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Research Article

Evaluation of Diethylhexyl phthalate (DEHP) Leached from Packaging Bags into Different Brands of Physiological Saline Solutions Obtained in Ibadan, South-Western Nigeria.

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ABSTRACT

Diethylhexyl phthalate (DEHP) is used as plasticizer in plastics and other polymer materials which are found in medical devices, blood bags, physiological saline bags and infusion tube. Phthalates do not bind to these polymers; but leach extensively into the fluids in them. This had been proposed as a means of DEHP exposure to patients who had physiological saline infused into them. However, there had been paucity of data to support this argument. Hence this study examined the level of DEHP in five brands of physiological saline solution and their packaging bags. For each brand, Soxhlet extraction using DCM: n-hexane (23:6) was used to extract the saline solution bag, while DCM was used to extract the saline solution followed by cleanup with silica gel in open column. Each cleanup extract was reconstituted into acetonitrile and analysed with HPLC-UV at 226 nm. The solution bags had a mean DEHP of 4.14 ± 0.76 µg/g; while the saline solutions had concentration range of DEHP between ND – 0.46 µg/mL. This study established that DEHP leached from most of the polymer packaging bags into the physiological saline solutions.

Keywords: *Phthalate, leaching, polymer materials, saline solutions, human exposure.*

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INTRODUCTION

Phthalate esters are esters of benzene 1,2 dicarboxylic acids and are used as plasticizers in plastics, blood bags, infusion tubes, saline solution bags, catheters and other polymer products (Inuoe *et al*., 2005) They are added to improve the workability, pliability and softness and they constitute about 40% of these polymer products (Gimeno *et al*., 2014). However, phthalate esters do not bind to these materials, as they are not chemically bound to the polymers but are physically included in the matrix and hence leach into the content or immediate environment (Strac *et al*., 2011). Diethylhexyl phthalate (DEHP) is the most commonly used plasticizers in plastics and polymer materials and has been found to be an endocrine disruptor in humans (Al Salloum *et al*., 2016). DEHP in physiological saline solution was found to increasingly leach when stored for a prolonged time. DEHP was found to increase in normal physiological saline solution from 0.220, to 4.930 to 3.588 to 6.450 µg/L in 1, 2, 7, and 9 months of storage respectively (Strac *et al*., 2011).

Blood bags also have been found to leach DEHP into the blood content. The DEHP concentration was increased from 100 to 275 mg/L when stored for one week in a refrigerator at (– 4^{o}C) (Al-Salloum *et al*., 2016). Also, the DEHP concentration increased from 200 to 300 mg/L when stored at ambient temperature for 3 days (Al Salloum *et al*., 2016). A study in Japan also confirmed the leaching of DEHP from blood bags and found that 83.2 µg/mL of DEHP leached in 21 days of storage. Exposures to DEHP in women have been linked with decreased birth weight, reduced anogenital distance in offspring, inability to sustain healthy pregnancy, and increased head circumference in offspring and increase in chances of preterm birth (Watkins *et al*., 2016).

Many studies have examined the phthalate in marine animals, sediments, water, plastics, toys and even in human subjects (Adeogun *et al*., 2015). Also, many have examined leaching from blood bags and infusion tube to blood as well as to human subjects. (Al Salloum *et al*., 2016; Onipede *et al*, 2018; Onipede *et al*., 2019). However, there is dearth of information on the level of DEHP leached into physiological saline solution from the solution bags, as this may be a source of phthalate exposure to patients in the hospitals. Also, there have been conflicting information on leaching of phthalate esters from polymer packaging materials to their fluid contents. Hence, this study aimed to determine the levels of

DEHP in physiological saline solution and their packaging bags. This may help to estimate the level of DEHP exposure in patients to which physiological saline solution were administered.

MATERIALS AND METHODS

Description of the sampling area and sampling

The samples were purchased at different pharmacy stores located at Agbowo, Sango, University College Hospital, Mokola and Adamasingba all in Ibadan metropolis South-Western Nigeria. Ten (10) replicate samples of each of normal saline, dextrose saline, Darrow's solution, glucose solution and ringers lactate solution were purchased in the stores at the aforementioned sample points in Ibadan metropolis and were stored in freezer until the time of analysis.

Materials and standards: Diethylhexyl phthalate standard, n-butyl benzoate standard, dichloromethane, ethyl acetate, n-hexane, acetonitrile, sodium chloride, sodium carbonate, sodium sulphate, and alumina were purchased from Sigma-Aldrich Munich Germany.

