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

## 29 **Introduction**

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

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

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

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

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

73

## 74 **1. Material and methods**

### 75 1.1. Instruments and reagents

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

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

87 DEHP metabolites quantification was realized using a Prominence UFLC (Shimadzu, Marne  
88 la Vallée, France) equipped with a SIL-20AC XR autosampler, a LC-20AB module, two LC-  
89 20 AD XR pumps, two FCV-11AL reservoir switching valves, two FCV-12AH 6-port  
90 switching valves, a CTO-20AC column oven, two DGU-20A3 on-line solvent degasser and a  
91 CBM-20A system controller. A precolumn in-line filter (0.5 μm, Thermo Fisher Scientific,  
92 Illkirch, France) was inserted between the injector and the switching valve to extend the  
93 lifetime of the columns. Two TurboFlow® Cyclone 0.5 × 50 mm columns (Thermo Fisher  
94 Scientific) were placed at the pump outlet to avoid contamination between the LC-20AB  
95 pump outlet and the switching valve and between the LC- 20AD XR pump outlet (pump A)  
96 and the solvent mixer. Mass spectrometer was a hybrid triple quadrupole/linear ion trap  
97 QTRAP 5500 (Sciex, Framingham, MA, USA) with a Turbo V IonSpray ionisation source.

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

102 1.2. Methods and study design

103 To set up a model for estimating exposure to DEHP released by respiratory MDs, we started  
104 by validating a quantification method for DEHP present in a gaseous vector following the  
105 recommendations of the French National Research and Safety Institute (INRS) presented in  
106 supplementary data (SD) 1 (Guide méthodologique MétroPol - Publications et outils - INRS,  
107 2015). This method then allowed us to analyse the amount of DEHP released by a MD of  
108 respiratory therapy in the conditions closest to reality by considering surrounding factors  
109 such as humidification and flow rate used in clinical situations. We then designed a  
110 simulation model of respiratory therapy to estimate released DEHP doses. Finally, the model  
111 application was realised on two patients undergoing two different respiratory assistance  
112 procedures, to estimate DEHP inhaled quantities. These quantities were then correlated to  
113 DEHP metabolites quantified in the patients' urines.

### 114 1.3. Validation of DEHP quantification in air

115 For DEHP quantification, we used the gas chromatography coupled to mass spectroscopy  
116 (GC-MS) method developed and validated by Bourdeaux *et al* (Bourdeaux et al. 2016), which  
117 presents a mean accuracy of  $98.4 \pm 2.8$  %, repeatability's relative standard deviation (RSD) of  
118 6.47 % and intermediate precision's RSD of 7.5 %, with a limit of detection of  $0.03 \mu\text{g mL}^{-1}$   
119 and a limit of quantification (LOQ) of  $0.1 \mu\text{g mL}^{-1}$ . It allows DEHP quantification from 0.1 to  
120  $10 \mu\text{g mL}^{-1}$  with a determination coefficient  $R^2$  higher to 0.997. We also used this method to  
121 verify DEHP absence in reagents. To avoid contaminations, glassware was cleaned before use  
122 through repeated washing steps with water and hexane. For each experiment, we realised the  
123 exact same experiment process using DEHP free ethanol to verify the absence of potential  
124 DEHP contamination coming from materials or other reagents. Blank samples processed in  
125 identical way as real samples did not show any signs of contamination. All experiments of the  
126 validation process were realised at  $23 \pm 1^\circ\text{C}$  in triplicate in one experiment per day during 3  
127 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<sup>®</sup> 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<sup>-1</sup> 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<sup>-1</sup>. The addition of the BBP  
150 results in a 9/10th dilution which will be considered when calculating the quantities of DEHP

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

154 • Verification of the air used during validation of the DEHP air quantification

