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# New generation of bags (DEHP excluded) in light of reports presented at the 33rd ISBT Regional Congress in Göteborg, held June 17–21, 2023

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The most common raw material for the production of containers for collection, preparation and storage of blood and blood components is poly(vinyl chloride) (PVC), in which bis (2-ethylhexyl) phthalate (DEHP) is used as plasticizer. Plasticizers make up a significant portion of PVC's composition, with a typical concentration of DEHP in PVC being 30% (wt/wt). Plasticizers render containers for collecting and storiage of blood and blood components flexible and transparent, as well as resistant to temperature fluctuations and pressures generated during preparation and storage. DEHP is a bipolar, lipophilic molecule that binds to PVC in a non-covalent manner. The DEHP — PVC interaction is weak, and so the compound migrates from the plastic to the stored blood component. DEHP builds into the lipid bilayer membrane of red blood cells with a stabilizing effect, preventing changes in cell shape, reducing the number of released microvesicles and reducing hemolysis. Phthalates, including DEHP, are classified as xenoestrogens, the harmful effects of which on the endocrine system and thus also on reproductive functions have been demonstrated on animal models. Sensitive patients (newborns, children, pregnant women) are particularly vulnerable to the harmful effects of DEHP.

Pursuant to the proven toxicity of DEHP, EU Regulation 2017/745 on medical devices banned its use in medical devices, including containers for blood and blood components above a maximum concentration of 0.1% (wt/wt). It therefore became necessary to replace DEHP with another non-toxic plasticizer with no loss to the quality of red blood cells and other blood components.

The regulation was originally scheduled to go into effect in 2025, but due to problems encountered in finding DEHP replacements, the implementation date was postponed until 2030.

Extensive research has long been launched on the impact of alternative plasticizers on blood component quality, as reflected at the 33rd ISBT Regional Congress in Göteborg, Sweden, held June 17–21, 2023. A session in the main scientific program, "DEHP bags in transition… are we ready?" was devoted to the topic of alternative plasticizers and 5 oral reports were presented. DEHP-free containers were also addressed in an oral presentation during the session "Factors affecting quality of red blood cell products" In addition, 6 posters on the topic were presented during the poster session.

During the session "DEHP bags in transition… are we ready?" L. Larsson of the Karolinska Institute recalled that so far research on replacing DEHP has not been satisfactory. The storage of red blood cells in containers made from plastic and plasticizers other than DEHP has been asociated with unacceptable levels of hemolysis, intensive microvesicle release from red blood cell and shortened red blood cells survival time in the

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recipient's circulation. To date, studies have been conducted using plasticizers such as 1,2-cyclohexanedicarboxylic acid diisononyl ester (DINCH), bis(2-ethylhexyl) terephthalate (DEHT) and tri-n--hexyl n-butryl citrate (BTHC). The author of the presentation stressed the fact that when changing the plasticizer it may also be necessary to change the additive solution in order to ensure the quality of red blood cells. All these changes would require numerous tests and validation procedures [1].

A. Chen presented the results of Terumo BCT. The purpose of their study was evaluation of RBCs, platelet concentrate (PCs) and plasma obtained with the Reveos® automated blood separation system (Terumo BCT, Inc., Lakewood, CO, USA) which uses Reveos LR EXT containers with no DEHP. A hybrid set of plasticizers was used. In containers for whole blood, plasma, PCs and residual leukocytes DINCH was used as plasticizer, Containers for storage of RBCs were made from ErythroMate, which does not contain DEHP the composition of which is however, secret.

With the DEHP-free container system, leukoreduced RBCs in additive solution, (PAGGSM) were obtained and maintained adequate quality until the 42nd day of storage. Also the values of all evaluated platelet parameters were within the acceptable range (EDQM) on day 5 and day 7 of storage in DEHP-free containers. Plasma samples evaluated after a minimum of 30 days of storage at –30°C ± 5°C also met all quality requirements. The feasibility of switching to a DEHP-free container system was thus confirmed [2].

L. Aoustin presented the results of the French research team. The aim of their study was to evaluate the effect of alternative plasticizers and additive solutions (PVC-citrate/PAGGSM and PVC-DINCH, respectively) on the quality of blood cells and plasma during storage, and to determine whether the quality of blood components is maintained in DEHP-free containers, with particular focus on the quality of blood cells due to the well-known effect of DEHP on the stability of the red cell cell membrane.

