using UV-A lamps at 365 nm (PHILIPS, 18w/10 BL, 25 W, G 13), at the distance of 10 cm for 12 hours. After radiation treatment, the samples were stored in shade.

Determination of DEHP in solid samples from PVC medical devices

A PVC sample (0.01 g) was dissolved in 10 ml of THF by soaking overnight at room temperature. Totally dissolved plastic polymer was precipitated by addition of 10 ml of hexane. Obtained solutions showed high level of turbidity and they had to be filtered through the 0.45 μm PTFE filter. After filtering, samples still had a certain level of turbidity and they were centrifuged at 6000 rpm for 3 min to remove it. Then, sample solutions were put into 2 ml vials and DBA was added as internal standard commonly used in phthalate determination. Samples were analyzed by GC-MS.

Determination of DEHP in liquid samples

Individual solutions from investigated bags, usually present in formulations for peritoneal dialysis and blood transfusion were stored in PVC bags at room temperature. The analyzed samples were: 2000 ml solution for peritoneal dialysis (Dianeal® Low Calcium Peritoneal Dialysis Solution with 1.5% dextrose, 538 mg sodium chloride; 448 mg sodium lactate; 18.3 mg calcium chloride; 5.08 mg magnesium chloride; pH 5.2), 63 ml CPD solution from Quadruple blood bag 450 ml (0.299 g citric acid (anhydrous); 2.63 g sodium citrate (dihydrate); 0.222 g monobasic sodium phosphate (monohydrate); 2.55 g dextrose (monohydrate); 100 ml water for injection) and 100 ml solution from SAG-M transfer bag (0.877 g sodium chloride; 0.0169 g adenine; 0.900 g dextrose (monohydrate); 0.525 g mannitol; 100 ml water for injection). Liquid samples were collected in glass flasks and stored at 4 ºC until analysis. Since the usual shelf-life of investigated solutions is three years, migration rates of DEHP from plastic containers were measured after a period of 36 months in order to determine the maximum possible leached concentration of DEHP before expiration period of the medical product. Each sample with 5 ml of hexane was mixing for 24 hours. The organic layers were transferred to glass vials, internal standard was added and aliquots were injected into GC-MS directly with no clean up stage.

RESULTS AND DISCUSSION

The chromatogram in Fig. 2 shows the separation of DEHP and DBA, as internal standard. Retention times for DBA and DEHP were 9.94 and 18.27 min, respectively.

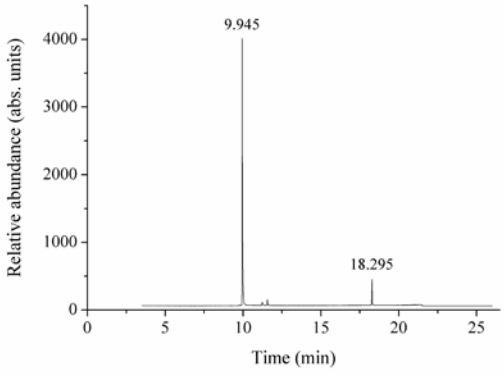

Fig. 2. GC-MS chromatogram of a standard solution containing DEHP (conc. 0.25 μg/ml) and DBA (conc. 1.0 μg/ml); abundance in arbitrary units is given for TIC.

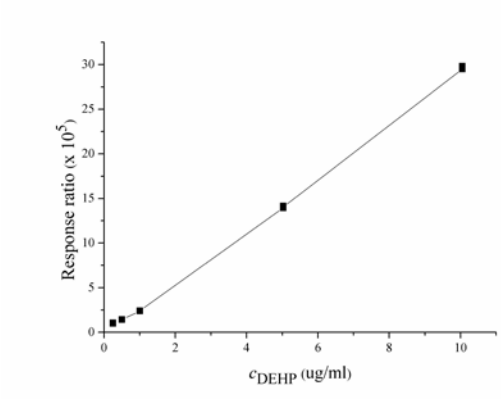

Fig. 3. Analytical curve for DEHP for concentration range 0.25 – 10 µg/ml.

