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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

Federica Chiellini [a,*], Marcella Ferri [a], Giuseppe Latini [b,c]

[a] Laboratory of Bioactive Polymeric Materials for Biomedical and Environmental Applications (BIOlab), Department of Chemistry & Industrial Chemistry, University of Pisa, Pisa, Italy
[b] Division of Neonatology, Perrino Hospital, Brindisi, Italy
[c] Clinical Physiology Institute, National Research Council of Italy (IFC-CNR), Italy

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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.,

* Corresponding author at: Laboratory of Bioactive Polymeric Materials for Biomedical and Environmental Applications (BIOlab)-UdR INSTM, Department of Chemistry & Industrial Chemistry, University of Pisa, Via Vecchia Livornese, 1291, 56010 San Piero a Grado, Pisa, Italy. Tel.: +39 050 2210305; fax: +39 050 2210332.
E-mail address: federica@dcci.unipi.it (F. Chiellini).

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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.

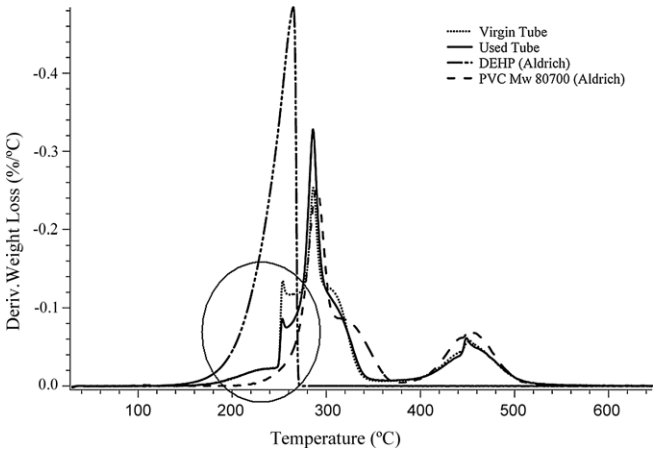

Fig. 1. Traces of the first derivative of weight loss (%ΔW) of virgin tube, used tube, DEHP (Aldrich) and virgin PVC (Aldrich).

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).