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# STUDY OF DI-*N*-ETHYL HEXYL PHTHALATE MIGRATION FROM PLASTIC MATERIALS

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## Abstract

*The aim of this paper was to examine the migration of the most frequently used phthalate, di-n-ethyl hexyl phthalate (DEHP), from the most commonly used plastics: polyethylene terephthalate (PET), high-density polyethylene (HDPE), polyvinyl chloride (PVC), low-density polyethylene (LDPE), polypropylene (PP), polystyrene (PS) and polycarbonate (PC). The investigation was performed through leaching of phthalates from plastic into n-hexane for three different leaching duration periods (6, 15 and 30 days). DEHP determination was carried out by gas chromatography coupled with mass spectrometry (GC/MS). The results showed that PVC samples contain DEHP in signaficient amount, from 5.19 to 28.76% by weight of samples after 30 days of leaching, which is related to the fact that phthalates are always added to PVC to be come softer, more elastic, with a longer lifetime. Bearing in mind results and that tested PVC is medical equipment, that is used in important medical procedures (peritoneal dialysis, transfusion and infusion), presence of DEHP in investigated samples is a potential risk to human health. The GC/MS analysis found that other types of plastics do not contain DEHP and that food and beverages packaging are safe to use.*

**Keywords**: DEHP, plastic materials, migration, GC-MS

## INTRODUCTION

Plastic materials that characterize suitable properties and relatively low cost are more commonly used as compared to other materials in the production of food packaging, cutlery, children toys, cosmetic packaging. Some plastics from an environmental aspect are appropriate and safe to use, while other plastics contain harmful chemicals and pollute the environment during production and usage. There are recyclable codes that are stamped on the underside of plastic products to indicate to consumers which plastic is safer for use. The most usually used plastics are marked with numbers from 1 to 7. In Table 1 the names of plastics, their abbreviations and recyclable codes are noted. Harmless plastics are marked with numbers 1, 2, 4 and 5. Plastic marked with numbers 3, 6 and 7 could be potentially harmfull because it contains toxic chemicals which affect human health. PVC releases chemicals known as phthalates which inhibit hormone activity in the human body. In spite of this, PVC is still the most commonly used bottle plastic.

***Table 1*** *The most commonly used plastics*

| Recyclable codes | Name of plastic | Abbreviations |
|---|---|---|
| 1 | Polyethylene terephthalate | PET |
| 2 | High-density polyethylene | HDPE |
| 3 | Polyvinyl chloride | PVC |
| 4 | Low-density polyethylene | LDPE |
| 5 | Polypropylene | PP |
| 6 | Polystyrene | PS |
| 7 | Polycarbonate | PC |

Esters of phthalic acid, known as phthalates, are added in the PVC during production process, in order to achieve its flexibility, softness and transparency. Given that phthalates are not bound by chemical bonds to plastics, they can be easily migrated from PVC under the influence of external factors, making the plastic inelastic and breakable. The most commonly used plasticizer is di-*n*-ethyl hexyl phthalate (DEHP) and plastics may content up to 40 % (w/w) of DEHP [1]. Annual production of DEHP is about 2 million tons worldwide [2]. DEHP is used in many products, such as medical equipment (peritoneal dialysis bag with tubing, transfusion set with blood bag and tubing, infusion tubing, *etc*.), consumer products (food packaging, beverages packaging, *etc*.), toys, cosmetics [3-4]. Considering the already mentioned nature of the bonding between phthalate and plastics, DEHP can be easily migrated from plastics in the environment, especially in fatty foods, because of its physicochemical properties (DEHP is soluble in non-polar solvents and poorly soluble in polar solvents). In this way, DEHP can come into human by ingestion, inhalation and through the skin. Scientific studies have shown harmful effect of DEHP on human health, making it one of endocrine disruptors [5]. In Europe tolerable daily intake (TDI) value for DEHP is 50 μg/kg bodyweight [6]. Bearing in mind impact of DEHP on human health, it is important to determine phthalates in different plastics.

There are different techniques for determining DEHP in plastics, but the most common technique is gas chromatography mass spectrometry analysis (GC/MS). GC/MS analysis is rapid and reliable and requires appropriate preparation of the samples.

The aim of this work is determination of DEHP in samples of plastics, marked with number 1 to 7, which is used for food and beverages containers and medical equipment, by GC/MS analysis. DEHP determination is carried out by leaching of DEHP in a non-polar *n*-hexane which is related to its lipophilic character. Leaching of DEHP was monitored during 6, 15 and 30 days. In this way, the potential pollution of food and beverage samples by DEHP is demonstrated.

## MATERIALS AND METHODS

### Reagents and materials

*Chemical reagents*

HPLC grade *n*-hexane was purchased from Carlo Erba (France). Di-*n*-ethyl hexyl phthalate (DEHP) was purchased, in the highest available purity, from Sigma–Aldrich (USA). Dibutyl adipate (DBA) was purchased from Fluka (Switzerland) and used as an internal standard.

*Investigated samples*

Samples used for this analysis was plastics used for food containers marked with number 1 to 7. All plastic samples were purchased at local store at Niš (Serbia), except medical equipment made of PVC which were taken from local Clinical Center Niš (Serbia).

