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Physical–chemical assessment of di-(2-ethylhexyl)-phthalate leakage from poly(vinyl chloride) endotracheal tubes after application in high risk newborns

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ABSTRACT

Poly(vinyl chloride) (PVC) is extensively used in the production of medical devices including endotracheal tubes. In order to make PVC flexible extensive quantities of plasticizers are added to the virgin matrix and among these, di-(2-ethylhexyl)-phthalate (DEHP) is the most used in PVC medical devices. DEHP is not covalently bound to PVC and during the use of medical devices, it tends to migrate out and accumulate in tissue.

To the best of our knowledge, limited literature data are available on the DEHP release from PVC medical devices as a consequence of applications in humans.

Aim of the present study was to verify through a physical–chemical characterization the occurrence of DEHP leakage from endotracheal tubes and to determine the correlation between the leaching of the plasticizer and the time of intubation of the tubes in high risk newborns. Thermogravimetric Analysis (TGA), Differential Scanning Calorimetry (DSC) and High-Performance Liquid Chromatography (HPLC) analyses were performed and the results show the effective release of DEHP from tubes. Moreover the study reveals that the release of DEHP occurs within the first 24 h of employments of the tubes.

1. Introduction

For a long time medical devices have been essential, effective, and often lifesaving tools for many patients and are frequently used in life-support procedures. Poly(vinyl chloride) (PVC) is one of the most widely used polymeric material in the medical field. Phthalates, a family of industrial compounds with a common chemical structure (dialkyl or alkyl/aryl esters of 1,2-benzenedicarboxylic acid), are added to an otherwise rigid PVC items to impart softness, flexibility and durability. Di-(2-ethylhexyl)-phthalate (DEHP) is the most commonly used plasticizer in PVC medical devices, including endotracheal tubes. In flexible PVC grades, DEHP is obviously not covalently bound to PVC and it is released into the external environment. The prolonged contact of medical devices with body fluids or tissue is associated to severe health risks due to the acute and chronic exposure to phthalate, which accumulate in the fat tissue of human beings and animals (Hill et al., 2001; Tickner et al., 2001; Matsumoto et al., 2008).

In this regards phthalates have shown to be reproductive and developmental toxicants in animal models, and are suspected of having endocrine disrupting or modulating effects in humans (Latini et al., 2010). In particular shorter pregnancy duration and reproductive disorders in human population have been associated to phthalate exposure (Skakkebaek, 2002; Sharpe, 2001; Latini et al., 2003, 2006; Huang et al., 2009; Zhang et al., 2009). Additionally, DEHP leaching from medical devices appears to determine side effects on human health such as bronchopulmonary dysplasia (BPD), deep venous and cholestasis (Latini, 2005; Von Rettberg et al., 2009). It is important to underline that, according to what has been reported by the Food and Drug Administration (FDA), the DEHP potential exposure risk is higher for infants, particularly for premature and critically ill neonates in the Neonatal Intensive Care Unit (NICU) due to their small body size, a weak physical condition and the need of a multitude of medical interventions, each increasing exposure levels and compromising their recovery (Latini et al., 2010).

In order to determine the actual leaching of DEHP from medical devices, previous studies concerning the quantification of plasticizer to which human are exposed have been reported in the literature (Latini, 2005). In particular two different approaches were described: in the first one the quantity of DEHP and its metabolites were directly determined from blood, urine or tissues of patients treated with medical devices made of PVC (Faouzi et al.,

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1999; Kambia et al., 2001; Calafat et al., 2004; Green et al., 2005; Weuve et al., 2006). The second one measured the amount of DEHP that is released from medical devices into the physiologic medium with which the devices come into contact (Hanawa et al., 2003; Kambia et al., 2003; Han et al., 2005; Ito et al., 2005; Takehisa et al., 2005; Takatori et al., 2008).

So far, however, no literature data are available on the DEHP release from endotracheal tubes as a consequence of their *in vivo* applications in high risk newborns.

The purpose of this study was to verify through a chemical–physical characterization the occurrence of DEHP leakage from endotracheal tubes and the correlation between the release of DEHP and the time of intubation in high risk newborns.

2. Materials and methods

2.1. Materials

DEHP and PVC (Mw = 80.7 kDa) were purchased from Sigma Aldrich.

