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Short communication

SHORT COMMUNICATION

A study of the influence of ultraviolet radiation on di(2-ethylhexyl) phthalate leaching from poly(vinyl chloride) medical devices

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Abstract: The influence of ultraviolet (UV) radiation on the leaching of di(2--ethylhexyl) phthalate (DEHP) from 8 different parts of plastic medical devices made of poly(vinyl chloride) (PVC) that are used in two important medical procedures (peritoneal dialysis and transfusion) was investigated. The investigation was performed for three different extraction times (6, 15 and 30 days). DEHP determination was realized by gas chromatography–mass spectrometry (GC-EI––MS). All the investigated samples contained a significant amount of DEHP. The results showed that some of the set for peritoneal dialysis contained DEHP in higher amounts than samples from the transfusion set. All samples of tubing material showed higher concentration levels of DEHP than the coupled bags. Results obtained after UV treatment showed that UV radiation has a certain influence on DEHP leaching from samples of PVC medical devices. The smallest difference was in the case of the quadruple blood bag from the transfusion set (about 73 % remained), while the biggest difference was obtained for the SAG-M transfer bag, also from the transfusion set, where just 25 % of total content of DEHP remained. The results obtained for DEHP leaching from investigated samples by time showed that most of the samples showed significant differences in the amounts of DEHP leached after 6 and 30 days.

Keywords: plastic bags; plastic tubings; phthalate amount.

INTRODUCTION

Poly(vinyl chloride) (PVC) is the third-most widely produced polymer and it has widespread usage in the production of many products, *e.g.*, food packaging, medical devices, children toys, personal care products, *etc*. This polymer contains

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additives, plasticizers, which are added to modify its physical properties, softness and flexibility.[1,2] Phthalates (esters of phthalic acid) are the most common used plasticizers.[3] Plasticizers do not possess polymeric structure and therefore, they can migrate from a plastic material to the surrounding media. The most commonly used plasticizer for PVC products is di(2-ethylhexyl) phthalate (DEHP).[4–6]

There are many medical devices made from DEHP-plasticized PVC and used in clinics, such as, infusion and transfusion tubings, blood bags, parenteral nutrition tubings, various tube systems for blood cell separation, parts of systems for peritoneal dialysis, *etc*. In general, PVC products used in medicine contain DEHP in amounts up to 40 % by weight.[7–9] Moreover, DEHP can easily migrate from plastic materials to the environment in contact with them. It means that it can be released from the medical device during contact with blood, enteral or total parenteral nutrition mixtures, or lipophilic drugs, which might lead to unwanted patient exposure.[10] Due to its highly lipophilic nature, DEHP tends to migrate into fat-containing surrounding environments. Different physicochemical factors (temperature, radiations, pH, presence of solvents, organic compounds, *etc*.) may increase the rate of phthalate migration.[11]

DEHP is classed as carcinogenic, mutagenic or toxic to reproduction (CMR1B) under CLP Regulations because of its potential toxicity to fertility and reproduction.[12] Many experimental studies (*in silico*, *in vitro* mechanistic, pre-clinical and clinical) have shown that DEHP behaves as endocrine disruptor *in vivo*, apart from causing various health problems, including hepatomegally, osteoporosis, peroxisome proliferation, feminization of boys, reduction in body weight, skin and breast cancers, *etc*.[13]

Humans can be exposed to phthalates *via* dermal, inhalation, oral, and intravenous routes.[11] In the organism, DEHP become rapidly metabolized, first by hydrolysation to the primary metabolite mono(2-ethylhexyl) phthalate (MEHP) followed by different oxidation steps, which create several secondary metabolites including mono(2-ethyl-5-hydroxyhexyl) phthalate (5-OH-MEHP), mono(2-ethyl-5-oxohexyl) phthalate (5-oxo-MEHP) and mono(2-ethyl-5-carboxypentyl) phthalate. These metabolites could then be found in detectable amounts in human urine.[14]

