**PHTHALATES RELEASED AFTER ERYTHROCYTE SUSPENSION: DO THEY POSE A RISK?**


## SUMMARY

**Aims:** It is commonly known that stored blood and blood products are heated before transfusion in order to prevent hypothermia, which leads to increased di-(2-ethylhexyl) phthalate content leaching into the blood and blood products and thereby causes greater conversion of di-(2-ethylhexyl) phthalate to mono (2-ethylhexyl) phthalate. However, there has been no study in the literature reporting on the amount of toxic phthalates in blood following the erythrocyte suspension transfused via warming. In this study, we aimed to investigate the di-(2-ethylhexyl) phthalate and mono (2-ethylhexyl) phthalate content in blood following the ES transfusions administered by di-(2-ethylhexyl) phthalate -containing and di-(2-ethylhexyl) phthalate -free infusion sets.

**Methods:** The study included 30 patients that were randomly divided into 2 groups with 15 patients each: group I underwent erythrocyte suspension transfusion via di-(2-ethylhexyl) phthalate -containing infusion sets warmed with blood-fluid warmers and group II underwent erythrocyte suspension transfusion via di-(2-ethylhexyl) phthalate-free infusion sets warmed with blood-fluid warmers. Di-(2-ethylhexyl) phthalate and mono (2-ethylhexyl) phthalate levels were measured both before and after transfusion.

**Results:** Di-(2-ethylhexyl) phthalate-free infusion sets led to no increase in the phthalate content, whereas di-(2-ethylhexyl) phthalate-containing infusion sets significantly increased the di-(2-ethylhexyl) phthalate and mono (2-ethylhexyl) phthalate levels, where the di-(2-ethylhexyl) phthalate level increased almost four times ($p$=0.001).

**Conclusion:** Di-(2-ethylhexyl) phthalate-containing products lead to toxicity. Therefore, using di-(2-ethylhexyl) phthalate-free products may prevent toxicity in patients undergoing erythrocyte suspension transfusion.


**What's already known about this topic?: Most of the blood infusion sets and blood bags are made of polyvinylchloride (PVC) containing di- (2-ethylhexyl) phthalate (DEHP). The transfusion by heating turns into MEHP, which is more toxic than other DEHP**

**What does this article add?: This study examines whether there is a difference between DEHP and MEHP levels between DEHP-containing sets and DEHP-free sets in heated erythrocyte suspension transfusions.**


**Key words:** Erythrocyte suspension, toxicity, di (2-etilheksil) phthalate, mono (2-etilheksil) phthalate


## INTRODUCTION

Blood-fluid warmers are commonly used in clinical practice particularly in the emergency service and the operating rooms for transfusing blood, blood products, and fluids by preventing the adverse effects of hypothermia. Line-type blood-fluid warmers are the most commonly used types since they are highly practical, inexpensive, and also do not require an additional kit for administration (1-3). In Turkey, most of the blood infusion sets and blood bags are made of polyvinylchloride (PVC) that contain di-(2-ethylhexyl) phthalate

(DEHP) as a plasticizer C It is commonly known that DEHP leaches from the bag into the blood and blood products as a result of warming (4,5). Moreover, DEHP converts to a more toxic metabolite known as mono (2-ethylhexyl) phthalate (MEHP) during the warming process [4-6]. It has been previously shown that exposure to DEHP-containing substances through inhalation or skin contact may result in gonadal injury or toxic effects on the reproductive system and kidneys (2). It has also been recently reported that there is an association between exposure to DEHP and atopic dermatitis (7).

Literature indicates that although the DEHP content in blood stored in plastic bags has been extensively investigated, there has been no study reporting on the DEHP and MEHP content in blood following the erythrocyte suspension (ES) transfused via warming. Therefore, the present study aimed to evaluate the DEHP and MEHP content in blood following ES transfusions administered by commonly used DEHP-containing infusion sets and less commonly used DEHP-free infusion sets and also investigate whether the DEHP and MEHP content poses a risk factor.