155 Medical air was chosen preferentially to oxygen because of the explosive risk of the latter and  
156 because medical air is the first-line gas used in NCIU (Olasveengen et al., 2017). We  
157 therefore used medical air for all validation and simulation stages. To verify air quality and  
158 ensure that it was DEHP free, we directly connected in series two cartridges to two different  
159 air supply points: a tapered air tap (TAT) with a conical connection allowing the direct  
160 connection of a glass tube which will be later used for the validation DEHP capture by the  
161 cartridge; and a secured air valve (SAV) with secure connection connectivity, similar to  
162 connectivity available in clinical units, for connection to a ball air flow meter and humidifier.  
163 The sampling media were mounted at these two feeding points for six days (see SD 2a and  
164 2b). For the SAV, we checked the air with and without a humidifier at flow rate of 5 L min<sup>-1</sup>  
165 measured using a ball flow meter. The choice of flow was based on the fact that the flow rate  
166 used for ventilation in premature infants and neonates starts at 1 L kg<sup>-1</sup> min<sup>-1</sup> and can vary  
167 from a flow rate of 0.5 L min<sup>-1</sup> to 5 L min<sup>-1</sup> in function of the pulsed oxygen saturation (SpO<sub>2</sub>).  
168 The DEHP captured was subsequently extracted from the cartridges using the method B  
169 described previously, since it has better extraction yield.

170 • Verification of DEHP sorption from the air

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

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

175 deposited in the middle of a glass tube (see SD 2c). The tube was connected on one side  
176 directly to the TAT for 6 days during which the air passed through the tubing and on the other  
177 side to the 2 cartridges mounted in series. The second cartridge was intended to collect the  
178 DEHP which could be released by the first cartridge, in accordance with the recommendations  
179 of INRS. At the sixth day, an extraction using method B and DEHP quantification was  
180 performed. In order to evaluate the amount of non-volatilised DEHP, a chloroform rinse of  
181 the glass tube was performed and the potential residual DEHP was researched and quantified.  
182 We performed a 1/100<sup>th</sup> dilution of the rinsing solution in a chloroform solution of BBP at  
183 2 µg mL<sup>-1</sup> so that the measured concentration fell within the calibration range. The amount of  
184 DEHP volatilized was estimated by calculating the difference between the amount deposited  
185 into the glass tube before and after volatilisation and this quantity was compared to the  
186 amount of DEHP trapped in the cartridge.

187 - Verification of the absence of DEHP diffusion through the sampling cartridges implying a  
188 loss of DEHP: this step was carried out using a cartridge loaded with a known DEHP amount.  
189 We used the SAV on which a ball flow meter was connected. Two quantities were studied:  
190 1.20 and 20.00 µg of DEHP contained in 20 µl of ethanol. The choice of the 1.20 µg quantity  
191 of DEHP was linked to the LOQ of the method: it is the minimum quantifiable concentration  
192 (0.1 µg mL<sup>-1</sup>) multiplied by the volume of extraction with chloroform (10 mL) and divided by  
193 the dilution factor of the internal standard solution is 0.9, the result (1.11 µg) finally rounded  
194 up to 1.2 µg. The loaded cartridges were mounted directly on the SAV and coupled with an  
195 unloaded cartridge in series. The second cartridge was intended to collect the DEHP which  
196 could be released by the loaded cartridge. In order to check if the humidification of the air  
197 caused a loss of retention of DEHP on the cartridges, we carried out these experiments with  
198 and without a humidifier. For the non-humidifier tests, we mounted an guard cartridge before  
199 the DEHP loaded cartridge to remove any DEHP contamination that could occur from the

200 SAV (SD 2d). The medical airflow passed through the cartridges for 3 days at a rate of 5 L  
201  $\text{min}^{-1}$ . After 3 days, an extraction was performed on each cartridge followed by DEHP  
202 quantification.

#### 203 1.4. Validation of the DEHP exposure model by respiratory device

- 204 • Design of the DEHP Exposure Model via Respiratory Support Devices

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

- 212 • Simulation of respiratory assistance

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

- 220 • Expression of results and exposure model presentation

221 DEHP quantification made it possible to measure, at each time (day 1, day 3 and day 6), the  
222 amount of DEHP released by 1 meter of PVC tubing corresponding to a contact area of 0.942  
223  $\text{dm}^2$ . The quantity of DEHP released by  $\text{dm}^2$  will be equal to:

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

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 Ve: 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 ( $0.942 \text{ dm}^2$ ).