When a patient undergoes peritoneal dialysis treatments and transfusion procedure, human body is exposed to a certain quantity of DEHP through contact with medical plastic pipes. The concentration of DEHP is increased when liquids cross through a dialysis or transfusion apparatus.[5]

Photochemical phthalate degradation is a natural process, usually occurring in aqueous environments. Direct absorption of UV radiation by phthalates is possible or indirectly through an aqueous environment, resulting in the formation of highly reactive oxygen species, *i.e*., singlet oxygen or hydroxyl radicals. It is possible that the content and transfer properties of phthalates may be influenced by optical radiation and temperature change during storage.[13–16]

The analysis of phthalates is mostly realized by high-performance liquid chromatography (HPLC),[17] gas chromatography-mass spectrometry (GC––MS),[17–19] HPLC–MS, HPLC–MS–MS,[20–23] and other analyses.

The aim of this work was the determination of DEHP in medical devices, that is a dialysis set, and a transfusion set, based on PVC, and an investigation of the influence of UV-A (ultraviolet radiation) on the leaching of DEHP over time. Determination of DEHP was performed by GC-EI-MS as the one of the most common methods for phthalate quantification, due to its specificity and sensitivity, and the availability of the instrumental technique. The results of this study provided novel information relevant to risk assessment of inadequate storage of PVC medical devices.

EXPERIMENTAL

Chemical reagents

Standards of DEHP and dibutyl adipate (DBA) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Used solvents, *n*-hexane and tetrahydrofuran (THF) were purchased from Fisher (Germany). Solvents were HPLC grade and screened to determine the phthalate background.

Standard preparation

All stock and working solutions were prepared in *n*-hexane. Amounts of DEHP and DBA standard were accurately weighed on an analytical balance with precision at ±0.00001 g (Kern, Germany) and diluted with *n*-hexane. These solutions were labelled as stock solutions and were stored in a refrigerator. The stock standard of DEHP was diluted stepwise with *n*-hexane to prepare at least 5 concentration levels of intermediate standards. Working solutions were prepared by dilution of intermediate solutions and by adding DBA at concentration 1 μg cm^{-3}. All solutions were stored in the dark at 4 °C.

Sample preparation

Plastic medical devices, consisting of filled plastic dialysis bags and tubing from a dialysis set (Baxter, USA) and bags and tubing from a transfusion set that contains three different bags (quadruple blood bag, SAG-M transfer bag and transfer bag) and coupled tubings (TIANHE Pharmaceutical, China) were collected from the local hospital (Niš, Serbia). Collected PVC medical devices were kept up in the dark at room temperature.

Individual solutions from the investigated PVC bags having the following contents: 2000 mL solution for peritoneal dialysis (Dianeal® dialysis low calcium peritoneal solution containing 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 (according to the composition of the solution, C, citrate, P, phosphate, D, dextrose) solution from the 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 the 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).

Parts of all the PVC samples were exposed to 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 h. After radiation, the samples were stored in the shade. Both plastic samples, exposed and not exposed, were cut

into pieces with area of about 1 cm^2. All samples were extracted for 6, 15 and 30 days with 5 ml of *n*-hexane in glass vials.

Determination of DEHP in both types of solid samples was performed by totally dissolving the samples in 10 mL of THF overnight at room temperature. The dissolved plastic polymer was precipitated by the addition of 10 mL of hexane and, due to the high level of turbidity of the obtained solutions, they were filtered through a 0.45 μm poly(tetrafluoroethylene) filter and centrifuged at 6000 rpm for 5 min.

Instrumental GC–MS analysis

The analysis was performed by gas chromatography coupled to a mass spectrometer (Agilent 6890 series GC System with an autosampler connected with Agilent 5973 mass selective detector (electron ionization MSD-EI, single quadrupole). The separation was achieved on a 30 m×0.25 mm×0.25 μm non-polar phenyl arylene-based Agilent DB-5MS column. The oven temperature was programmed from 65 °C (holding time 1 min) to 220 °C (1 min) at a rate of 20 °C min^{-1}, then to 280 °C at a rate of 5 °C min^{-1} (4 min). The sample (1 μL) was injected in the splitless mode. Helium (purity 99.999 %) was the carrier gas at a constant flow rate of 1.0 mL min^{-1}. The inlet temperature was 250 °C. The temperatures of MS quad and MS source were 150 and 230 °C, respectively. The energy of the ionizing electrons was 70 eV. The MSD was used in the single ion-monitoring (SIM) mode at *m*/*z* 149. Identification of the 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. Ion *m*/*z* 185 was chosen as the representative ion of the DBA internal standard.

