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► To cite this version:

Yassine Bouattour, Mathieu Wasiak, Lise Bernard, Jérémy Pinguet, Damien Richard Richard, et al.. Quantification of bis(2-ethylhexyl) phthalate released by medical devices during respiratory assistance and estimation of patient exposure. *Chemosphere*, 2020, 255, pp.126978. 10.1016/j.chemosphere.2020.126978 . hal-02870578

HAL Id: hal-02870578

<https://hal.science/hal-02870578>

Submitted on 20 May 2022

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Quantification of bis(2-ethylhexyl) phthalate released by medical devices during respiratory assistance and estimation of patient exposure

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1 **Abbreviations**

2	ANSM	French national drug safety agency
3	ARMED	Assessment and Risk Management of Medical Devices in plasticized
4		polyvinylchloride
5	BBP	Benzyl butyl phthalate
6	d	Day
7	DEHP	Bis(2-ethylhexyl) phthalate
8	ECMO	Extracorporeal Membrane Oxygenation
9	GC-MS	Gas chromatography coupled to mass spectroscopy
10	ICU	Intensive care unit
11	INRS	National Research and Safety Institute
12	Kc	Conservation yield
13	Kt	Recovery rate
14	LC-MS/MS	liquid-chromatography tandem mass spectrometry
15	LOD	Limit of detection
16	LOQ	Limit of quantification
17	MEHP	Mono(2-éthylhexyl)phthalate
18	MD	Medical device
19	NICU	Neonatal intensive care unit
20	NIOCH	The National Institute for Occupational Safety and Health

21	PVC	Polyvinyl chloride
22	SAV	Secured air valve
23	SCENIHR	Scientific Committee on Emerging and Newly Identified Health Risks
24	SD	Supplementary data
25	SpO2	Peripheral oxygen saturation
26	RSV	Relative standard deviation
27	TAT	Tapered air tap
28		

Introduction

Polyvinyl chloride (PVC) is a material widely used in medical devices (MDs). Plasticisers are added to improve its flexibility, which makes it possible to design soft, resistant and transparent tubings. The concentration of plasticizers generally varies between 20 and 40% of the total weight of PVC (Bernard et al., 2015a). However, since they are not covalently bonded to the PVC matrix, they can easily migrate from the MD and come into contact with patients during certain medical procedures (Eckert et al., 2016). The 2008 edition of the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) report, updated in 2015 (SCENIHR, 2016), highlighted the risks associated with the use of PVC containing bis- (2-ethylhexyl) phthalate (DEHP) as a plasticizer. Indeed, DEHP is classified as a CMR1b compound (carcinogenic, mutagenic or toxic for reproduction) according to the European regulation 1272-2008, with the hazard rating H360 (former EU 2 classification): “toxic for reproduction and development”. Its use in PVC devices has therefore been questioned by the European authorities (*European Union, DIRECTIVE 2007/47/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of September 5th, 2007*). The European Regulation 2017/745 of the European Parliament and the Council of April 5th 2017 on MDs specifies that, unless justified, MDs must not contain concentrations greater than 0.1% in mass fraction (m/m) of carcinogenic, mutagenic or toxic substances for reproduction category 1A or 1B, or substances with endocrine disrupting properties, for which it is scientifically proven that they may have serious effects on human health (*Regulation (EU) 2017/745 of the European Parliament and of the Council of 5 April 2017 on medical devices, 2017*). The specifications of this Regulation are applicable by 26th May 2020 at the latest.

DEHP toxicity is particularly dreaded in newborns because of their low metabolic and detoxification capacity, their low body weight and their organic immaturity, especially in premature infants (Ku and Smith, 2015). Several studies have been conducted to evaluate

newborns exposure to DEHP via MDs. Loff *et al* (Loff et al., 2000) concluded that the amount of DEHP released by DEHP plasticized PVC infusion lines is particularly at risk for these vulnerable patients. In 2018, a study showed that, despite the decrease in urinary concentration of DEHP metabolites in intensive care unit (ICU) infants compared with other earlier cohorts, these amounts remained higher than those of non-hospitalized newborns (Stroustrup et al., 2018). In another study, a significant correlation between DEHP metabolites and the use of ventilation devices was found, including non-invasive ventilation (Stroustrup et al., 2020). Strømme *et al* also showed (Strømme et al., 2016) that high levels of DEHP metabolites in hospitalized newborns are significantly correlated with the duration of mechanical ventilation.

The ARMED[®] study, coordinated by the University Hospital of Clermont-Ferrand and funded by the French national drug safety agency (ANSM), aimed to assess and manage the risks associated with PVC plastic MDs. While DEHP migration has been studied in infusion (Takehisa et al., 2005), transfusion (Buchta et al., 2005) and cardiopulmonary bypass MDs (Fernandez-Canal et al., 2018), no study has been able to quantitatively estimate neonates exposure to DEHP via respiratory assistance devices.

This work aims to develop an *ex vivo* model to quantify the doses of DEHP released by the plasticized PVC MDs. After validation, this model will be used to estimate exposure doses of newborns to DEHP via these MDs.