## MATERIALS AND METHODS

The study included 30 patients that were indicated for ES transfusion at YuzuncuYil University Medical School Emergency Service and Dokuz Eylul University Medical School Operating Rooms between December 2012 and May 2013. The study protocol was conducted in accordance with the 2,000 revision of the Helsinki Declaration and was approved by the local ethics committee as named Yüzüncü Yıl University Clinical Research Ethics Committee. The patients were randomly divided into 2 groups with 15 patients each:group I included patients that underwent ES transfusion via DEHP-containing infusion sets (Braun, Melsungen AG) warmed with blood-fluid warmers (Eczacibası-Baxter, Istanbul,Turkey) and group II included patients that underwent ES transfusion via DEHP-free infusion sets warmed with blood-fluid warmers (Braun, Melsungen AG). Each infusion set was used for transfusing a maximum of two units. Blood samples were obtained from each patient both before and after transfusion and DEHP and MEHP levels were analyzed at Alfagen Laboratory. The study was approved by YuzuncuYil University Ethics Committee.

### Blood Sampling and Storing

Venous blood samples were collected from the brachial wing vein and were transferred to plain blood tubes. After centrifuging the tubes at 1,200 g for 10 min, the top serum layer was placed in DEHP-free Eppendorf tubes and stored at -80° C until biochemical analysis.

### Biochemical Analysis

Serum DEHP and MEHP levels were measured using high-performance liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) technique.

### Statistical Analysis

Data were analyzed using SPSS 15.0 for Windows (SPSS Co., Chicago, IL, USA). Descriptive statistics included frequency tables and crosstabs for categorical variables and mean, median, standard deviation (SD), minimum,

and maximum for numeric variables. Chi-square test was used for comparing independent categorical variables, one-way ANOVA for comparing multiple groups of numeric variables with normal distribution, Kruskal Wallis for comparing multiple groups of numeric variables with nonnormal distribution, t-test for comparing binary categorical variables with normal distribution, and Mann Whitney U test for comparing binary categorical variables with nonnormal distribution. Spearman's rank correlation coefficient was used for analyzing the association between the numeric variables. Linear regression analysis with backward method was used for determining the risk factors. A p value of $<0.05$ was considered significant.

**RESULTS**

Table 1 and Figures 1 and 2 present the pre- and post-transfusion DEHP and MEHP levels in both groups.

The results indicated that pre-transfusion DEHP and MEHP levels were highly similar to each other in both groups ($p<0.05$) (Table 1, Figures 1, 2). However, post-transfusion DEHP and MEHP levels were significantly higher compared to their pre-transfusion levels in the group administered with DEHP-containing infusion sets ($p<0.0001$) and both levels were highly similar to their pre-transfusion levels in the group administered with DEHP-free infusion sets ($p=0.79$, $p=0.98$, respectively) (Figures 1, 2).

**DİSCUSSİON**

Technology is currently advancing at a dazzling speed and thereby provides numerous facilities for human life. However, human life has become under serious threat since technology has led to the development of new chemicals and their products. The 2002 European Union Commission Report revealed that among the chemical substances in the world, there are 60 toxic substances that have been clearly shown to have serious hazards for the environment and humans (8). One of these 60 substances is known as “phthalate”. Phthalateis used as a plasticizer in numerous consumer products such as cosmetics, wallpapers, flooring materials, automotive industry, synthetic curtains, clothing, food packaging, toys, baby care products, and medical devices (9,10). As it provides elasticity and durability for these products, phthalate is produced in millions of tons worldwide and can result in human exposures by inhalation, or oral or dermal contact (11-14). DEHP is one of the most common forms of phthalate used as plasticizers (15). MEHP, on the other hand, is the primary biodegradation product of DEHP. DEHP converts to MEHP through the lipase enzyme that is primarily available in the small bowel and pancreas and also in the liver, kidney, skin, lung, and plasma (16).

In light of the previous studies reporting on the toxic effects of DEHP, we performed ES transfusion after warming ES with blood-fluid warmers in order to avoid the risk of anemia and also the hypothermic effects of ES and the side effects of ES such acid-base imbalance. By doing so, we investigated the effects of normothermic ES transfusion on DEHP and MEHP in DEHP-containing and DEHP-free infusion sets warmed by blood-fluid warmers. The results indicated that DEHP and MEHP levels were not affected by the ES transfusions performed with heated DEHP-free infusion sets, whereas high amounts of DEHP leached from DEHP-containing infusion sets into the blood after warming, thereby leading to a significant increase in the MEHP levels as a result of DEHP metabolism.