RESULTS AND DISCUSSION

The calibration curve obtained for DEHP within the concentration range 0.25–10 μg mL^{-1} was linear with a coefficient of determination of $R^2 = 0.99629$ and linear equation $y = (3.623\pm0.0988)x - 0.452\pm0.4667$. The limit of quantitation (*LOQ*) was determined using a signal to noise ratio of 10 to 1, for repeated measurements with an *RSD* of less than 20 %. The obtained *LOQ* value was 0.05 μg mL^{-1}.

The amounts of DEHP extracted from parts of the peritoneal dialysis set before and after UV-A treatment for 6, 15 and 30 days extraction times are presented in Table I.

TABLE I. Amount of leached DEHP (mg) from PVC parts of the peritoneal dialysis set determined for different extraction times (6, 15 and 30 days) before and after exposure to UV-A radiation (compared to 1 g of sample); Standard deviations for $n = 3$; a–c: the same letter within a row are not statistically significantly different at the $p < 0.05$ level (Tukey's HSD test)

Sample	Before UV treatment			After UV treatment		
	6 days	15 days	30 days	6 days	15 days	30 days
Dialysis bag	25.82± 1.05[a]	301.00± 23.58[b]	324.98± 14.17[c]	9.69± 1.01[a]	11.68± 0.77[a,b]	12.45± 0.69[b]
Coupled tubing	319.13± 28.37[a]	325.33± 8.10[a]	351.18± 28.74[b]	9.52± 0.71[a]	10.68± 1.03[a,b]	12.04± 1.66[b]

The determined concentration levels of leached DEHP in the parts of peritoneal dialysis set before UV-A treatment and after 6, 15 and 30 days extraction times were high but expected, bearing in mind that a PVC type of plastic material was used for the production of the medical device and its softness and flexibility.

The amount of DEHP extracted from parts of the transfusion set before and after UV-A treatment for 6, 15 and 30 days extraction times are presented in Table II. The obtained results were also high, as in case of the parts of the peritoneal dialysis set. The lowest amount was obtained from the SAG-M transfer bag.

TABLE II. Amount of leached DEHP (mg) from PVC parts of the transfusion set determined for different extraction times (6, 15 and 30 days) before and after exposure to UV-A radiation (compared to 1 g of sample); Standard deviations for $n = 3$; a,b: the same letter within a row are not statistically significantly different at the $p < 0.05$ level (Tukey's HSD test)

Sample	Before UV treatment			After UV treatment		
	6 days	15 days	30 days	6 days	15 days	30 days
Quadruple blood bag	193.71± 1.61^{a}	217.23± 4.81^{b}	229.02± 3.03^{b}	9.07± 0.87^{a}	9.93± 0.89^{a,b}	11.46± 0.49^{b}
Tubing coupled to quadruple blood bag	22.01± 2.21^{a}	25.71± 1.96^{a,b}	29.39± 2.63^{b}	13.45± 0.34^{a}	16.03± 1.23^{b}	17.02± 0.97^{b}
SAG-M transfer bag	1.77± 0.04^{a}	3.79± 0.19^{b}	4.06± 0.12^{b}	1.14± 0.09^{a}	1.14± 0.07^{a}	2.73± 0.51^{b}
Tubing coupled to SAG-M transfer bag	22.73± 2.02^{a}	26.52± 2.17^{a,b}	29.43± 2.56^{b}	13.04± 0.92^{a}	14.92± 0.40^{b}	15.84± 0.75^{b}
Transfer bag	18.46± 1.73^{a}	22.63± 0.59^{b}	26.43± 2.20^{b}	11.78± 1.06^{a}	12.67± 0.64^{a}	13.75± 0.43^{a}
Tubing coupled to transfer bag	23.89± 0.42^{a}	26.07± 2.54^{a,b}	27.89± 0.61^{b}	14.75± 0.69^{a}	15.24± 0.72^{a,b}	16.41± 0.53^{b}