1. Material and methods

1.1. Instruments and reagents

For DEHP quantification in air, we used ORBO[™] -23 2-HMP cartridges topped with XAD2[®] resin (20/40) 120/60 mg manufactured by Supelco[®] (Ref: 20257-U, Clermont-

Ferrand, France). Extraction was realised with Branson 1200 ultrasonic (Emerson, Rungis, France) and Visiprep™ Supelco cartridge vacuum manifold (reference 57030U Sigma-Aldrich, St. Louis, USA). For the simulation, we used a ball flow meter (reference IU 024, Air Liquide Santé, France), humidifier (Reference 2221, Covidien, France) and a 1 m length oxygen cannula containing DEHP with internal diameter of 3 mm (Reference: 13320, Covidie, France). To quantify DEHP, we used a Clarus 500 GC–MS chromatograph (Perkin Elmer, USA) using electronic impact ionisation tuned to 70 eV and an Optima 5 Accent, 5% diphenyl 95% dimethylpolysiloxane (30 m × 0.25 µm × 0.25 mmID) capillary column (Macherey-Nagel, Germany).

DEHP metabolites quantification was realized using a Prominence UFLC (Shimadzu, Marne la Vallée, France) equipped with a SIL-20AC XR autosampler, a LC-20AB module, two LC-20 AD XR pumps, two FCV-11AL reservoir switching valves, two FCV-12AH 6-port switching valves, a CTO-20AC column oven, two DGU-20A3 on-line solvent degasser and a CBM-20A system controller. A precolumn in-line filter (0.5 µm, Thermo Fisher Scientific, Illkirch, France) was inserted between the injector and the switching valve to extend the lifetime of the columns. Two TurboFlow® Cyclone 0.5 × 50 mm columns (Thermo Fisher Scientific) were placed at the pump outlet to avoid contamination between the LC-20AB pump outlet and the switching valve and between the LC-20AD XR pump outlet (pump A) and the solvent mixer. Mass spectrometer was a hybrid triple quadrupole/linear ion trap QTRAP 5500 (Sciex, Framingham, MA, USA) with a Turbo V IonSpray ionisation source.

As for reagents, we used 99.99% purity chloroform (CAS N° 67-66-3 Fisher Scientific, Illkirch-Graffenstaden, France), DEHP (CAS N° 117-81-7, Honeywell Fluka, Illkirch-Graffenstaden, France), 99.8% purity ethanol (CAS N° 64-17-5, VWR, Briare, France), benzylbutylphthalate (BBP) (CAS N° 85-68-7 Sigma Aldrich, Saint-Louis, United States).

1.2. Methods and study design

To set up a model for estimating exposure to DEHP released by respiratory MDs, we started by validating a quantification method for DEHP present in a gaseous vector following the recommendations of the French National Research and Safety Institute (INRS) presented in supplementary data (SD) 1 (Guide méthodologique MétroPol - Publications et outils - INRS, 2015). This method then allowed us to analyse the amount of DEHP released by a MD of respiratory therapy in the conditions closest to reality by considering surrounding factors such as humidification and flow rate used in clinical situations. We then designed a simulation model of respiratory therapy to estimate released DEHP doses. Finally, the model application was realised on two patients undergoing two different respiratory assistance procedures, to estimate DEHP inhaled quantities. These quantities were then correlated to DEHP metabolites quantified in the patients' urines.

1.3.Validation of DEHP quantification in air

For DEHP quantification, we used the gas chromatography coupled to mass spectroscopy (GC-MS) method developed and validated by Bourdeaux *et al* (Bourdeaux et al. 2016), which presents a mean accuracy of $98.4 \pm 2.8 \%$, repeatability's relative standard deviation (RSD) of 6.47% and intermediate precision's RSD of 7.5% , with a limit of detection of $0.03 \mu\text{g mL}^{-1}$ and a limit of quantification (LOQ) of $0.1 \mu\text{g mL}^{-1}$. It allows DEHP quantification from 0.1 to $10 \mu\text{g mL}^{-1}$ with a determination coefficient R^2 higher to 0.997 . We also used this method to verify DEHP absence in reagents. To avoid contaminations, glassware was cleaned before use through repeated washing steps with water and hexane. For each experiment, we realised the exact same experiment process using DEHP free ethanol to verify the absence of potential DEHP contamination coming from materials or other reagents. Blank samples processed in identical way as real samples did not show any signs of contamination. All experiments of the validation process were realised at $23 \pm 1^\circ\text{C}$ in triplicate in one experiment per day during 3 days to verify the intermediate precision as expressed by the RSD, in percentage.

128 • Selection of the sampling media

129 The sampling of DEHP in the air during the validation process and during the exposure
130 simulation was performed using XAD[®] cartridge. The choice of this support was based on the
131 work of Das *et al* (Das et al., 2014) and Cecinato *et al* (Cecinato et al., 2012)

132 • Selection of the desorption solvent

133 Chloroform extraction was chosen based on the work of Bernard *et al* (Bernard et al., 2015b)
134 showing the superiority of chloroform in extracting DEHP from solid polymers compared to
135 other solvents.

136 • Validation of DEHP extraction from cartridges

137 In order to validate the extraction method of DEHP from the collection cartridges, we
138 proceeded to load the cartridge with 5 µg of DEHP using 20 µL of a 250 µg mL⁻¹ solution of
139 DEHP in ethanol, introduced with a micropipette. The cartridges were then dried under
140 vacuum using a cartridge vacuum manifold for 10 hours. After drying, these cartridges were
141 immersed in hermetically sealed glass tubes filled with 10 ml of chloroform. Next, we tested
142 two extraction methods:

143 - Method A: Heat Extraction: The tubes containing the cartridges with 10 mL of chloroform
144 were heated at 55° C for 72 hours.