In the present study, a total of 10 endotracheal tubes [PORTEX, Smith Medical Intl. Ltd., Hythe, Kent, UK; internal diameters (I.D.) of 3.0 mm, 3.5 mm, and 4.0 mm] employed in high-risk newborns were examined. Infants were admitted to the Neonatal Intensive Care Unit (NICU) of the "Perrino" Hospital (Brindisi, Italy) for respiratory distress syndrome (RDS) ($N = 10$; males: 7, females: 3; intubation duration: median 56 h; range: 18–168 h). Virgin ($N = 3$) and used tubes were compared.

2.2. Thermogravimetric Analysis (TGA)

TGA evaluations were performed on about 10 mg of PVC endotracheal tube from 30 °C to 650 °C at 2 °C/min under an 60 mL/min nitrogen flow by using a Thermogravimetric Analyzer TGA Q500 (TA Instruments-Waters Division, Milan, Italy).

All measurements were carried out in triplicate and average data were used for statistical analysis.

2.3. Differential Scanning Calorimetry (DSC)

The DSC analyses were performed by using a Mettler DSC-822 (Mettler Toledo, Milan, Italy) under an 80 mL/min nitrogen flow. The amount of PVC tubes employed was about 10 mg. All samples were subjected to a double cooling–heating cycle at 10 °C/min between −60 °C and 130 °C. The glass transitions temperatures (Tg) were taken at the inflection point of sample devitrification.

All measurements were carried out in triplicate and average data were used for statistical analysis.

2.4. High Performance Liquid Chromatography (HPLC)

The amount of DEHP in used and virgin tubes was measured with HPLC using a reverse phase C18 column (Discovery C18 25 mm × 4.5 mm) operated at room temperature. The eluent was monitored at 270 nm with a Waters 486 Tunable Absorbance Detector. The separation was performed with a mobile phase consisting of acetonitrile/methanol mixture (9:1, v/v) pumped at a flow rate of 0.8 mL/min and chromatograms were analyzed with IgorPro software (Wavemetrics, USA).

A calibration curve was constructed using the area under the curve (AUC) method. Standard solutions containing graded amounts of commercial DEHP in hexane were used to prepare the calibration curve.

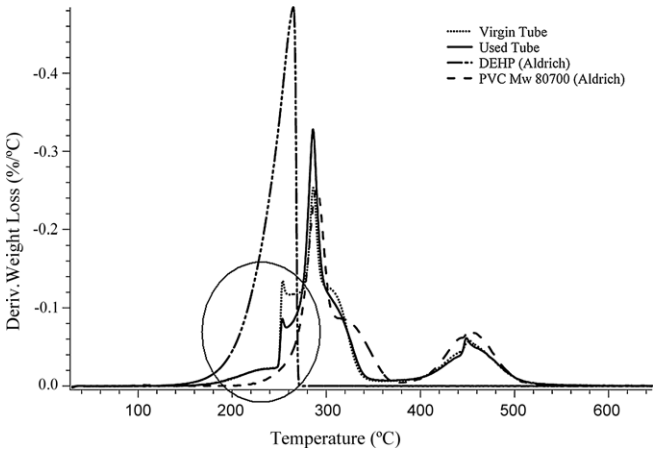

Fig. 1. Traces of the first derivative of weight loss (%ΔW) of virgin tube, used tube, DEHP (Aldrich) and virgin PVC (Aldrich).

2.4.1. Sample preparations

Samples were prepared according to the European Pharmacopoeia Guidelines as described by the literature (Aignasse et al., 1995): 1 g of finely cut pieces of material was introduced into a glass tube (15 mL) and extracted at room temperature with 10 mL of hexane for 15 min.

2.5. Data analysis

Results of DSC and TGA investigations were tested for statistical significance by calculating standard deviation at 95% confidence intervals using IgorPro software (Wavemetrics, USA). Relative weight loss percent (%ΔW) associated to DEHP as calculated by TGA was also analyzed by the *t*-test for unpaired data.

3. Results and discussion

3.1. Thermogravimetric Analysis

The TGA analyses were employed to determine the weight change of decomposition reactions, which allowed for quantitative composition analyses.

In a previous study (Latini et al., 2009) we reported the results of TGA analyses of PVC-DEHP tubes performed with a heating rate of 10 °C/min. In the present work, in order to improve the resolution of TGA traces, analyses were performed with a slower heating rate of 2 °C/min. Under this condition a better separation of the decomposition peaks of endotracheal tubes components was achieved, thus allowing for a more accurate determination of tubes composition.