A DEHP-free container system, (FQ422FR; Fresenius Kabi) with PAGGSM, was used for blood collection. The quality of the red cells was assessed on day 1 before and after preparation, then on storage days: 21, 28, 35 and 49. The obtained plasma units were filtered (DEHP-free filter; FS013FR, Fresenius Kabi) for leukoreduction, to the level required by the French legislation ($\leq 1 \times 10^4$/L). Plasma quality was assessed before filtration, before freezing, and after 14 days, 6 and 12 months of storage. Depending on the type of blood component, various quality control parameters were analyzed and compared with historical data. In the case of the RBCs the results for: hemoglobin, hematocrit, ATP, glucose, lactate, degree of hemolysis, potassium, pH, $pO_2$ and $pCO_2$ were comparable to the control group (leukoreduced RBCs prepared in DEHP containers). No DEHP in containers for collection and storage had no adverse effect on the quality of leukoreduced RBCs stored for up to 49 days, as confirmed by cell membrane stability parameters such as: degree of hemolysis (%), ATP, glucose and lactate concentrations. Plasma evaluation included: total protein, albumin, IgG, IgM and IgA, complement factors (C3a and C5a), prothrombin time, APTT, coagulation factors (fibrinogen, FVIII, FII, FV, FVII, FX, FIX, FXI, VWF and ADAMTS13), coagulation inhibitors (antithrombin, protein S and C), fibrinolytic factors (plasminogen, α2-antiplasmin, TAT), thromboelastometric parameters. The results were comparable to those obtained in the control group (plasma collected, processed and stored in DEHP containers).

All of the quality control results for RBCs prepared in PVC-citrate/PAGGSM containers as well as plasma prepared and stored in PVC-DINCH containers met French quality requirements [3].

Another presentation, demonstrated the results of the qualitative evaluation of a set of containers for collection, preparation and storage of blood and blood components manufactured from modified PVC plastic without DEHP (Prototype RENOLIT BLOODPROTECT 42Plus). The evaluation was based on the qualitative study of buffy coat removed RBCs in additive solution.

Whole blood was collected into DEHP-free plastic container sets. Each time, at least 2 WB units ABO- and Rh-compatible were tested. 2 units of blood group-compatible RBCs were pooled, and the pool was divided into two units. The control group was the unit transferred into a standard DEHP container, and the test group was the unit transferred into the tested plastic container. SAGM additive solution was added to both containers. Samples for quality control were taken on the first and forty-second days of storage. The quality of no buffy coat RBCs in additive solution stored until the 42nd day in containers made of modified PVC Prototype RENOLIT BLOODPROTECT 42Plus plastic was comparable to that of no buffy coat RBCs in additive solution stored up to 42nd day in conventional containers with DEHP. The average values of the tested quality control parameters

in the control and test groups met the criteria of Polish legislation [4].

The aim of the next presentation was comparison of the quality of RBCs stored in PAGGSM additive solution in three types of containers in which DEHP was replaced by BTHC, DEHT and DINCH respectively.

Whole blood was collected in containers in which DEHP was replaced with DINCH plasticizer (Fresenius Kabi, GQ422NL). Additive solution (PAGGSM) was added to whole blood. RBCs were subjected to filtration through a filter connected to a receipt container for RBC storage with BTHC used as a plasticizer. Two units of ABO-compatible RBCs were pooled, then separated and transferred to containers with BTHC and DEHT or DINCH. On the 35$^{th}$ and 42$^{nd}$ day of storage, samples were collected for metabolic and hematology tests. Red blood cell counts remained stable throughout the storage period in all three types of containers. A slight increase in MCV was observed during the storage of the RBCs. The increase in red blood cell volume was greater in containers with DEHT as compared to containers with BTHC and DINCH. The degree of hemolysis increased during RBC storage but on day 42 all the RBC units met the EDQM requirements of hemolysis < 0.8%. Hemolysis was significantly higher in DEHT and DINCH containers compared to BTHC containers. Glycolytic activity was measured by glucose consumption and lactate production. It was significantly higher in containers with BTHC compared to the glycolytic activity found in containers with DEHT and DINCH. Although ATP production was more stable in containers with BTHC and DINCH as compared to those in containers with DEHT, RBC 24-hour *in vivo* recovery measured after 42 days of storage in all units was acceptable.

RBCs in additive solution (PAGGSM) obtained from whole blood collected into DINCH containers and stored in either BTHC, DEHT or DINCH containers up to 42 days met the quality requirements for acceptable hemolysis and ATP production. It was found that RBCs stability (assessed by the degree of hemolysis) and glycolytic activity were best maintained in BTHC containers. The Sanquin Blood Bank of Amsterdam is currently evaluating the incidence rate of adverse reactions following transfusion of RBCs in additive solution (PAGGSM) stored in containers with BTHC [5].