145 - Method B: Ultrasonic extraction: The tubes containing the cartridges with 10 mL of
146 chloroform were placed in an ultrasound bath for 14 hours. At the end of the extraction, the
147 bath temperature was of 35°C.

148 At the end of the extraction, a 900 µL sample was taken to which the internal standard was
149 added, i.e. 100 µL of a chloroform solution BBP at 20 µg mL⁻¹. The addition of the BBP
150 results in a 9/10th dilution which will be considered when calculating the quantities of DEHP

from the measured concentrations. We calculated the recovery rate from the initial loaded quantity (5 µg). The extraction method was considered valid if the recovery rate was greater than 90%.

- Verification of the air used during validation of the DEHP air quantification

Medical air was chosen preferentially to oxygen because of the explosive risk of the latter and because medical air is the first-line gas used in NCIU (Olasveengen et al., 2017). We therefore used medical air for all validation and simulation stages. To verify air quality and ensure that it was DEHP free, we directly connected in series two cartridges to two different air supply points: a tapered air tap (TAT) with a conical connection allowing the direct connection of a glass tube which will be later used for the validation DEHP capture by the cartridge; and a secured air valve (SAV) with secure connection connectivity, similar to connectivity available in clinical units, for connection to a ball air flow meter and humidifier. The sampling media were mounted at these two feeding points for six days (see SD 2a and 2b). For the SAV, we checked the air with and without a humidifier at flow rate of 5 L min⁻¹ measured using a ball flow meter. The choice of flow was based on the fact that the flow rate used for ventilation in premature infants and neonates starts at 1 L kg⁻¹ min⁻¹ and can vary from a flow rate of 0.5 L min⁻¹ to 5 L min⁻¹ in function of the pulsed oxygen saturation (SpO₂). The DEHP captured was subsequently extracted from the cartridges using the method B described previously, since it has better extraction yield.

- Verification of DEHP sorption from the air

In order to verify that all DEHP carried by the medical air was captured in the sampling cartridge, two types of experiments were performed:

- Volatilisation of a known quantity of DEHP and capture by the cartridges: during these experiments, 20 µL of an ethanolic solution containing 10 mg mL⁻¹ (200 µg) of DEHP were

deposited in the middle of a glass tube (see SD 2c). The tube was connected on one side directly to the TAT for 6 days during which the air passed through the tubing and on the other side to the 2 cartridges mounted in series. The second cartridge was intended to collect the DEHP which could be released by the first cartridge, in accordance with the recommendations of INRS. At the sixth day, an extraction using method B and DEHP quantification was performed. In order to evaluate the amount of non-volatilised DEHP, a chloroform rinse of the glass tube was performed and the potential residual DEHP was researched and quantified. We performed a 1/100th dilution of the rinsing solution in a chloroform solution of BBP at 2 µg mL⁻¹ so that the measured concentration fell within the calibration range. The amount of DEHP volatilized was estimated by calculating the difference between the amount deposited into the glass tube before and after volatilisation and this quantity was compared to the amount of DEHP trapped in the cartridge.

- Verification of the absence of DEHP diffusion through the sampling cartridges implying a loss of DEHP: this step was carried out using a cartridge loaded with a known DEHP amount. We used the SAV on which a ball flow meter was connected. Two quantities were studied: 1.20 and 20.00 µg of DEHP contained in 20 µl of ethanol. The choice of the 1.20 µg quantity of DEHP was linked to the LOQ of the method: it is the minimum quantifiable concentration (0.1 µg mL⁻¹) multiplied by the volume of extraction with chloroform (10 mL) and divided by the dilution factor of the internal standard solution is 0.9, the result (1.11 µg) finally rounded up to 1.2 µg. The loaded cartridges were mounted directly on the SAV and coupled with an unloaded cartridge in series. The second cartridge was intended to collect the DEHP which could be released by the loaded cartridge. In order to check if the humidification of the air caused a loss of retention of DEHP on the cartridges, we carried out these experiments with and without a humidifier. For the non-humidifier tests, we mounted an guard cartridge before the DEHP loaded cartridge to remove any DEHP contamination that could occur from the

SAV (SD 2d). The medical airflow passed through the cartridges for 3 days at a rate of 5 L min⁻¹. After 3 days, an extraction was performed on each cartridge followed by DEHP quantification.

1.4. Validation of the DEHP exposure model by respiratory device

- Design of the DEHP Exposure Model via Respiratory Support Devices

In order to simulate the use of respiratory therapy devices in clinical situations, an experimental model comprising a medical air source with SAV, a ball flow meter, a PVC oxygen cannula containing DEHP and a cartridge for DEHP collection at the end of the tubing was set-up. This experimental apparatus was tested with and without a humidifier connected between the flow meter and the respiratory therapy cannula. When no humidifier was used, we put a guard cartridge between the flow meter and the PVC tubing to remove DEHP contamination from the SAV (SD 3).

- Simulation of respiratory assistance

All experiments were performed in an air-conditioned room at a temperature of 23 ± 1 °C. The designed model was implemented during 6 days. DEHP quantification was performed after 1, 3 and 6 days of contact between the air flow and a PVC cannula, by removing the cartridge from the device and replacing it with a new one until the end of the experiment. DEHP was desorbed from the removed cartridge using method B as previously described. Two airflow rates were tested, 2 and 4 L min⁻¹ and for each flow, experiments were performed with and without humidifier, in triplicate.