In particular, the obtained curves showed four steps of decomposition (Fig. 1): the first and the second peak indicate DEHP evaporation as confirmed by the degradation trend of commercial DEHP taken as control. The other two steps correspond to PVC decomposition. In agreement with the decomposition trace of virgin PVC, initially dehydrochlorination forms HCl and polyene structures. During this phase, benzene and some naphthalene and phenanthrene are also formed through Diels Alder reactions and successive dealkylation of polyene molecules. Then, when Cl has been quantitatively released from the melt, the polyene molecules rearrange and through cyclization and cross-linking reactions, form alkyl aromatic hydrocarbons and char residues (Marongiu et al., 2003).

By comparing the area of the two peaks of evaporation of DEHP of used and virgin sample, it was evident that the second peak of evaporation of DEHP in case of virgin tube was more intensive and showed a different trend to that of the used tubes (Fig. 1).

Table 1
TGA value of virgin (control 1–3 samples) and used (1–10 samples) tubes.

Sample	T_{onDEHP}[a] (°C)	$T_{en\ DEHP}$[b] (°C)	DEHP amount ΔW[c] ± S.D. (%)	Diameter (mm)	Intubation time (h)
1	160	258	10.4 ± 0.9	3.0	18
2	160	259	13.7 ± 0.7	3.0	19
3	160	257	11.8 ± 0.2	3.0	52
Control 1	**160**	**270**	**17.9 ± 1.5**	**3.0**	**–**
4	160	261	13.7 ± 0.5	3.5	22
5	160	258	10.2 ± 0.6	3.5	29
6	160	259	10.9 ± 1.0	3.5	29
7	160	259	10.0 ± 0.2	3.5	96
S	160	260	12.5 ± 0.5	3.5	127
9	160	260	10.2 ± 0.9	3.5	168
Control 2	**160**	**277**	**24.4 ± 1.9**	**3.5**	**–**
10	160	258	9.6 ± 0.5	4.0	100
Control 3	**160**	**270**	**23.4 ± 2.4**	**4.0**	**–**

[a] T_{onDEHP} = onset temperature of the first derivative of the peak of DEHP.
[b] $T_{en\ DEHP}$ = end temperature of the first derivative peak of DEHP.
[c] ΔW = weight loss.

The different behaviour can be explained by assuming that the used tubes have undergone a partial dehalogenation of PVC during the use, due to the contact with biological fluids but also to the oxygenation to which the used sample were subjected. The partial dehalogenation results in double bond formation and as a consequence in a low interaction between PVC and DEHP. DEHP would be less tied to PVC in used tube in relation to the virgin one, and it evaporates more quickly.

On the assumption made, the weight loss percent of DEHP from used and virgin tubes was calculated. The obtained values were reported in Table 1, where they were classified according to the diameters of tubes.

The data obtained showed that the used tubes had a weight loss percent of DEPH on average lower than the corresponding virgin tubes. In particular the used tubes contained a quantity of DEHP corresponding to an average 11.3% DEHP, while the virgins tubes had an average of 22% with a difference between the two means statistically significant at $p < 0.001$. These results indicate the occurrence of plasticizer release from tubes as a consequence of their medical application.

Taking into account the intubation time as a variable, the obtained data showed (Table 1) that the release of DEHP occurs within the first 24 h of application of the endotracheal tubes, probably due to a partial alteration of the material following intubation thus leading to a weaker interaction between PVC and DEHP. The lipophilic nature of DEHP and its weak interaction with the polymer matrix make the release of DEHP and its accumulation in tissue a favourable and rapid process.

3.2. Differential Scanning Calorimetry analysis

Additional information about the release of DEHP from PVC tubes has been obtained by DSC analysis.

DSC analyses were employed to determine the glass transition temperature (Tg) of the samples. Tg is a characteristic temperature for each virgin polymer that is essential for our studies since it decreases with the addition of plasticizer into the virgin polymer. An increase in the Tg values of used tubes should be observed as a result of a loss of plasticizer from tubes.

The Tg values were determined at the inflection points of sample devitrification (Fig. 2) and are reported in Table 2, according to the diameter of tubes.

The obtained results showed an increase in the Tg value of tubes after the use, thus indicating a release of plasticizer from tubing after their application.