*Preparation of calibration standards*

All stock, intermediate and working solutions were prepared in *n*-hexane. The stock solutions of DEHP and DBA were prepared at a concentration of 1000 µg $mL^{-1}$ of each. The stock standard was diluted stepwise with *n*-hexane to prepare working solutions of DEHP. The calibration standard series is obtained with DEHP concentration range 0.25, 0.50, 1.00, 1.50 and 2.50 µg $mL^{-1}$ with DBA at a concentration of 1 µg $mL^{-1}$.

### GC-MS technique

Gas chromatographic analysis was performed by gas chromatograph 6890 (Hewlett-Packard, USA) equipped with a mass selective detector (MSD) 5973 (Hewlett-Packard, USA) and Autosampler 7683 (Agilent, USA) and SGE 25QC2/BPX5 0.25 capillary column (25 m×0.22 mm×0.25 µm, non-polar). The gas chromatograph was operated in the split less injection mode. The oven temperature was programmed from initial temperature 90 °C (hold time 0 min) to 280 °C at a rate of 20 °C $min^{-1}$ with hold time of 4 min, and post run 300 °C (2 min). Helium was the carrier gas (flow rate of 1.0 mL $min^{-1}$). The operating temperature of the MSD was 280 °C with the electron impact ionization (EI) voltage of 70 eV. The dwell time was 100 ms. The MSD was used in the single ion-monitoring mode (SIM), the quantification ion is *m/z* 149 for DEHP and ion *m/z* 185 was chosen as representative ion of DBA. Analyte response was normalized to DBA as internal standard. The identification and quantification of target compound was based on the relative retention time, the presence of target ions and its relative abundance. Both data acquisition and processing were accomplished by Agilent MSD ChemStation® D.02.00.275 software.

### Sample preparation

In this study a total of 44 samples of plastics marked with number from 1 to 7 were analysed. Seven different types of medical equipment made of PVC was examined: dialysis bag with coupled tubing which constitute a set for peritoneal dialysis (Baxter, USA), three bags (quadruple blood bag, SAG-M transfer bag and transfer bag) and coupled tubing (TIANHE Pharmaceutical, China) and tubing for infusion set (Mediaset, Spain). Plastic packaging used for dairy products and water, made of PET and PP, was investigated on content of DEHP. Also, these include 28 different samples: one milk bottle, two plastic bags for milk, eight yoghurt bottles, three fruit yogurt bottles, seven yogurt cups, cap for sour milk, one bottle for non-carbonated mineral water, one bottle for carbonated mineral water, one disposable plastic foods packaging, one cellophane, one freezer bag and one cutting board. An egg box made from PS and disposable platter of the same content were examined. One bag from HDPE plastic and HDPE granulate from which further packaging are produced for different uses were analysed. Bottle for infusion (Hemofarm, Serbia), bottle with Ringer's solution for parenteral nutrition (Zdravlje, Serbia), granulate and zip bag for food made of LDPE was examined. One packaging bag made of PC was examined. At the end, dialysis bag (Biofine, Germany) made of a substance from the group of polyolefins were analysed.

On the analytical balance with accuracy of ±0.00001 g, was measured about 0.02 g of each plastic sample (each plastic sample was cut into pieces with area about 0.5 $cm^2$). *n*-Hexane (5 ml) was added in each plastic sample in glass vial. DEHP migration into *n*-hexane was monitored within 6, 15 and 30 days. The analysis of each sample was done three times, to obtain reliable data covered by standard deviation (SD). After 6, 15, and 30 days, a certain amount of *n*-hexane (900 µL) was removed from *n*-hexane solution, DBA (100 µL from solution of DBA 10 µg $mL^{-1}$) was added and recording was performed by GC/MS.

In order to avoid laboratory phthalates contamination, only glassware was used. The glassware was prepared in the manner described in the reference [8].

## RESULTS AND DISCUSSION

### GC-MS analysis

The chromatogram of a standard solution of DEHP and DBA, is given in Figure 1. The given chromatogram shows good separation of DEHP and DBA occurred within a running time of 13.5 min with retention times for DBA and DEHP 6.413 and 10.078 min, respectively.

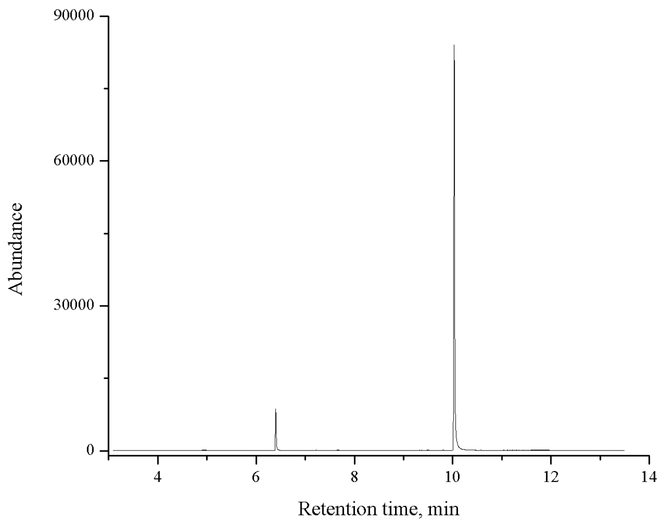


***Figure 1*** *GC/MS chromatogram of a standard solution of DEHP and DBA in a concentration of 2.5 µg $mL^{-1}$ and 1.00 µg $mL^{-1}$, respectively*

The calibration curve was linear in the range from 0.25 to 2.5 µg $mL^{-1}$ with correlation coefficient of $R^2$ = 0.99 and linear equation y = (2488.2 ± 17947.8) x + (358701.3 ± 14027.4).