- Expression of results and exposure model presentation

DEHP quantification made it possible to measure, at each time (day 1, day 3 and day 6), the amount of DEHP released by 1 meter of PVC tubing corresponding to a contact area of 0.942 dm². The quantity of DEHP released by dm² will be equal to:

$$Q = \frac{(C \times V_e)}{(K \times S \times d)}$$

224

225 With Q: quantity of DEHP released by surface of PVC expressed in $\mu\text{g per dm}^2$

226 C: concentration of DEHP extracted from the cartridge obtained by GC-MS in $\mu\text{g mL}^{-1}$

227 d: 9 / 10th dilution with BBP internal standard solution

228 V_e : Chloroform extraction volume equal to 10 mL

229 K: Extraction coefficient, if K higher than 0.95, it will be rounded up to 1

230 S: Area corresponding to the MD surface used during the simulation (0.942 dm^2).

231 The quantities dosed were cumulated on day 3 and day 6 with those of the previous days to
 232 estimate the total quantity released at each measuring time in order to determine flow rate and
 233 humidification impact on the release kinetics. By plotting the cumulated quantity per surface
 234 in function of time ($f(t)$), we established the quantity release curve variation per area and per
 235 time.

236 1.5. Model application

237 As a model application to estimate the DEHP amount inhaled, we applied the equation
 238 corresponding to the humidified condition at 2 L min^{-1} to two randomly chosen patients
 239 hospitalized in the NICU in the hospital of Clermont-Ferrand, one was under invasive
 240 ventilation procedure through an endotracheal tube, and the other was undergoing a non-
 241 invasive ventilation using an oxygen canula. These patients were included in the ARMED
 242 clinical study which had been approved by the ethical committee (CPP Sud-Est VI, AU
 243 1082). The total surface of PVC containing DEHP and the use time of the MD was collected

using a case report form (SD 4). Plasticizers' concentration in the used MDs was determined by GC-MS using Bourdeaux et al method (Bourdeaux et al. 2016). DEHP metabolites quantification in the correspondent two patients' urine was realised using Pinguet et al liquid-chromatography tandem mass spectrometry method (Pinguet et al., 2019) as following: each day at 8:00 AM, a urine sample was withdrawn from a non-PVC bottle into which the collection bag used to collect the standard urinary drainings was emptied 6 times a day for each patient. We estimated the inhaled amount of DEHP, following this formula:

$$A = f(t) \times S$$

In which

A: Estimated inhaled amount of DEHP in μg

S : PVC surface to which the patient was exposed via the respiratory assistance circuit, expressed in dm^2

t : Use duration in day.

The calculated amount was then compared to the sum of DEHP specific urinary metabolites for these two patients, i.e. Mono(2-ethylhexyl)phthalate (MEHP), 5-oxoMEHP, 5-HydroxyMEHP, 5-CarboxyMeMEHP and 2-CarboxyMeMEHP, in order to correlate exposure with the metabolites.

1.6. Statistical tests

Statistical analyses were performed using Stata statistical software (version 13, StataCorp, College Station, US). The tests were two-sided with type I error set at 5%. Continuous parameters were expressed as mean and standard-deviation according to the statistical distribution. The assumption of the normality was studied using the Shapiro-Wilk test. To

carry out longitudinal analyses, correlated repeated data were analysed using linear mixed models for (1) quantity of plasticizers released during migration tests from PVC tubing and (2) migration kinetics of DEHP. Such statistical approach was preferred and considered as more relevant, because the data independence assumption was not satisfied. Residuals normality was studied as aforementioned. If appropriate, a logarithmic transformation was proposed to achieve dependent variable normality.

2. Results

2.1. Validation of the quantification method of DEHP in air

Results of the validation of DEHP quantification in air are summarised in SD 5

- Validation of DEHP extraction from cartridges

An improvement in DEHP extraction was noted with the use of the ultrasonic bath compared to the thermal extraction at 55° C. As well as allowing an average extraction efficiency of more than 98%, the ultrasonic method was shorter than the heat method (14 hours versus 72 hours). Method B was therefore used for all subsequent experiments. The blank test showed no DEHP contamination.

- Verification of the medical air used during validation of the DEHP quantification

SAV analysis showed that the medical air was contaminated with DEHP and the use of a guard cartridge or a humidifier eliminated all contaminating DEHP.

- Verification of DEHP sorption from the air

After volatilisation for 6 days of 200 µg of DEHP using air, cartridge showed effectiveness of DEHP trapping of 102.6%. The amount of DEHP deposited revealed a volatilised DEHP level of 1.73% with an RSD of 8.61%. Estimated loss from the cartridges after 3 days was of 4.63%

for a 20 µg spiked quantity when a humidifier was used, while it was of about 1% for the 20 µg loaded cartridges without humidifier and for the 1.2 µg loaded cartridges, with and without humidifier.

2.2. Validation of the DEHP exposure model by respiratory device

- *Ex vivo* model: quantification of DEHP released by PVC tubing

The analysis of the air passing through the PVC tubing of an oxygen canula showed that the release of DEHP varied depending on the flowrate and the presence or absence of humidification. Figure 1A shows the different amounts of DEHP released over time during 6 days, depending on the parameters studied. Based on these results, the lowest released amount was of 29.61 µg in 6 days (cumulative quantity) and was found for an experimental setup using a humidifier with a flowrate of 4 L min⁻¹. DEHP amount in the air at 4 L min⁻¹ without humidifier was the largest, reaching 122.87 µg on the sixth day. For the flow rate of 2 L min⁻¹, we note that the use of a humidifier caused a decrease in the quantities of DEHP released on day (D) 1 and D 3, but the quantities on D 6 remains comparable. Regarding the impact of the flow, we note that the increase in the flow rate led to a decrease in the quantities of DEHP in the presence of a humidifier, whereas this quantity increases considerably without humidifier if we go from 2 L min⁻¹ to 4 L min⁻¹. The statistical analysis of the impacts of flow and humidification on released quantities are presented in SD 6a.