During the session entitled "Factors affecting quality of red blood cell products" a study was presented highlighting the impact of transfusing preterm neonates with RBCs from adult donors. Adult RBCs contain > 95% HbA, while RBCs of preterm infants contain > 90% HbF. HbA affinity for oxygen is lower than that of HbF, therefore there occurs an increased oxygen release after transfusion of adult donor red blood cells. Higher intraretinal oxygen partial pressure may be associated with a higher risk of retinopathy. Red blood cells (RBCs) from cord blood contain fetal hemoglobin that is predominant in newborns and, therefore, may be more appropriate for neonatal transfusions than currently transfused adult RBCs. The purpose of this study was to evaluate the quality of RBCs from cord blood stored in PVC containers containing DINCH as a plasticizer. Cord blood donations were filtered to remove leukocytes and platelets, followed by centrifugation and plasma removal. Red blood cells were diluted with SAGM additive solution to a hematocrit (Ht) value of 50–65%. Cord blood RBCs were stored in DINCH-PVC containers for up to 21 days. Samples were collected weekly for analysis of quality parameters. The red blood cell count remained constant throughout the storage period. Hemolysis on the 21st day of storage in all RBC units did not exceed 0.8%. During storage, a decrease of glucose concentration with a concomitant increase in the concentration of lactate was observed in the RBCs. Despite the decrease in ATP concentration and increase in the concentration of ADP and AMP, on the 21 st day of storage the total adenylate level, (an indicator of the energy status of red blood cells) was still 93% ± 12% of the level of day 1. Accordingly, it was concluded that cord blood can be prepared to obtain the RBCs in additive solution (SAGM), then stored for 21 days in DEHP-free containers without loss of red cell integrity as measured by the degree of hemolysis and parameters determining the energy status of red cells. There is a relatively high loss of red blood cells during cord blood preparation. Therefore, future research, in addition to prolonging the shelf life of cord blood RBCs, should be aimed at increasing the recovery of red blood cells during preparation. In addition, the use of second-generation additive solutions is advisable to increase ATP concentration [6].

During the poster session, 6 posters were presented, 3 of which evaluated the effect of DEHP-free containers on the quality of inactivated blood components.

The purpose of one paper was to evaluate the quality of plasma inactivated with methylene blue (THERAFLEX MB-Plasma; Macopharma) in DEHP-free containers.

Whole blood was collected in standard DEHP containers, plasma was separated and further processed. 8 plasma containers were combined with a DEHP-free inactivation container system. Inactivation was performed using a Macotronic B2 device. After exposure, plasma was filtered using a Blueflex filter. Samples for the determination of plasma clotting factors were collected before inactivation, after filtration through a Blueflex filter and after one month of storage.

Inactivation with methylene blue had a significant effect on the activity of plasma clotting factors. Activated partial thromboplastin time (APTT) was prolonged by 7% and thrombin time (TT) by 13%. A reduction in the concentration of clotting factors was observed, with the exception of factor VII and protein S. Although significant reductions in fibrinogen (25%), FV (10%), FVIII (22%) and FXI (18%) were observed, the quality requirements of the European guidelines were met for all plasma units. It was found that plasma inactivated with the THERAFLEX MB-Plasma system using DEHP-free PROSDV1 preparation containers showed the expected levels of plasma factors. The results were comparable to those obtained for plasma inactivated with the above system using standard DEHP-containing containers [7].

The purpose of the next study was to investigate the effectiveness of virus inactivation with THERAFLEX MB-Plasma system (Macopharma) in plasma stored in a DEHP-free container. Four different virus models were used during the test: the Suid Herpes Virus (SHV-1), Bovine Viral Diarrhea Virus (BVDV), Feline Calici Virus (FCV) and Vesicular Stomatitis Virus (VSV), which differ in structure and biophysical characteristics.

The reduction factor for all viruses tested in this study was ≥ 4 log10 at a final irradiation intensity (power density) of 120 J/cm$^2$, and the degree of virus reduction was comparable to that obtained with DEHP THERAFLEX MB containers [8].

The ability to eliminate bacteria from plasma with methylene blue (MB) was also investigated using the DEHP-free version of the THERAFLEX MB-Plasma container system (Macopharma).

The study investigated the important steps in the inactivation process with THERAFLEX MB-Plasma system, including two filtration steps: to remove leukocytes (Plasmaflex filtration) and to remove methylene blue and its photoproducts (Blueflex filtration), and their effect on the elimination of two different bacterial species (*Klebsiella pneumoniae* and *Brevundimonas diminuta*). Removal of *Klebsiella pneumoniae* and *Brevundimonas diminuta* from plasma stored in THERAFLEX System MB PROSDV1 (DEHP-free) containers was effective. Suspensions with bacterial titers that were higher than those used during the study with THERAFLEX System MB-Plasma containers with DEHP, so even higher bacterial reduction rates were achieved in this study [9].