Administration of acute blood loss in trauma patients and patients under anesthesiais of vital importance for the survival of the patients. The recent trend of transfusing essential blood components instead of whole blood has led to the avoidance of numerous harmful effects of whole blood transfusion. Among these blood components, erythrocyte suspension (ES) is currently the most common blood product used in the treatment of acute anemia. To enable long-term preservation of blood products such as ES, the products are frozen at 4-6° C with the addition of various preservative solutions. Transfusion of ES prevents mortality and morbidity by enabling necessary oxygen for the tissues and by maintaining hemodynamic stability; however, blood-fluid warmers are needed to prevent the hypothermia caused by the transfusion of frozen ES (17, 18). Nevertheless, it has been reported that blood-fluid warmers may lead to erythrocyte injury and also to increased DEHP toxicity as a result of the warming of the PVC product (5). The rate at which DEHP leaches from the PVC product to the blood is dependent upon numerous factors including the type of the plastic product and its temperature, storage time, the extent of shaking, and the amount of DEHP in the PVC product. DEHP is highly lipophilic and does not chemically bind to the PVC polymer, and it also can easily leach into lipid-containing solutions when heated. Line-type blood-fluid warmers are the most common warmers used for heating blood products and fluids and are designed to deliver fluid sat 39° C to maintain normothermia. The phthalates found in PVC products have low migration rates at low temperatures but their migration rates increase with temperature, particularly at temperatures above 24° C (19, 20). In our study, we also found that the DEHP and MEHP levels significantly increased with temperature, which suggests that such an increase may not lead to acute toxicity but may probably lead to chronic toxicity.

Animal studies have indicated that exposure to DEHP may lead to harmful effects on the reproductive and endocrine systems (21). Moreover, some other studies have reported that DEHP may have similar effects in humans and MEHP may have negative inotropic effects, decrease the concentrations of total T3 and free T4, and result in inflammation (1, 2, 22). However, although the toxic effects of DEHP have been shown in animal models, there is insufficient evidence regarding the toxic effects of DEHP on humans. It has also been hypothesized that the use of DEHP for medical purposes may have deleterious effects on certain cell groups and may accumulate in the organism (1, 5). In massive transfusions, large amounts of DEHP may enter the organism and exceed the tolerance limit (3, 4). Moreover, exposure to DEHP via blood transfusion may lead to increased DEHP toxicity (5).

Phthalate has been associated with endocrine disorders in numerous epidemiological studies (8). A longitudinal Taiwanese study extending over 12 years reported that prenatal exposure to phthalates may have negative effects on pubertal development (23). Another study reported that plasma-free hemoglobin levels were 90.3 mg/dl after 35 days of storage in DEHP-containing plastic packs and 181.7 mg/dl in polyolefin bags, suggesting that the stability of red cells was lower in the DEHP-containing packs compared to DEHP-free packs (5). Leuenbergeret al. also revealed that DEHP was detected in urine samples on day 1 after the transfusion of autologous blood stored in DEHP-containing bags (24).

The exact burden of blood bags, infusion sets, and other plastic products on patients in emergency services, operating rooms, and intensive-care units remains unknown. Our results indicated that although they can be relatively more costly, DEHP-free infusion sets can result in lower chronic toxicity in ES transfusions.

Therefore, alternative infusion sets that contain no plastic products such as polyethylene and polyurethane sets can be preferred for ES transfusions. Although there are several studies in the literature reporting on the toxic effects of DEHP and MEHP, further larger studies are needed to investigate the acute and chronic toxicity caused by DEHP and MEHP. We believe that our results will provide guidelines for further studies.

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**Data Availability**: The analyzed data sets generated during the present study are available from the corresponding author on reasonable request.

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**Competing Interests**. The author's declare no conflict of interest.

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| | **Group I** (n=15) | **Group II** (n=15) | ***p*** |
|---|---|---|---|
| **[1] DEHP (g/ml)** | 1.20±0.28 (0.9-1.8) | 1.21±0.41 (0.5-1.9) | 0.97 |
| **[2] DEHP (g/ml)** | 4.27±0.41 (3.7-4.8) | 1.21±0.40 (0.5-2) | <.0001 |
| **[1]MEHP (g/ml)** | 1.08±0.37 (0.3-1.7) | 1.11±0.39 (0.3-1.7) | 0.80 |
| **[2] MEHP (g/ml)** | 2.64 ±0.72 (1.7-3.9) | 1.12±0.33 (0.4-1.7) | <.0001 |

**Table 1.** Pre- and post-transfusion DEHP and MEHP levels in both groups (mean±SD) (min-max)

[1]Pre-transfusion, [2] Post-transfusion*$p$=0.0001

**Figure Legends:**

**Figure 1.** Comparison of pre- and post-transfusion DEHP levels

**Figure 2.** Comparison of pre- and post-transfusion MEHP levels