The comparison of DEHP concentration in air over time shows that concentrations decrease for all the experiments except for the 4 L min⁻¹ air humidification test where a stability of DEHP concentrations is observed, beginning from 0.87 µg m⁻³ at D 1 up to 0.92 µg m⁻³ at D6. Impacts of flow and humidification on DEHP concentrations in air are presented in Figure 1B and SD 6b (statistical analysis).

314

- 315 • DEHP release model from PVC respiratory devices

316 For all experiments, we established a polynomial correlation between the quantity released by
317 decimetre square of MDs area and the exposure time, with a coefficient of determination R^2
318 higher to 0.9, except 4 L min⁻¹ flow experiment using humidifier in which R^2 was equal to
319 0.86, as shown in Figure 2.

320 2.3. Model application

321 The application of the 2 L min⁻¹ with humidifier model to the two patients in the neonatal
322 intensive care units (NICU) and quantification of DEHP metabolites showed the results
323 presented in Table 1.

324

325 3. DISCUSSION

326 Our study contributes to the evaluation of the risks associated with the use of PVC respiratory
327 MDs in NICU by estimating newborns exposure to DEHP, a plasticizer known for its
328 reprotoxic and endocrine disrupting effects.

329 The validation of DEHP quantification in the gas passing through respiratory MDs is the key
330 step that determines the success of the *ex vivo* experiments and therefore the exposure
331 estimation. We followed the INRS methodology for the development of methods for sampling
332 and analysis of organic gases and vapours in the air. The use of ultrasounds coupled with the
333 chemical extraction allowed us to obtain a yield of 98.5% (compared to only 59.3% by
334 heating at 55° C) for DEHP doped cartridges. This method also allowed a reduced extraction
335 time. Various extraction methods have been described in the literature such as Soxhlet
336 extraction (Net et al., 2015), thermal desorption (Hill et al., 2003) or ultrasounds. The

National Institute for Occupational Safety and Health (NIOSH) reference method advocates the use of a cellulose ester filter as a support coupled with carbon disulfide extraction for 30 minutes using ultrasounds. However, carbon disulfide is toxic and does not allow complete desorption of DEHP. Although Soxhlet extraction is recognized as a traditional method for extracting phthalates from solid samples, it is a tedious and laborious procedure, which lasts between 4 to 72 hours, and which requires large amounts of solvent (100 to 500 mL). On the other hand, Hill *et al* found it difficult to extract all of the DEHP from the Tenax[®] adsorbent cartridges when trying the extraction using a thermal desorption, resulting in higher LOQ, due to the low volatility of DEHP (Hill et al., 2003). Szewczyńska *et al* (Szewczyńska et al., 2020) had a recovery rate ranging between 66% and 88% when extracting plasticizers from XAD-2 resin using 3 mL of dichloromethane/acetone (1:1) as an extraction solvent in an ultrasonic cleaner for 30 min, when in our experiments, we obtained 98.5% recovery rate with chloroform and 14 hours ultrasonic extraction. XAD-2[®] resins advantage is their ability to collect particulate and gaseous DEHP (Das et al., 2014; Szewczyńska et al., 2020).

The verification of two air supply points proved that the conical feeding point did not release any DEHP. In contrast, the secure feed point released DEHP as a contaminant with an average of 1.04 µg day⁻¹ when using a flow rate of 5 L min⁻¹. This contamination could be suppressed by using a humidifier containing sterile water. The effect of a “water trap” for DEHP suppression has been described by Roth *et al* (Roth et al., 1988) who used this method of DEHP trapping via a water reservoir which was subsequently used to estimate exposure to DEHP by respiratory MDs of 3 newborns. The solubility of DEHP in water could vary from 0.0006 to 1.3 mg/L at 20-25°C (Bis(2-ethylhexyl) phthalate (DEHP) - Summary Risk Assessment Report - ECHA, 2008), a solubilisation of 6 µg in a volume of 330 ml of water seems possible. However, this proves that exposure to DEHP may be increased from some air feed line exit if no humidifier is used.

DEHP migration tests in the air passing through the respiratory devices was performed by varying the airflow and using or not a humidifier. The comparison of the released quantities of DEHP after 24 hours of simulation in the different tested situations showed a clearly higher level of DEHP released at 4 L min⁻¹ without humidifier. We note that the total amount of DEHP released continues to increase during the six days of the experiment for all conditions studied. If we look at the flow rate effect, we note that the flow rate increase leads to an increase in the quantities released in the absence of a humidifier, whereas in the presence of a humidifier, increasing the flow rate decreased the quantity released, especially at day 6. The effect of the humidifier however is similar for both flow rates, resulting in a decrease in the amount each time the humidifier is used. Despite the DEHP quantities released, none exceeded the Derived No Effect Level of 130 µg m⁻³ (Bui et al., 2016). The variation study of the DEHP air concentration over time shows a drop in the concentration for all the conditions except for the 4 L min⁻¹ humidified air test. After six days, except for experiment with 4 L min⁻¹ with humidifier, the concentrations were comparable. In clinical conditions, no study was conducted to evaluate plasticizers concentration variations in air as function of the airflow. Flow rate impact on the migration of DEHP and other plasticizers concentrations over time has been studied for a perfusion model of liquid solutions (Bagel-Boithias et al., 2005; Bernard et al., 2018) showing that their concentration in sodium chloride 0.9% remained constant regardless of the flow rate used over 24 hours. For the respiratory model, the decrease in DEHP concentration in the air over time could be explained by a limited exchange between the gas and the surface of the inner lumen of the PVC cannula. One can hypothesise a simple desorption of the DEHP on the surface of the PVC whereas at the time of the contact between a liquid and the PVC the exchanges can be done in depth of the tubes as well as with the surface. The best explanation of the concentration variation in air over time in these experiments was reported by Wei *et al* (Wei et al., 2019), claiming that plasticizer