From DSC investigation an other important information regarding the nature of endotracheal tubes was also obtained. On the basis

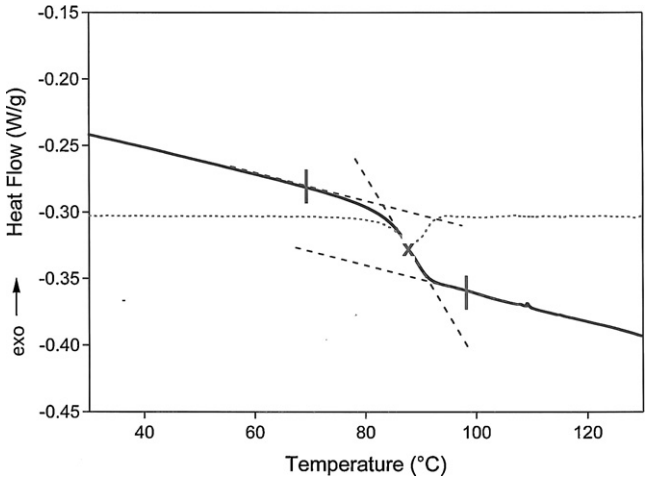

Fig. 2. DSC trace of a PVC sample (Aldrich) and determination of Tg.

of TGA investigation it was indicated that the composition of virgin tubes was about 77% of PVC and 23% of DEHP. Taking into consideration this particular type of composition of virgin tubes, the experimental Tg values were compared with the theoretical values, calculated using Fox equation. The experimental Tg value were in general 35 °C lower than the theoretically ones (Table 3).

In agreement with the data reported by the literature (Brandrup and Immergutand, 1989), results indicated that DEHP did not form a simple miscible intimate blend with PVC, but instead gave rise to a heterogeneous mixture.

Table 2
Tg values of used and virgin endotracheal tubes.

Sample	Tg ± S.D. (°C)	Diameter (mm)	Intubation time (h)
1	−6.5 ± 1.1	3.0	18
2	−13.4 ± 1.2	3.0	19
3	−13.0 ± 1.8	3.0	52
Control 1	**−18.0 ± 1.4**	**3.0**	**–**
4	−12.9 ± 1.1	3.5	22
5	−9.5 ± 1.6	3.5	29
6	−10.1 ± 1.3	3.5	29
7	−9.4 ± 1.5	3.5	96
8	−5.6 ± 1.9	3.5	127
9	−11.2 ± 1.2	3.5	168
Control 2	**−15.5 ± 1.2**	**3.5**	**–**
10	−4.9 ± 1.8	4.0	100
Control 3	**−16.8 ± 1.1**	**4.0**	**–**

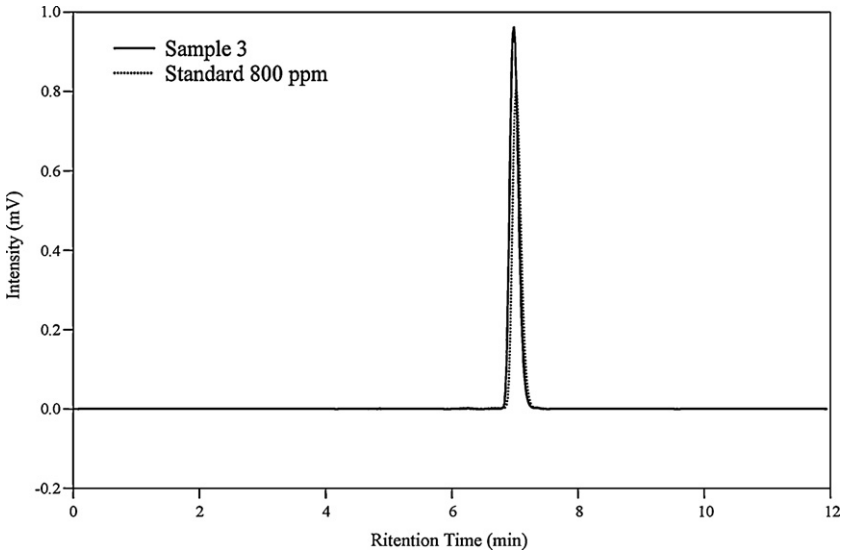

Fig. 3. Typical HPLC chromatograms of endotracheal tube and DEHP standard.

The presented result further confirms that DEHP is not effectively dispersed and bound to PVC, thus underlining once again the high capacity of plasticizer molecules to diffuse throughout the polymer matrix. This justifies, once again, the occurrence of DEHP release in the early hours of intubation.