Sample extraction

Extraction of physiological saline solution bag: All soft sections of the bags were cut into pieces to less than 2 mm with scissors. A 5 g of the cut component were wrapped properly with 12.5 mm Whatman filter paper before being placed in soxhlet extractor. The soxhlet apparatus was set up with 115 mL of dichloromethane and 30 mL of n-hexane mixture and was allowed to run at 60^{o}C for 7 hours. The extract was concentrated to 5 mL and the kept for the cleanup procedure (Adewuyi and Olowu, 2012).

Extraction of physiological saline solution: Physiological saline solution of about 200 mL was poured into a separating funnel, 3g of NaCl was added to it to emulsify its fat. This was then extracted three (3) times, each with 10 mL of dichloromethane. The extracts were pooled for further extraction with three (3) times washing, each with 5 mL of 0.1 M of Na_2CO_3 solution to remove free fatty acid interference. The dichloromethane extract after alkaline washing was then dried over anhydrous Na_2SO_4 and then kept for the cleanup procedure (Ogunfowokan *et al*., 2006).

Cleanup: Dichloromethane extract was made into slurry with 3 g of the activated alumina and was allowed to dry. A 10 mL column was packed with the already dried alumina slurry of the extract. The column was conditioned with 2 mL of n-hexane and thereafter extracted with 11 mL of 6: 5 of ethyl acetate in n-hexane. The extract was reconstituted into 1 mL of acetonitrile for HPLC analysis (Ogunfowokan *et al*., 2006).

Recovery study: A 6 mL of ultrapure water was spiked with diethylhexyl phthalate (DEHP) to a concentration of 100 mg/L. . (Onipede et al., 2019). The solution was then extracted and cleanup with the same procedure used for physiological saline solution sample (Ogunfowokan et al., 2006).

Blank study: Unspiked ultrapure water was used as blank in this study (Adewuyi, 2012). This was also extracted and cleanup with the same procedure as the saline solution sample (Ogunfowokan et al., 2006).

Limit of detection: The limit of detection was three (3) times the noise to signal ratio in the sample. While the limit of quantification is nine (9) times the noise to signal ratio in the sample. Limit of quantification represents the lowest quantifiable concentration of the analyte, with acceptable accuracy and precision in the real sample. The limits of detection (LOD) of the HPLC-UV for diethylhexyl phthalate was 0.005 mg/L. While the limit of quantification (LOQ) for was 0.015 mg/L, as shown in Table 2.

HPLC analysis: The samples were analysed with HPLC CECIL 4900 chromatographic system with UV detector, the column was Nucleosil 120 – 10 C_{18} (250 x 4.6 mm). The gradient elution of DEHP was done with acetonitrile: water (75:25) and the mobile phase were gradually increased to 100% acetonitrile over 7 minutes. The temperature of operation was ambient and the injection volume was 10 µL and the flow rate was 1 mL/minute, the wavelength of detection was 226 nm of DEHP was, the mode of quantification was peak area. Microsoft excel 2010 was used to get the plot of concentration against peak area and to obtain the concentration in samples (Onipede *et al*., 2018).

RESULTS AND DISCUSSION

Table 1:
Percentage recovery

Phthalate ester	% Recovery
DEHP	70.46

It could be observed from Table 1 that the percentage recovery of our study was found to be 70.46%; this could be an indication that the method of extraction and determination of DEHP employed in this study was efficient and effective. It could also suggest that there was no extraneous source of contamination or positive interference to the method employed in this study. DEHP was not detectable in the blank examined in this study as shown in Fig 1.

Table 2:
Showing the parameters of the HPLC and their respective value

Parameter	Value
Limit of detection	0.005mg/L
Limit of quantification	0.015 mg/L
Retention time of DEHP	4.27 min
Absorbance wavelength	226nm

It could be observed from Table 2; that the detection limit of the DEHP analysed was very low, an indication that a robust method and excellent instrument were used in the determination of the phthalate ester in the physiological saline solution and the bags.