migration is controlled by two phenomenon, diffusion and evaporation. In fact, they described DEHP volatilisation could be either diffusion-controlled (if diffusion is slower than evaporation) or evaporation-controlled (when evaporation is slower than the diffusion). In our experiments, the high DEHP concentration at day one followed by the remarkable decrease with non-humidified air at 2 and 4 L min⁻¹ and with humidified air at 2 L min⁻¹ suggested that, in these conditions, DEHP extraction is diffusion controlled. On the other hand, the low concentration of DEHP and its steadiness over time with humidified air at 4 L min⁻¹ suggest that in this condition volatilisation is evaporation controlled. The decreasing concentration in air with humidifier could be due to the hydrophobic and low polar property of DEHP (Hsu et al., 2017), limiting its evaporation in presence of water vapor. As reported by Fechter et al (Fechter et al., 2006), humidity could enhance evaporation of polar component such as hexanal, or limit it for non-polar component such as 3-Carene or α -Pinene. Besides, low concentrations found particularly with humidified 4 L min⁻¹ airflow could also be explained, in addition to the humidification, by the affinity law (Forsthoffer, 2011) increasing flow rate leads to increasing pressure in the tubing which could limit DEHP evaporation. However, this effect was not observed in the absence of humidifier, suggesting that airflow impact is only significant when a humidifier is used. Overall, our experiments showed that respiratory DEHP plasticized MD are not a major source of contamination compared to other MD used in clinical unit such as infusion (Takehisa et al., 2005) or transfusion (Buchta et al., 2005).

Model application to two patients in NICU showed that inhaled amount was higher for the patient undergoing non-invasive ventilation, due to the use of a large surface of DEHP plasticized PVC and the long duration of use. The urinary specific biomarkers total amount was also in concordance with the estimated DEHP exposure using the model, knowing that no other DEHP plasticised MDs was used for these patients. However, it is hard to confirm that no other materials containing DEHP were used during hospitalisation in NICU.

Our study validated a method for the sorption and quantification of DEHP volatilised in air and developed an *ex vivo* model for estimating the exposure to DEHP *via* respiratory devices. Although this model considered two parameters that can influence DEHP exposure by respiratory devices such as flow and humidification of the gaseous vector, other parameters could not be studied such as temperature, composition of the air, notably its oxygen concentration which can vary in a clinical situation depending on the patients' oxygen saturation. Indeed, we carried out the extrapolation of the models for tests carried out at 23° C to avoid temperature variation during experiments which could distort results, since air coming from the valve is already at this temperature. Non-invasive ventilation is realised at room temperature, however when used during intubation procedures the respiratory assistance is often performed at 37° C. A temperature of 37° C can significantly increase the amount of DEHP volatilized from the MDS which will then expose more neonates to inhalation of this plasticizer. A correction of the model therefore seems necessary according to the temperature in order to get closer to the quantity inhaled during the clinical situations of respiratory assistance. The realisation of simulation experiments using medical air as a simulant was based on the use of medical air as first line for ventilation of newborns. However, it is common to adjust the oxygen concentration for newborns in case of hypoxia or SpO₂ disturbance. No studies have examined DEHP volatilisation variation as a function of oxygen concentration. In our study we could not perform oxygen tests because of its explosive risk and the inadequacy of the laboratory for this type of test. Since the use of alternative PVC plasticizers, many industries have begun to replace DEHP in respiratory MDs with other plasticizers. It will be useful to test this developed method with respiratory MDs plasticized with other plasticizers such as Tris (2-Ethylhexyl) Trimellitate.

4. Conclusion

This work presents an experimental model for measuring a phthalate, DEHP, released by MDs, in the gas intended to ventilate the neonates in an ICU. We validated a rapid and simple method of determination of DEHP volatilised in a gas matrix. The method was used to study DEHP release from MDs to the medical airway in an experimental setup mimicking the ventilation of newborns, according to the flow and humidification. Our work did not reveal a determining role of the airflow rate on DEHP migration. In contrast, air humidification induced a decrease in DEHP volatilisation in the air passing through the simulation MD, probably due to DEHP low polarity, and therefore air humidifiers could help limit pulmonary exposure to DEHP at room temperature. Overall, our experiments showed that respiratory DEHP plasticized MD are not a major source of DEHP contamination compared to other MD used in clinical situation. Since the increase in temperature could increase DEHP release, it will be necessary to integrate this variable into the model optimisation in order to accurately estimate newborns exposure to DEHP by respiratory MDs.

Acknowledgement

This study is a part of the project ARMED-NEO (Assessment and Risk management of MEDical devices in plasticized polyvinylchloride in NEOnatology) which has received the financial support of the French Medicine Agency (ANSM).