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  $2 \text{ L min}^{-1}$  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

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

$$251 \quad A = f(t) \times S$$

252

253 In which

254 A: Estimated inhaled amount of DEHP in  $\mu\text{g}$

255 S : PVC surface to which the patient was exposed via the respiratory assistance circuit,  
256 expressed in  $\text{dm}^2$

257 t : Use duration in day.

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

## 262 1.6. Statistical tests

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

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

273

## 274 **2. Results**

### 275 2.1. Validation of the quantification method of DEHP in air

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

- 277 • Validation of DEHP extraction from cartridges

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

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

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

- 286 • Verification of DEHP sorption from the air

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

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

## 293 2.2. Validation of the DEHP exposure model by respiratory device

294

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

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

309 The comparison of DEHP concentration in air over time shows that concentrations decrease  
310 for all the experiments except for the 4 L min<sup>-1</sup> air humidification test where a stability of  
311 DEHP concentrations is observed, beginning from 0.87 µg m<sup>-3</sup> at D 1 up to 0.92 µg m<sup>-3</sup> at D6.  
312 Impacts of flow and humidification on DEHP concentrations in air are presented in Figure 1B  
313 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<sup>-1</sup> 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<sup>-1</sup> 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

337 National Institute for Occupational Safety and Health (NIOSH) reference method advocates  
338 the use of a cellulose ester filter as a support coupled with carbon disulfide extraction for 30  
339 minutes using ultrasounds. However, carbon disulfide is toxic and does not allow complete  
340 desorption of DEHP. Although Soxhlet extraction is recognized as a traditional method for  
341 extracting phthalates from solid samples, it is a tedious and laborious procedure, which lasts  
342 between 4 to 72 hours, and which requires large amounts of solvent (100 to 500 mL). On the  
343 other hand, Hill *et al* found it difficult to extract all of the DEHP from the Tenax<sup>®</sup> adsorbent  
344 cartridges when trying the extraction using a thermal desorption, resulting in higher LOQ, due  
345 to the low volatility of DEHP (Hill et al., 2003). Szewczyńska *et al* (Szewczyńska et al.,  
346 2020) had a recovery rate ranging between 66% and 88% when extracting plasticizers from  
347 XAD-2 resin using 3 mL of dichloromethane/acetone (1:1) as an extraction solvent in an  
348 ultrasonic cleaner for 30 min, when in our experiments, we obtained 98.5% recovery rate with  
349 chloroform and 14 hours ultrasonic extraction. XAD-2<sup>®</sup> resins advantage is their ability to  
350 collect particulate and gaseous DEHP (Das et al., 2014; Szewczyńska et al., 2020).

351 The verification of two air supply points proved that the conical feeding point did not release  
352 any DEHP. In contrast, the secure feed point released DEHP as a contaminant with an average  
353 of 1.04 µg day<sup>-1</sup> when using a flow rate of 5 L min<sup>-1</sup>. This contamination could be suppressed  
354 by using a humidifier containing sterile water. The effect of a “water trap” for DEHP  
355 suppression has been described by Roth *et al* (Roth et al., 1988) who used this method of  
356 DEHP trapping via a water reservoir which was subsequently used to estimate exposure to  
357 DEHP by respiratory MDs of 3 newborns. The solubility of DEHP in water could vary from  
358 0.0006 to 1.3 mg/L at 20-25°C (Bis(2-ethylhexyl) phthalate (DEHP) - Summary Risk  
359 Assessment Report - ECHA, 2008), a solubilisation of 6 µg in a volume of 330 ml of water  
360 seems possible. However, this proves that exposure to DEHP may be increased from some air  
361 feed line exit if no humidifier is used.