The data reported in Table 3 were obtained using Fox equation (Eqs. (1)–(3)).

$$\frac{1}{Tg_c} = \frac{W_{PVC}}{Tg_{PVC}} + \frac{W_{DEHP}}{Tg_{DEHP}} \quad (1)$$

$$W_{PVC} = 1 - W_{DEHP} \quad (2)$$

$$Tg_c = \frac{Tg_{PVC} \cdot Tg_{DEHP}}{W_{PVC} \cdot Tg_{DEHP} + W_{DEHP} \cdot Tg_{PVC}} \quad (3)$$

3.3. High Performance Liquid Chromatography analysis

HPLC technique was used to assess the content of DEHP in used and virgin tubes according to the protocol reported by the International Pharmacopoeia Guidelines (European Pharmacopoeia, 1993).

To obtain the solutions of DEHP from the endotracheal tubes as indicated by the protocol of the International Pharmacopoeia, the tubes were cut into small pieces, then they were transferred into a glass tube containing hexane as solvent for extraction. In particular, 10 mL of hexane were employed per 1 g of endotracheal tube pieces and the mixture was kept under stirring at room temperature for 15 min.

Hexane was chosen as solvent for the extraction not only because it is a good solvent for DEHP, but also because it is a non-solvent for PVC. The extract was diluted and then injected into the column.

In agreement with the procedure describe by the literature (Aignasse et al., 1995), the use of methanol/acetonitrile (1:9, v/v) mixture as eluent allowed for the direct injection of the hexane extracts, which greatly simplified the handling of the sample. Moreover no evaporation step was needed thus limiting the risk of DEHP loss.

A typical chromatogram of the extracted solution from endotracheal tube is reported in Fig. 3.

As shown in the figure, the peak of extraction from endotracheal tube appeared at the same retention time of commercial DEHP taken as standard, thus confirming that the only species extracted from the tubes was DEHP. The analyses were repeated for each used endotracheal tubes and virgin tubes taken as control.

Results showed a lower content of DEHP (average 1039.0 ppm) in the used tubes in relation to the amount found in virgin tubes (average 1138.0 ppm), in agreement with the results obtained through thermal analysis (Table 4).

Table 3
Theoretical value of Tg calculated using the Fox equation related to the change in the amount of DEHP.

% PVC	% DEHP	Tg (°C)
10	90	−74.7
20	80	−64.4
30	70	−52.9
40	60	−40.2
50	50	−25.8
60	40	−9.6
70	30	8.8
75	25	19.1
80	20	30.1
90	10	54.8

Table 4
DEHP concentration in used (1–10 samples) and virgin (control 1–3 samples) endotracheal tubes calculated by HPLC analysis.

Sample	DEHP concentration (ppm)	Diameter (mm)	Intubation time (h)
1	1082.67	3.0	18
2	1117.51	3.0	19
3	1038.07	3.0	52
Control 1	**1140.27**	**3.0**	**–**
4	1013.74	3.5	22
5	1107.19	3.5	29
6	973.02	3.5	29
7	1033.63	3.5	96
8	1085.55	3.5	127
9	899.97	3.5	168
Control 2	**1138.40**	**3.5**	**–**
Control 3	**1135.33**	**4.0**	**–**

4. Conclusion

The present work was devoted to the determination of DEHP release from endotracheal tube as a consequence of their applications in high risk newborns and to evaluate the correlation between the intubation time and the leaching of the plasticizer.

A chemical–physical investigation was carried out. The TGA analysis showed a weight loss percent of DEHP from used tubes lower than the corresponding value of virgin tubes taken as control, indicating a release of DEHP from the tubes as a consequence of their *in vivo* application. Moreover, the analysis displayed that the evaporation of DEHP from used tubes occurred with a different speed in comparison to that of virgin samples, indicating the occurrence of a possible material alteration as a consequence of their use.

The above results were also confirmed by the increase of the glass transition temperature (Tg) of tubes after their application, indicated by DSC analysis. The DEHP concentration in tubes was also evaluated. The HPLC analysis showed a content of DEHP in the virgin tube greater than the value of used tubes, displaying a trend in agreement with that observed by thermal analysis.

Another important aspect of the release of DEHP from the tubes was assessed. In particular, a correlation between the time of intubation of tubes in high risk newborns and the amount of DEHP released from the tubes was evaluated. The thermal analysis showed that the release of DEHP occurs within the first 24 h of intubation. This result is in agreement with the observation that DEHP is weakly linked to PVC and it does not form a homogenous blend with the polymer matrix but a heterogeneous mixture as detected by TGA and DSC analysis respectively and consequently the release process is enhanced and rapid.

In conclusion, the results of this study confirm the occurrence of DEHP release from endotracheal tubes after their application in high risk newborns and at the meantime reveal that the time of intubation is an important factor that can be harmful to the high risk newborn since the tubes start to release the DEHP immediately after their employment.

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