The analytical curve obtained for DEHP within concentration range 0.25 – 10 µg/ml is linear with coefficient of determination of R^2 = 0.99853 and linear equation y = (2.94352±0.03017)x – 0.18073±0.15241 (Fig. 3). Limit of quantitation (LOQ) was determined using signal to noise ratio of 10 to 1, for repeated measurements with RSD less than 20%. The obtained LOQ value was 0.05 µg/ml.

The determined DEHP concentration levels, approx. 35% by weight of bag and approx. 37% by weight of tubing from peritoneal dialysis set, are high but expected, bearing in mind that the PVC type of plastic material is used for medical device production. Obtained results are given in Table 1.

Results obtained for samples from transfusion set are given in Table 2 and showed that Quadruple blood bag and transfer bag contain almost the same amount of DEHP by weight (25.63% and 26.92%, respectively) while SAG-M transfer bag contains lower amount of DEHP (16.07%). The determined DEHP concentration levels in tubing material from transfusion set showed that all samples contain more than 30% DEHP by weight. All samples of tubing material from transfusion set showed the higher concentration of DEHP than coupled bags. The most significance difference was found between SAG-M transfer bag and coupled tubing.

Results obtained after UV treatment showed that UV radiation has a certain influence on leaching of DEHP from samples of PVC medical devices. All investigated samples contained smaller amount of DEHP after UV-A treatment than samples which were not treated by UV radiation. Obtained results are given in Table 3.

Table 1. Percentage of DEHP in solid PVC samples of dialysis set before and after UV treatment.

Dialysis set (Baxter)	Total content of DEHP (%)	Total content of DEHP after UV-A treatment (%)
Dialysis bag	35.81±1.55	15.89±0.12
Tubing from dialysis set	37.58±0.78	14.01±0.33

Table 2. Percentage of DEHP in solid PVC samples of transfusion set before and after UV treatment

Transfusion set (Tianhe Pharmaceutical)	Total content of DEHP (%)	Total content of DEHP after UV-A treatment (%)
Quadruple blood bag	25.63±1.34	18.91±0.77
Tubing coupled to Quadruple blood bag	31.27±1.59	17.12±0.55
SAG-M transfer bag	16.07±0.98	4.02±0.15
Tubing coupled to SAG-M transfer bag	31.64±1.33	22.29±0.82
Transfer bag	26.92±0.85	18.72±0.24
Tubing coupled to transfer bag	35.66±1.87	13.27±0.14

Table 3. Amount of leached DEHP from solid samples after UV treatment (%)

Sample	Amount of leached DEHP after UV treatment (%)
Dialysis bag	55.63
Tubing from dialysis set	62.72
Quadruple blood bag	26.22
Tubing coupled to Quadruple blood bag	45.25
SAG-M transfer bag	74.98
Tubing coupled to SAG-M transfer bag	29.55
Transfer bag	30.46
Tubing coupled to transfer bag	62.79

Samples from dialysis set showed that UV radiation caused greater leaching of DEHP from samples of tubing than samples of bag. Transfusion set showed that UV-A radiation had greater influence on DEHP leaching from samples of SAG-M transfer bag (> 74%) than from samples of Quadruple blood bag and transfer bag (< 27% and 31%, respectively). Also, UV-A radiation has greater influence on DEHP leaching from samples of tubing coupled to transfer bag (62%), than tubing coupled to Quadruple blood bag (45%) and the lowest influence on DEHP leaching from samples of tubing coupled to SAG-M transfer bag.

The results obtained for DEHP from Peritoneal Dialysis Solution, CPD solution from Quadruple blood bag and solution from Transfer bag are given in Table 4. Very low amount was leached by Peritoneal Dialysis Solution from PVC dialysis bag, despite the fact that dialysis bag contains DEHP in high concentration level. Concentration of DEHP in CPD solution is higher than concentration in Peritoneal Dialysis Solution about 10 times. DEHP was not detected in solution from SAG-M transfer bag.

Table 4. DEHP concentrations (μg L^{-1}) in Peritoneal Dialysis Solution, CPD solution from Quadruple blood bag and solution from transfer bag stored in PVC bags.

Sample	DEHP concentration (μg L^{-1})
Dialysis solution	3.72±0.21
CPD solution from Quadruple blood bag	37.04±0.25
Solution from Transfer bag	n.d.