362 DEHP migration tests in the air passing through the respiratory devices was performed by  
363 varying the airflow and using or not a humidifier. The comparison of the released quantities  
364 of DEHP after 24 hours of simulation in the different tested situations showed a clearly higher  
365 level of DEHP released at  $4 \text{ L min}^{-1}$  without humidifier. We note that the total amount of  
366 DEHP released continues to increase during the six days of the experiment for all conditions  
367 studied. If we look at the flow rate effect, we note that the flow rate increase leads to an  
368 increase in the quantities released in the absence of a humidifier, whereas in the presence of a  
369 humidifier, increasing the flow rate decreased the quantity released, especially at day 6. The  
370 effect of the humidifier however is similar for both flow rates, resulting in a decrease in the  
371 amount each time the humidifier is used. Despite the DEHP quantities released, none  
372 exceeded the Derived No Effect Level of  $130 \mu\text{g m}^{-3}$  (Bui et al., 2016). The variation study of  
373 the DEHP air concentration over time shows a drop in the concentration for all the conditions  
374 except for the  $4 \text{ L min}^{-1}$  humidified air test. After six days, except for experiment with  $4 \text{ L}$   
375  $\text{min}^{-1}$  with humidifier, the concentrations were comparable. In clinical conditions, no study  
376 was conducted to evaluate plasticizers concentration variations in air as function of the  
377 airflow. Flow rate impact on the migration of DEHP and other plasticizers concentrations  
378 over time has been studied for a perfusion model of liquid solutions (Bagel-Boithias et al.,  
379 2005; Bernard et al., 2018) showing that their concentration in sodium chloride 0.9%  
380 remained constant regardless of the flow rate used over 24 hours. For the respiratory model,  
381 the decrease in DEHP concentration in the air over time could be explained by a limited  
382 exchange between the gas and the surface of the inner lumen of the PVC cannula. One can  
383 hypothesise a simple desorption of the DEHP on the surface of the PVC whereas at the time  
384 of the contact between a liquid and the PVC the exchanges can be done in depth of the tubes  
385 as well as with the surface. The best explanation of the concentration variation in air over time  
386 in these experiments was reported by Wei *et al* (Wei et al., 2019), claiming that plasticizer

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

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

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

#### 435 **4. Conclusion**

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

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452 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<sup>-1</sup>, 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<sup>-1</sup> with humidifier, B) 2 L min<sup>-1</sup> without humidifier, C) 4 L min<sup>-1</sup> with humidifier and D) 4 L min<sup>-1</sup> without humidifier.