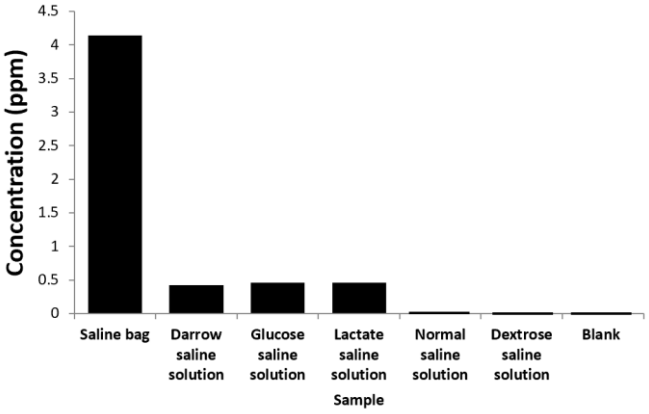

Figure 1
Concentration of DEHP in saline bag and saline solution

DEHP in physiological saline bag was found to be 4.14 ± 0.76 µg/g, this seems to be far lower than that obtained in in France study, which found a migration of DEHP to be 2 mg/mL in ethanol (Al Salloum *et al*., 2016). This suggests that DEHP migration in our study was slow and was not as significant as that found in other studies. The frequency of detection of DEHP in the ten (10) saline bag samples examined in this study was 100%; this could be an indication that the DEHP was used as plasticizer used in physiological saline bag examined in this study. The DEHP release in this study was lower than that obtained in China study which had a DEHP migration of 2.84 mg in ethanol-water mixture (Luo *et al*., 2014). This seems to suggest that ethanol could be better extraction solvent than dichloromethane used in our study. The level of DEHP in our study was found to be lower than what was reported in a Japan study which found a concentration ranged of 6.8 – 36.5 µg/mL leaching in blood (Inoue *et al*., 2005). The DEHP measured in our study is however higher than that obtained in a study in Italy which found DEHP to be ND (not detectable) in medical devices (Gosetti *et al*., 2018). The level of DEHP in our study was found to be lower than that obtained in Germany study, which found 23.7 µg/g of DEHP leaching from tubing (Nikolaus Khun-Velten *et al*., 2001), as shown in Table 3.

DEHP in Darrows physiological saline solution was 0.42 ± 0.03 mg/L, this seems to be far lower than that obtained in a China study, which had a migration of 2.8 mg in ethanol-water mixture (Luo *et al*., 2014). The frequency of detection of DEHP in the ten (10) Darrows physiological saline solution samples examined in this study was 100%. This suggests that phthalate leached also into physiological saline solution, which may be transferred to patients who received it during medical treatment. It may be an affirmation to many scientific researches findings that phthalate esters leach extensively from medical devices to their content, which in turn leach to the system of recipients of materials from these devices. The DEHP found in our study was found to be lower than concentration obtained in a Japan study which found DEHP leaching in whole blood product stored in blood bags to be 15.0 to 83.2 µg/mL (Inoue *et al*., 2005) as shown in Table 3. This suggests that blood in blood bags could leach more phthalate esters from polymer materials than physiological saline solution. In the same vein, the blood transfusion recipient could have more phthalate esters leached into their system more than recipient of physiological solution.

The mean DEHP concentration in glucose physiological saline solution is found to be 0.46 mg/L. This is lower than that obtained in a Germany study which had 14.5 µg/mL in serum (Nikolaus Khun-Velten, *et al*., 2001). This suggests that blood could leach more DEHP from their polymer containers than glucose physiological saline solution. The frequency of detection of DEHP in the ten (10) glucose physiological saline solution samples examined in this study is 50%. This is an indication that the DEHP was used as plasticizer in glucose physiological saline bag. Hence, DEHP leached extensively into the glucose physiological saline solution examined in this study. The mean DEHP in our study is higher than that obtained in a Croatia study, which had DEHP concentration of 3.481 ± 3.196 µg/L (mean ± standard dev) in normal saline solution (Strac *et al*., 2011). This is in addition to the other phthalate esters such dimethyl phthalate (DMP), diethyl phthalate (DEP), dibutyl phthalate (DBP) and di-n-octyl phthalate (DnOP) determined in the study. This could possibly suggest that the glucose physiological saline solution was better at leaching phthalate esters than normal physiological saline solution used in the Germany study. The level of DEHP in our study is found to be higher than that found in a Japan study, which found DEHP leached during hemodialysis at zero (0) circulation and was found to be 248.9 ± 123.6 ppb (Haishima *et al*., 2004). This could be an indication that glucose physiological solution is a veritable source of DEHP exposure, that is comparable to exposure through other medical devices encountered during treatment in the hospital. The mean DEHP concentration in lactate physiological saline solution is found to be 0.46 mg/L. This is higher than that obtained in a Croatia study which had DEHP concentration of 3.481 ± 3.196 µg/L (mean ± standard dev) in normal saline solution (Strac *et al*., 2011).

Table 3:
Concentration of DEHP in samples as compared to the literature

Sample	Concentration	Lit. value	Reference	Place of study
Saline bag	4.14±0.76 µg/g	23.7µg/g	Khun-Velten *et al* 2001	Germany
Darrows saline sol.	0.42±0.03 mg/L	15 – 83.2mg/L	Inoue et al., 2005	Japan
Glucose sal. sol.	0.46 mg/L	248.9±123.6 µg/L	Haishima *et al*., 2004	Japan
Lactate sal. sol.	0.46 mg/L	13.17 mg/L	Luo *et al*., 2014	China
Normal sal. sol.	nd	3.481±3.196 µg/L	Strac *et al*., 2011	Croatia
Dextrose sal. sol.	nd	100 – 1000 mg/L	Gimeno *et al*., 2014	France