Results presented in Tables I and II showed that the amount of DEHP leached from the investigated samples before UV-A treatment and after 6 days was in the range 70–90 % compared to the amount of DEHP leached after 30 days, except in case of bag from peritoneal dialysis set and the SAG-M transfer bag from the transfusion set. For these two samples DEHP leaching contents after 6 days were about 8 and 43 %, respectively. The results of the DEHP leaching investigation after 15 days showed that more than 85 % of the DEHP had been leached compare to amount after 30 days for all the investigated samples before UV-A treatment.

The obtained results for the investigated samples after UV-A treatment showed that after 6 days of leaching, the amount of DEHP leached from investigated samples was more than 77 % compared to the amount of DEHP leached after 30 days, except for the SAG-M transfer bag that was about 41 %. The results obtained after 15 days of leaching showed that the amount of DEHP leached was increased for all samples except for the SAG-M transfer bag, which

was more than 86 %. The samples of the SAG-M transfer bag presented the results presented the same values as recorded for 6 days, *i.e.*, 41 %.

By comparing results of total content of DEHP in the investigated samples (Fig. 1), it could be concluded that there is a large difference in the DEHP content in the investigated samples before and after UV-A treatment. The smallest difference was in case of quadruple blood bag from transfusion set (about 73 % remained), while the biggest difference was obtained for SAG-M transfer bag, also from transfusion set, where just 25 % of total content of DEHP remained.

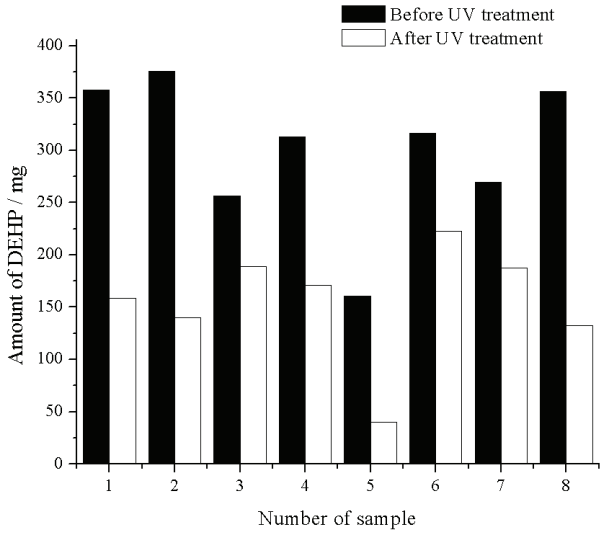

Fig. 1. Total content of DEHP in 1-g samples before and after UV treatment (1 – peritoneal dialysis bag; 2 – tubing coupled to peritoneal dialysis bag; 3 – quadruple blood bag; 4 – tubing coupled to quadruple blood bag; 5 – SAG-M transfer bag; 6 – tubing coupled to SAG-M transfer bag; 7 – transfer bag; 8 – tubing coupled to transfer bag).

Differences between the leached amounts of DEHP after different extraction times were compared to a critical value to see if the differences were significant. The *post-hoc* Tukey's test was performed and the test compares the difference between each pair of mean values with appropriate adjustment for multiple testing. The critical value of q was obtained from the Table values, and it is the point when a mean difference becomes statistically significantly different. The critical value for the investigated samples was 3.95.[24] Values of *SSD* (statistically significant difference) for each pair were computed in the Origin© program. The comparison was performed at the $p < 0.05$ level.