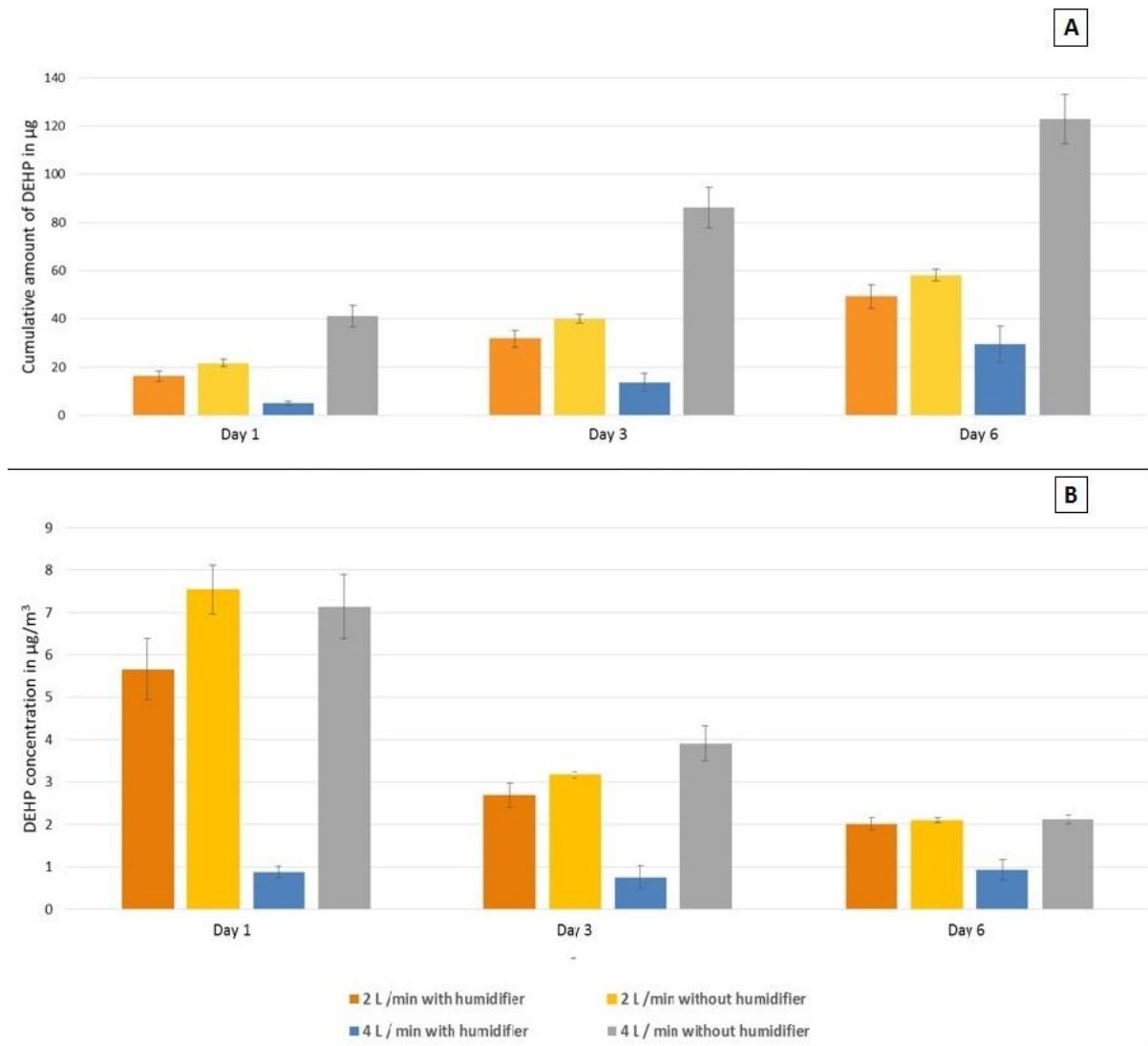


Fig 1

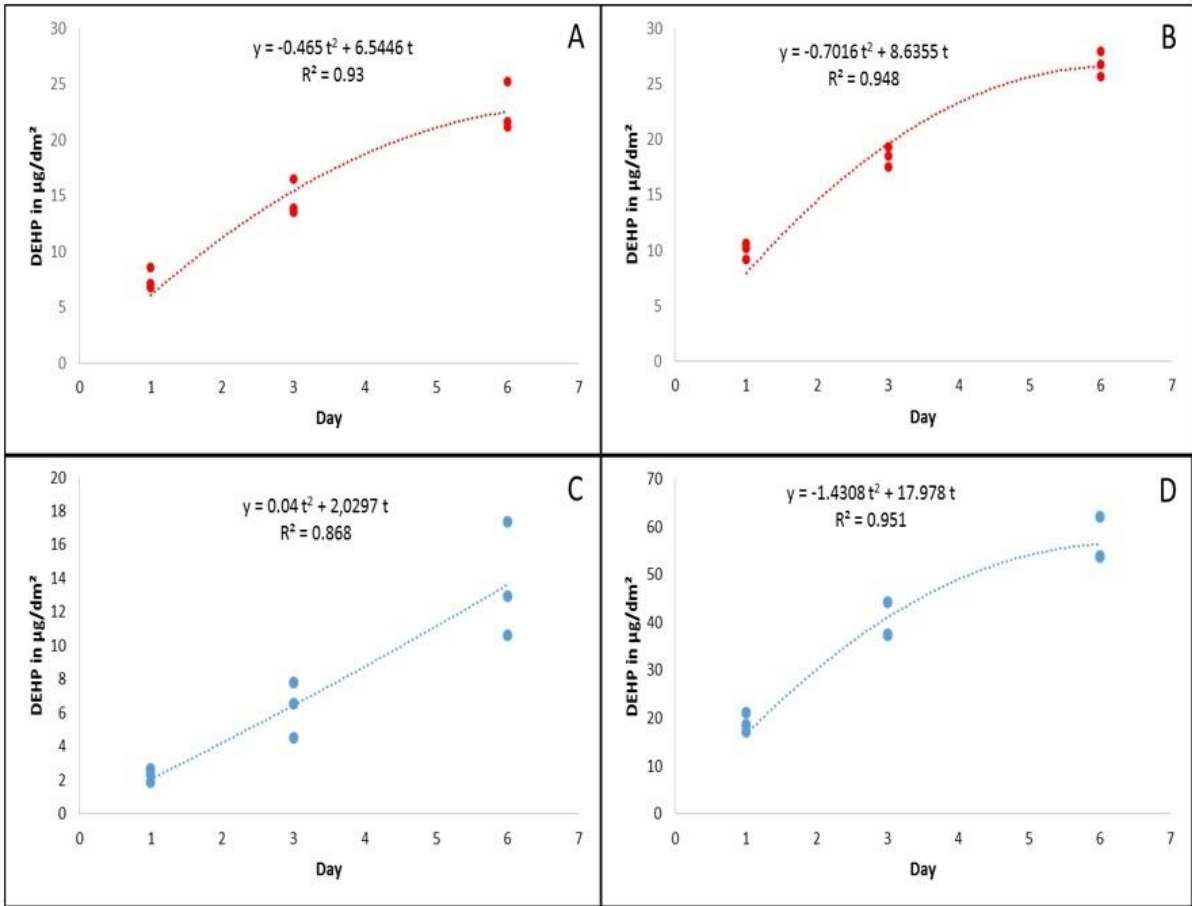
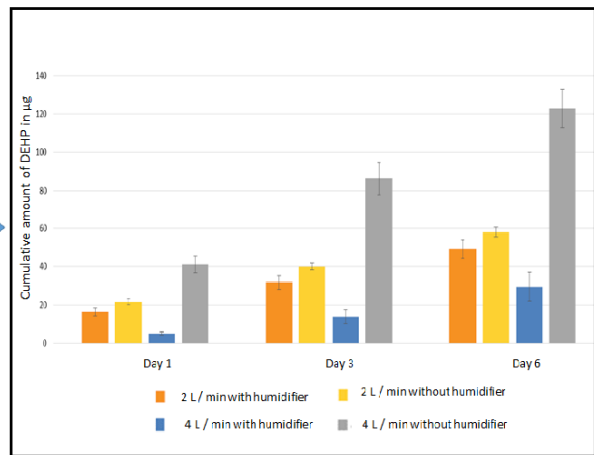
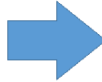
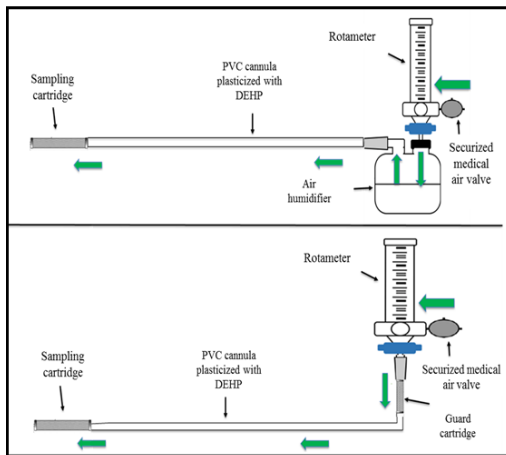


Fig 2

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