Keys: *Concentration mean ± standard deviation, nd; not detectable. Sal. Sol.; Saline solution.*

This could be an indication that lactate physiological saline solution was better at leaching phthalate esters from polymer bags than the normal physiological saline solution. The frequency of detection of DEHP in the ten (10) lactate physiological saline solution samples examined in this study is 50%. This suggests that the DEHP leached from the lactate physiological saline bag into the lactate physiological saline solution examined in this study. The mean DEHP in our study is lower than that obtained in a study in France, which found a concentration in PVC material to be between 100 to 1000 ppm (Gimeno *et al*., 2014). This may seem to support the fact which have been observed by scientific study, that phthalate esters leaching by physiological saline solution could be low (Strac *et al*., 2011). The level of DEHP obtained in our study was lower than that obtained a China study, where the DEHP extracted with ethanol-water mixture was found to be 13.17 µg/mL (Luo *et al*., 2014). This could suggest that leaching with lactate physiological solution may not be as effective as extraction with a solvent.

The mean DEHP concentration in normal physiological saline solution in our study is found to be below the detection limit (ND) in all the samples analysed. The none detection of DEHP in the normal physiological saline solution could be an indication that there was no contamination in our method of extraction or analysis by HPLC. The DEHP concentration obtained in normal physiological saline solution is far lower than that obtained in a Croatia study, which had DEHP concentration of 3.481 ± 3.196 µg/L (mean ± standard dev) in normal saline solution (Strac *et al*., 2011). This seems to suggest that the leaching of phthalate from the physiological saline bag may not have been substantial within the period it was analysed. However, the Croatia study was allowed to leach for up to nine (9) months into the normal saline solution. The level of DEHP in the normal physiological saline solution was found to be lower than that obtained in the study in a Germany. In which DEHP is found to be 14.5 µg/mL in serum (Nikolaus Khun-Velten *et al*., 2001). This could be an indication that blood seems to leaches phthalate esters more from the polymer bag materials than physiological saline solution. The level of DEHP found in our study was found to be lower than that obtained in the study in a China study, which DEHP concentration is found to be 13.17 µg/mL, in blood bag extracted with ethanol-water. (Luo *et al*., 2014). This may seem to suggest that DEHP is leached faster with organic solvent-water mixture than physiological saline solution alone.

The mean DEHP concentration in dextrose physiological saline solution obtained in this study is below detection limit (ND) in all the samples analysed. This is by far lower than that obtained in a France study in PVC blood bag which obtained DEHP of 2 mg/mL after four (4) weeks of storage. (Al Salloum *et al*., 2016). This could add evidence to the fact that, dextrose physiological saline solution may not easily leach phthalate esters from the PVC bags than when organic solvent is employed. The mean DEHP in our study is lower than that obtained in a Croatia study, which obtained a DEHP concentration of 3.481 ± 3.196 µg/L in the normal saline solution (Strac *et al*., 2011). This could be suggestive that, leaching of phthalate esters to saline solution could definitely occur if the adequate time is given for the leaching to occur. This is suggestive that the DEHP is present in PVC bags, but the dextrose saline solution used in our study had not leached enough into the dextrose physiological solution to the extent that could be detected by the HPLC employed in this study.

The DEHP is about a factor of ten (10) higher in the extracted polymer saline bag, than the highest DEHP concentration in physiological saline solution. This could be an indication that DEHP leaches from the saline bags to the physiological saline solution. The general trend of DEHP concentration observed in the physiological saline solution examined in this study was Glucose saline solution ≡ Lactate saline solution > Darrow saline solution > Normal saline solution ≡ Dextrose saline solution. This seems to suggest that the physiological saline solutions had variable level of leached DEHP in them; which could be as a result of duration of the physiological saline solution in the polymer bag or the leaching capability of the various physiological solutions as a result of their contents.

HPLC was employed to determine DEHP in physiological saline bag and five different brands of physiological saline solution. It showed a robust method, which could be attested in the recovery study. However, the bag indicated the presence of DEHP, but out of the five brands of physiological saline solution analysed in this study, three showed traceable value of DEHP in them. While the other two were below the detection limit; this could be an indication that DEHP is a major plasticizer used in PVC, blood bags and physiological saline bags. And that DEHP leached extensively into the three physiological saline solution they were detected. This could be a viable source of exposure to phthalate esters to the recipients of the physiological saline solution, during treatment in the health services system. It could be said that the concentration was relatively small yet the effect of phthalate esters in the human system have been scientifically proven to be high risk. This study established that DEHP leached from physiological saline bag to the physiological saline solution. Hence a better substitute could be sought to replace phthalate esters as plasticizers in polymer products.

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