The limit of determination (LOD) and limit of quantification (LOQ) were calculated from the signal/noise ratios which were multiplied with the factor 3 and 10 respectively [9]. The LOD and the LOQ values for DEHP are 0.04 μg $mL^{-1}$ and 0.12 μg $mL^{-1}$ with relative standard deviation (RSD) value 3.6 % (n = 3).

**Leaching of DEHP**

In order to determine the amount of leached DEHP in samples, the GC/MS analysis was performed after 6, 15 and 30 days of leaching. Chromatograms were recorded after addition of internal standard. On the basis of the performed calibration, the quantification of the samples was carried out. The amount of leached DEHP in the investigated PVC samples is given in Table 2. DEHP was found in extracts from all PVC samples (medical equipment) in concentration levels of 5.19-28.76% by weight of PVC samples. These are the expected results since it is known that phthalates are added to the PVC to soften it.

***Table 2*** *Concentration of leached DEHP (mg $g^{-1}$) from PVC material determined for different extraction times (6, 15 and 30 days) SD – standard deviation (n = 3)*

| | Concentration of leached DEHP (mg $g^{-1}$) | | |
|---|---|---|---|
| PVC samples | 6 days | 15 days | 30 days |
| Transfusion tubing | 139.40±1.27[a] | 177.80±3.44[b] | 287.58±7.67[c] |
| Tubing for infusion set | 127.35±1.20[a] | 161.43±1.76[b] | 263.60±6.36[c] |
| Quadruple blood bag | 119.45±1.48[a] | 152.60±2.21[b] | 257.21±5.95[c] |
| Transfer bag | 120.20±0.57[a] | 154.62±7.35[b] | 251.68±3.79[c] |
| Dialysis tubing | 101.45±1.34[a] | 109.91±4.41[a] | 205.71±7.23[b] |
| Dialysis bag | 91.50±2.97[a] | 100.33±4.98[a] | 188.14±6.87[b] |
| SAG-M transfer bag | 23.60±0.71[a] | 26.37±2.16[a] | 51.90±0.14[b] |

[a–c] values with the same letter within a row are not statistically significant different at the $p < 0.05$ level (Tukey's HSD test)

Difference between the leached concentration of DEHP obtained after different extraction times (6, 15 and 30 days) was determinated using the post-hoc test, Tukey's test. In order to determinate significant of difference, difference was compared to a critical value. The adjustment multiple testing was carried out with the aim of comparing difference between each pair of mean values. The critical value (q) is the point when a mean difference becomes honestly significantly different and it was obtained from table values. The critical value for the examined samples was 4.34 [9]. Values of HSD (honest significant difference) for each pair were computed by Origin© program. Comparing was performed in case $p < 0.05$.

Obtained results using Tukey's post-hoc test indicated significant difference between the obtained amounts of leached DEHP for 6 days extraction period and 30 days extraction period for all samples. There are significant diiferences between the obtained results for 6 days extraction period and 15 days extraction period for transfusion tubing, tubing for infusion set, quadrupole blood bag and transfer bag. For the remaining three samples, results showed no significant difference between these periods of leaching. Obtained amount of leached DEHP showed significant difference between the obtained amounts of leached DEHP for 15 days extraction period and 30 days extraction period for all samples.

Migration of DEHP from other plastic samples (PET, LDPE, HDPE, PP, PS and PC) did not occur even after 30 days, as determined by GC/MS analysis of *n*-hexane extracts of these plastic samples. From this it can be concluded that these types of plastics do not contain phthalates. Bearing in mind these results, the use of these investigated food and beverages packaging and mediqal equipment made from LDPE, can not negatively affect human health, as they don't contain DEHP. Otherwise, DEHP from the samples would tend to migrate to fatty foods and milk, which would endanger the human health.

In this work, the peritoneal dialysis bag made of Biofine materials, which does not contain phthalates, was also examined. Analyisis confirmed this fact. In view of this, these results should be kept in mind because using this bag does not cause harmful effects of DEHP on human health.

## CONCLUSION

Considering the technical and economic superiority of a variety of plastic materials, they have been used in the production of consumer products and in practically all areas of the technique. Given the nature of the bound between phthalate and plastics, DEHP migration from plastics under certain conditions was tested. In this study was found that PVC plastic contains DEHP in significant amount which may endanger the environment. In view of these results, it can be concluded that the tested samples for food and beverage packaging are safe to use.

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