The goal of the next study was to investigate the qualitative differences and metabolic parameters of PCs stored in DEHP and BTHC containers.

All units were evaluated for swirling, color change, and the presence of clots. PC volume, platelet and leukocyte counts were also evaluated. After the storage period, pH, oxygen partial pressure, carbon dioxide partial pressure, bicarbonate, glucose and lactate concentrations were measured. The stored PCs were also subjected to microbiological control. There was a marked reduction of pH in BTHC containers , while differences in $pCO_2$ and $pO_2$ concentrations were significantly greater in DEHP containers. Platelet loss during storage was greater in DEHP containers. There were no significant differences in either HCO3-, glucose- or lactate concentrations, or in mean platelet volume.

Quality parameters of PCs stored up to 5 days in both types of containers were comparable. BTHC containers were found to have higher gas permeability and lower platelet loss during storage and were suitable for collection and storage of PCs [10].

During the poster session another report presented the results of a multicenter study to evaluate Macopharma containers in which DEHP was replaced by DEHT and PAGGSM was used as an additive solution. The study was conducted in collaboration between the French blood service (EFS, *Etablissement Français du Sang*) and the Belgian blood service (SFS, *Service Francophone du Sang*). The solution proposed by Macopharma DEHT/PAGGSM was evaluated in terms of preparation efficiency and the compliance of the obtained RBCs and plasma with EDQM requirements.

RBCs and plasma were obtained from whole blood. For RBCs the following parameters were compared for compliance with EDQM guidelines: hemoglobin (Hb), hematocrit (Ht), residual leukocytes (rWBC). In addition, the following parameters were assessed: volume, glucose, lactate, $pO_2$, $pCO_2$, potassium and pH value. For plasma, the following were assessed: residual morphotic elements (platelets, red blood cells, white blood cells) and FVIII concentration was examined. In addition, the volume of plasma obtained and the concentration of fibrinogen were evaluated. The

tests were carried out on the first day. All the units of the blood cells met the quality requirements set by the EDQM. In the case of plasma, all units met the EDQM requirements in terms of residual white blood cells, red blood cells and platelet cell count. An abnormal FVIII result was obtained (below 70 IU/100 mL), in 7 plasma units, but the values obtained met the quality criteria defined locally by the ESF.

The authors concluded that preparation of whole blood in DEHT-PAGGSM containers did not affect the quality control results of the obtained blood components [11].

During the poster session the results of a study aimed at evaluating the release of alternative plasticizers from blood and blood component containers into the stored blood components were also presented.

Whole blood was prepared using PVC containers with DEHP, DINCH or DEHT as the plasticizer. The concentrations of DINCH and DEHT in the blood components were tested by liquid chromatography coupled to tandem mass spectrometry or with a UV detector and compared to the concentrations of DEHP. The concentration of the plasticizer to which the recipient is exposed during transfusion depends on the preparation of the labile blood components and their storage conditions (temperature, time).

After the first storage day, DEHP release in all labile blood components, was 5.0 and 8.5 times higher than for DINCH and DEHT, respectively. On the 49th day of storage, DEHP concentrations were statistically higher in the RBCs as compared to DINCH and DEHT concentrations.

DINCH and DEHT plasticizers showed lower toxicity than DEHP. In addition, patients who were transfused with RBCs stored in PVC-DINCH or PVC-DEHT containers are at lower risk of exposure to these plasticizers as compared to those with PVC-DEHP containers due to their lower release rate into blood components (from 38.9% to 87.3% compared to DEHP) [12].

## Summary

The ISBT congress presented the outcome of studies on various aspects related to the replacement of plasticizer in PVC used for manufacturing containers for collection and preparation of blood and blood components. All study results on the quality of blood components collected, prepared and stored in containers with alternative plasticizers seem very promising; all authors reported that the quality of blood components met EDQM or national quality criteria. Importantly, this also applied to the degree of hemolysis in the red blood cells. Studies were conducted on the effect of alternative plasticizer on the quality of blood components subjected to pathogen inactivation with methylene blue. The studies demonstrated the effectiveness of pathogen inactivation as well as no impact on the quality of plasma subjected to pathogen inactivation in novel containers.

Only one paper addressed the potential toxicity of alternative plasticizers. It showed that their release rate into blood components was much lower compared to that of DEHP. Prior to the introduction of containers with alternative plasticizers into routine use, this topic requires particularly intensive research.

The results of the studies presented at the ISBT Congress prove that work on replacing DEHP is well advanced, nevertheless intensive efforts are still necessary to cover all aspects that could result from plasticizer replacement.

**Conflict of interest:** none declared

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