Results obtained by Tukey's test indicated that most samples showed significant difference between the amounts of DEHP leached after 6 and after 30 days. Only samples of the bag from the Dialysis set before UV treatment showed

significant difference in the amounts of DEHP leached after all three times, while only samples of the transfer bag from the transfusion set after UV treatment showed no significant difference between any periods of leaching. By comparing the results of the amount of DEHP leached after 30 days and total content of DEHP in the investigated samples before UV treatment, it could be observed that after this period approximately 90–93 % of total DEHP content was leached from samples of the peritoneal dialysis set, and the quadruple blood bag from the transfusion set, while in case of other investigated samples, less than 10 % was leached. Even in the case of the SAG-M transfer bag, the amount leached was just 2.5 % of total DEHP content.

The results obtained by comparing the amount of DEHP leached after 30 days and total content of DEHP in samples treated with UV-A radiation, it could be observed that after 30 days, less than 10 % was leached, except from the tubing coupled to the transfer bag from the transfusion set, when the amount was about 12 %.

CONCLUSION

The influence of UV radiation on DEHP leaching from 8 different parts of plastic medical devices, *i.e.*, a dialysis set (bags and tubing) and a transfusion set (bags and tubing), was investigated. The obtained results showed that UV radiation has a huge influence on the level of DEHP leaching from PVC materials. Most of investigated samples contained more than 20 % of DEHP by weight (up to 38 %) expect for the SAG-M transfer bag that contained lower amount of DEHP (16.07 %). All samples of tubing material showed higher concentration levels of DEHP than the coupled bags. The results obtained for the total DEHP content before and after UV treatment showed that the difference was the smallest for the quadruple blood bag from transfusion set (about 73 % remained), while the biggest difference was obtained for the SAG-M transfer bag also from the transfusion set, where just 25 % of total content of DEHP remained. The results obtained in the investigation of DEHP leaching from investigated samples showed that most samples showed significant difference between DEHP leached amounts after 6 and after 30 days. The amounts of DEHP leached after 30 days from the parts of the dialysis set and the quadruple blood bag from transfusion set before UV treatment were approx. 90–93 % of the total DEHP content. From the other investigated samples, less than 10 % of the total DEHP contents were leached after 30 days. By comparing the amount of DEHP leached after 30 days and total content of DEHP in the samples treated with UV-A radiation, it could be observed that after 30 days period, less than 10 % was leached, except from the tubing coupled to the transfer bag from the transfusion set from which about 12 % was leached.

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И З В О Д

ИСПИТИВАЊЕ УТИЦАЈА УЛТРАЉУБИЧАСТОГ ЗРАЧЕЊА НА ИЗЛУЖИВАЊЕ ДИ(2-ЕТИЛХЕКСИЛ) ФТАЛАТА ИЗ МЕДИЦИНСКЕ ОПРЕМЕ ИЗРАЂЕНЕ ОД ПОЛИВИНИЛ-ХЛОРИДА

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У овом раду испитиван је утицај ултраљубичастог зрачења на излуживање ди(2-етилхексил)-фталата (DEHF) из 8 различитих делова медицинске опреме израђене од поливинил хлорида и која се користи за време важних медицинских процедура, перитонеалне дијализе и трансфузије. Испитивање је извршено за три различита периода екстракције (6, 15 и 30 дана). Одређивање DEHF извршено је помоћу гасне хроматографије са масеном спектрометријом (GC–MS). Добијени резултати су показали да сви испитивани узорци садрже значајну количину DEFH, као и да делови опреме која се користи за перитонеалну дијализу садрже већу количину DEHF од делова сета за трансфузију. Сви испитивани узорци цевчица су садржали већу количину DEHF у односу на одговарајуће кесе. Резултати добијени након третмана зрачењем показали су да зрачење има значајан утицај на излуживање DEHF из медицинске опреме израђене од поливинил-хлорида. Најмања разлика у одређеној количини DEHF након третмана зрачењем утврђена је код једног узорка кесе из сета за трансфузију (око 73 % укупне количине DEHF је заостало), док је највећа разлика утврђена, такође код узорка из истог сета, али другог дела, где је заостало око 25% количине DEHF. Добијени резултати су показали да је значајна разлика у излуженој количини DEHF уочена између количина одређених након 6 и 30 дана.

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