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Figure captions:

Figure 1: Variation of (A) cumulative quantities and (B) concentration of bis(2-ethylhexyl) phthalate in the air passing through PVC tubing over time at 2 and 4 L min⁻¹, with and without humidifier (mean \pm Standard deviation, n = 3).

Figure 2: Bis(2-ethylhexyl) phthalate release over time in different conditions: A) 2 L min⁻¹ with humidifier, B) 2 L min⁻¹ without humidifier, C) 4 L min⁻¹ with humidifier and D) 4 L min⁻¹ without humidifier.

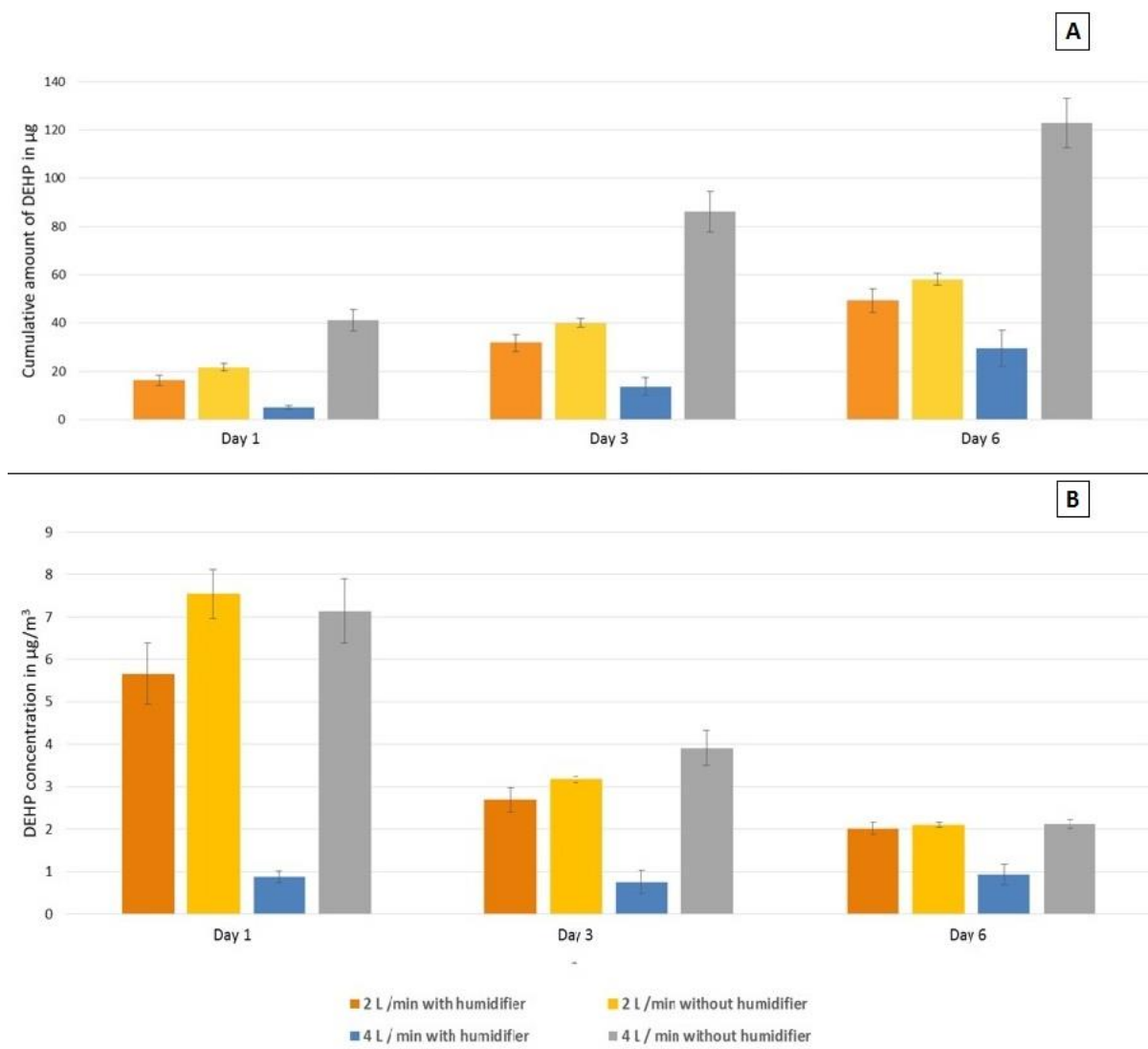


Fig 1

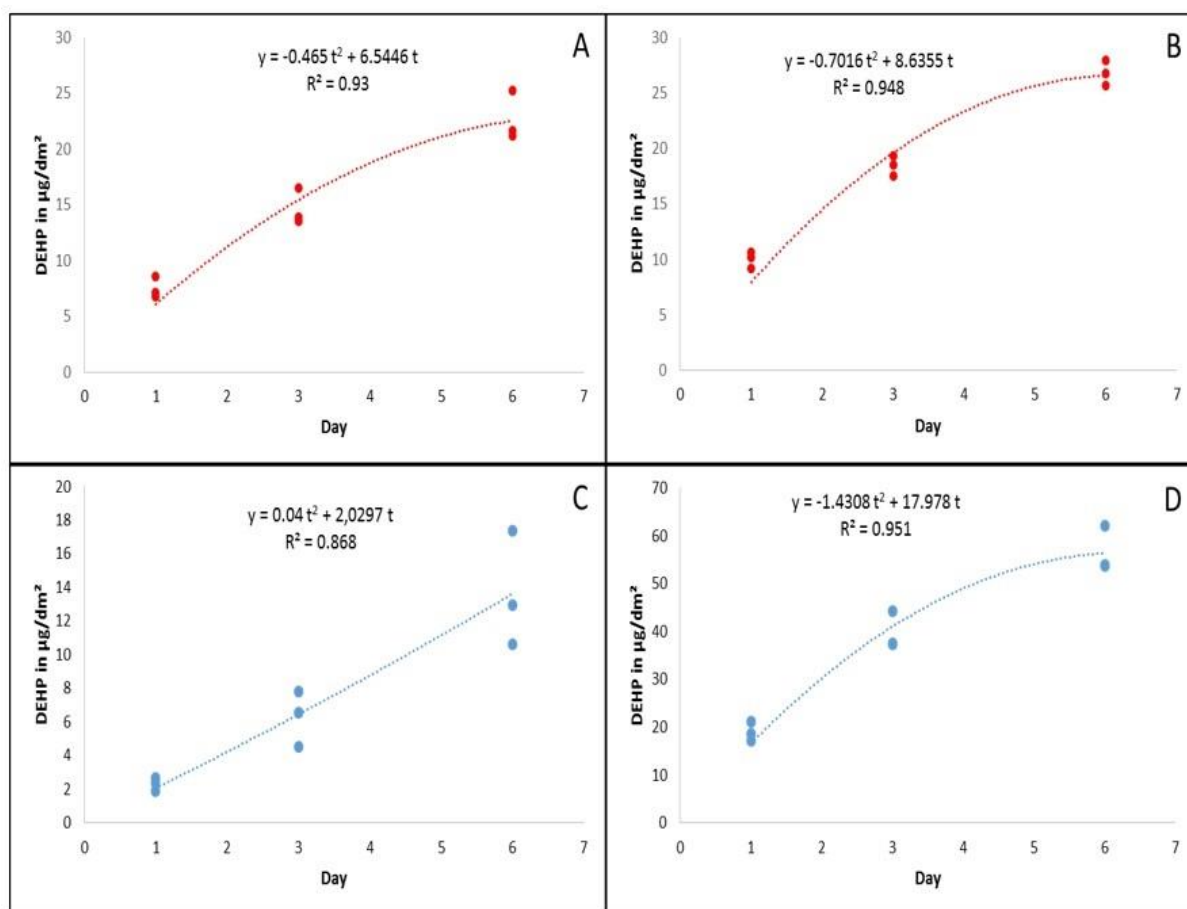
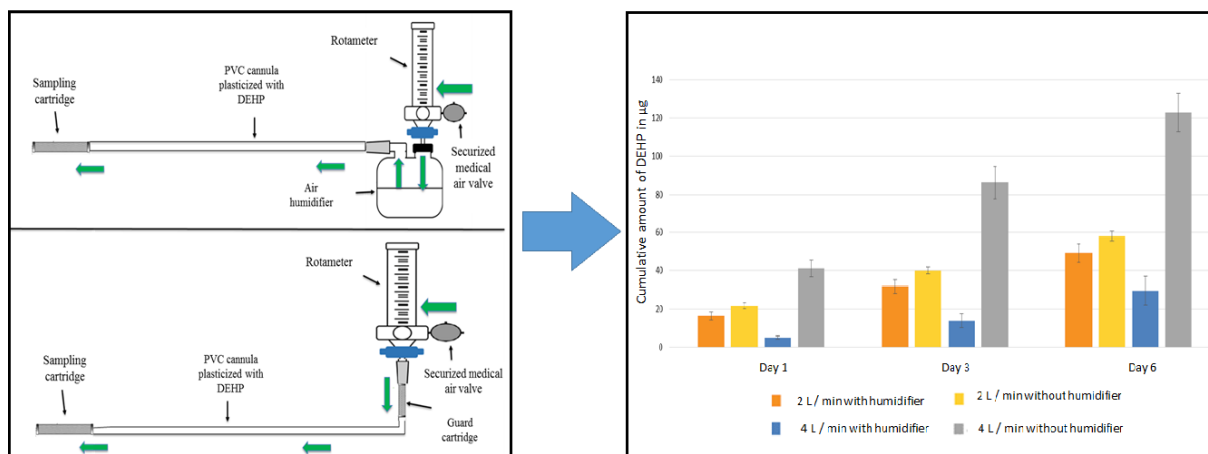


Fig 2

	<i>Patient 1</i>	<i>Patient 2</i>
<i>Inclusion number</i>	028LL	029MS
<i>Monitoring time (day)</i>	2	5
<i>Type of respiratory assistance</i>	Non-invasive	Invasive
<i>Respiratory medical device used</i>	Oxygen cannula EUROCARE reference 0559	Nasal probe COVIDIEN reference 11130
<i>DEHP concentration in medical device (g/g of PVC)</i>	0.238	0.243
<i>Interior surface (dm²)</i>	2.71	0.05
<i>Duration of use (mn)</i>	900	4920
<i>DEHP Release curve equation</i>	$- 0.465 t^2 + 6.5446 t$	$- 0.465 t^2 + 6.5446 t$
<i>Estimated inhaled amount of DEHP (μg)</i>	10.59	0.87
<i>Cumulative DEHP urinary metabolites quantity (ng / μmol of creatinine)*</i>	3516.27	716.43

Table 1 : Model application to two patients in the neonatal intensive care units undergoing two different respiratory assistance procedure. * Data given for comparaison



Graphical Abstract