On average, patient under peritoneal dialysis procedure receives about 8 L of Peritoneal Dialysis Solution a day and from this obtained result it means that human body receives about 30 μg DEHP in total. Obtained value is under estimated upper-bound dose of DEHP received by adult patients undergoing procedures of peritoneal dialysis 0.01 mg/kg/day (for adult with average body weight 70 kg).

Patient undergoing a routine, elective surgical procedure typically receives about two units of packed red blood cells, volume of 450 ml. Bearing in the mind that volume of CPD solution in Quadruple blood bag is 63 ml, it means that total amount of DEHP in each units of packed red blood cells is 2.33 μg. Obtained value is under estimated upper-bound dose of DEHP received by adult patients undergoing surgical procedures (8.5 mg/kg/day).

CONCLUSION

The presence of DEHP in 8 different parts of plastic medical devices, such as dialysis set (bags and tubing) and transfusion set (bags and tubing) were determined and UV-A effect on DEHP leaching was investigated. Obtained results showed that majority of investigated samples of medical devices contains DEHP > 30% by weight. All investigated tubing material contain DEHP in higher amount than coupled bags. Results obtained after UV-A treatment showed that UV-A radiation has a huge influence on leaching level of DEHP from PVC materials. UV-A radiation showed the biggest influence on DEHP leaching from samples of SAG-M transfer bag.

DEHP was determined in Peritoneal Dialysis Solution and CPD solution from Quadruple blood bag, while solution from SAG-M transfer bag did not contain DEHP. Concentration of DEHP in CPD solution is higher than concentration in Peritoneal Dialysis Solution about 10 times. Obtained values are under estimated upper-bound dose of DEHP received by adult patients undergoing procedures of peritoneal dialysis 0.01 mg/kg/day (for adult with average body weight 70 kg) and transfusion as part of surgical procedures 8.5 mg/kg/day.

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КОЛИЧЕСТВЕНО ОПРЕДЕЛЯНЕ НА DEHP В КОМПОНЕНТИ ОТ ИНТРАВЕНОЗНИ СИСТЕМИ И ПРИБОРИ ЗА ПЕРИТОНИАЛНА ДИАЛИЗА ОТ PVC ПРЕДИ И СЛЕД ТРЕТИРАНЕ С УЛТРАВИОЛЕТОВИ ЛЪЧИ

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(Резюме)

Изследвано е наличиието на ди-(2-етил-хексил) фталат (DEHP) в 8 различни части на пластмасови медицински съоръжения, използвани в два много важни процеса, както и влиянието на облъчването с ултравиолетови лъчи. Определянето на DEHP е извършвано с газ-хроматография/мас-спектрометрия (GC-MS). Резултатите показват, че уредите за перитониална диализа съдържат приблизително 35% тегл. от торбичката и около 37% от тръбите. Резултатите, получени за проби от системи за кръвопреливане показват, че квадруполните торбички за кръв и за пренасяне съдържат почти еднакво количество DEHP (съответно 25.63% и 26.92% тегл.), докато торбичките за пренос SAG-M съдържат по-малко от DEHP (16.07%). Всички проби от тръбичките показват по-висока концентрация отколкото при свързаните торбички. Извлечени са много малки количества във физиологичния разтвор за перитониална диализа (Peritoneal Dialysis Solution) от торбичките за диализа от PVC (3.72 μg L^{-1}), въпреки че самите торбички за диализа съдържат по-големи количества от DEHP. Получената концентрация на DEHP в разтвора на CPD от квадруполна кръвна торбичка е по-висока от концентрацията в разтвора за перитонеална диализа около 10 пъти (37,04 μg L^{-1}). DEHP не е открит в разтвор от SAG-M трансферна торбичкаа. Получените стойности са под пресметната горна доза DEHP, получена от възрастни пациенти, подложени на процедури на перитонеална диализа 0,01 mg / kg / ден (за възрастни със средно телесно тегло 70 kg) и трансфузия като част от хирургични процедури 8,5 mg / kg / ден. Резултатите, получени след UV обработка, показват, че ултравиолетовото лъчение оказва известно влияние върху излугването на DEHP от проби на медицинските изделия от PVC. Всички изследвани проби съдържат по-малко количество от DEHP след третиране с UV-A, отколкото проби, които не са третирани с ултравиолетова радиация.