



**HAL**  
open science

## How does continuous venovenous hemofiltration theoretically expose (ex-vivo models) inpatients to diethylhexyladipate, a plasticizer of PVC medical devices?

Lise Bernard, Mélanie Bailleau, Teuta Eljezi, Philip Chennell, Bertrand Souweine, Alexandre Lautrette, Valérie Sautou

### ► To cite this version:

Lise Bernard, Mélanie Bailleau, Teuta Eljezi, Philip Chennell, Bertrand Souweine, et al.. How does continuous venovenous hemofiltration theoretically expose (ex-vivo models) inpatients to diethylhexyladipate, a plasticizer of PVC medical devices?. *Chemosphere*, 2020, 250, pp.126241. 10.1016/j.chemosphere.2020.126241 . hal-02528010

**HAL Id: hal-02528010**

**<https://hal.science/hal-02528010v1>**

Submitted on 7 Mar 2022

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

1 **How does Continuous Venovenous Hemofiltration theoretically expose (ex-vivo models) inpatients**  
2 **to diethylhexyladipate, a plasticizer of PVC medical devices?**

3 Lise Bernard<sup>1</sup>, Mélanie Bailleau<sup>2</sup>, Teuta Eljezi<sup>1</sup>, Philip Chennell<sup>1</sup>, Bertrand Souweine<sup>3</sup>, Alexandre  
4 Lautrette<sup>3</sup>, Valérie Sautou<sup>1</sup>

5  
6 <sup>1</sup> Université Clermont Auvergne, CHU Clermont-Ferrand, CNRS, SIGMA Clermont, ICCF, f-  
7 63000 Clermont-Ferrand, France

8 <sup>2</sup> CHU Clermont-Ferrand, Service de réanimation chirurgie cardio-vasculaire, f-63000  
9 Clermont-Ferrand, France

10 <sup>3</sup> Université Clermont Auvergne, CHU Clermont-Ferrand, CNRS, LMGE «Laboratoire Micro-  
11 organismes: Génome et Environnement», f-63000 Clermont-Ferrand, France

12

13

14 Corresponding author: Lise Bernard

15 E-mail: [l\\_bernard@chu-clermontferrand.fr](mailto:l_bernard@chu-clermontferrand.fr)

16 Postal address: CHU Clermont-Ferrand, pôle Pharmacie, 58 rue Montalembert, 63000 Clermont-  
17 Ferrand Cedex 1

18 Phone: +33 4 73 75 17 69; Fax: +33 4 73 75 48 29

19

20 **Abstract**

21 Continuous venovenous hemofiltration (CVVH) is widely used in intensive care units to treat patients  
22 with acute kidney injury requiring renal replacement therapy. The medical devices (MD) used for  
23 CVVH include a hemofilter and tubings made of plasticized PVC. Due to its known reprotoxicity,  
24 diethylhexyl phthalate (DEHP) has been replaced by alternatives such as diethylhexyladipate (DEHA)  
25 in some of these tubings. The migration of DEHA from hemofiltration systems has not been assessed  
26 and thus the level of patient exposure to this DEHP-alternative remains unknown.

27 In this study, 2 CVVH models were used to evaluate the potential migration of DEHA from PVC  
28 tubings, allowing the determination of (1) the highest rates of DEHA able to migrate into a simulant  
29 flowing in a marketed adult CVVH circuit by disregarding any metabolism and (2) the clinical-  
30 reflecting exposure of patients to this plasticizer and its metabolites by assessing their migration into  
31 blood.

32 In the first model, we showed that patients undergoing a CVVH procedure may be exposed to high  
33 rates of DEHA. Moreover, DEHA is continuously hydrolyzed into its primary metabolite MEHA  
34 (monoethylhexyladipate), which may reach cytotoxic level in the patients' blood.

35 When looking from a « safer » MD perspective, DEHA might not be the best alternative plasticizer for  
36 CVVH tubings. However, to reflect clinical conditions, this study should be completed by an *in-vivo*  
37 evaluation (biomonitoring) of the oxidized metabolites of DEHA in urines of inpatients undergoing  
38 CVVH.

39 **Keywords**

40 Continuous venovenous hemofiltration, medical devices, plasticizer, diethylhexyladipate

41

42 **Highlights**

43 - Tubings used for CVVH can be made of DEHA plasticized PVC

- 44 - 2 different but complementary *ex-vivo* models aimed to evaluate DEHA migration from these
- 45 tubings
- 46 - During CVVH, patients may be exposed to DEHA and to its primary metabolite MEHA
- 47 - MEHA concentrations were high and match those that may induce effects *ex-vivo*
- 48 - A biomonitoring study should be performed to confirm these exposure doses

49        **1. Introduction**

50        Continuous venovenous hemofiltration (CVVH), a highly specialized therapy, is widely used in  
51        intensive care units to treat patients with acute kidney injury (1).

52        To perform a CVVH procedure, many medical devices (MD) are required, such as continuous renal  
53        replacement therapy (CCRT) machines and CCRT disposables (sets). A hemofilter set is made of a  
54        hemofilter and PVC tubings necessary to run the patient’s blood through the filter and deliver a  
55        replacement fluid either upstream or downstream of the hemofilter.

56        Numerous studies have reported that patients undergoing Renal replacement therapy (RRT),  
57        including CVVH, may be exposed to different leachables, especially Bisphenol A (BPA) released from  
58        components of the dialyzer itself, particularly polycarbonate housing and polysulfone dialysis  
59        membrane (2–5). Moreover, most of the tubings used in dialysis are designed with plasticized PVC  
60        for greater flexibility with the CVVH system. However, it is widely accepted that these plasticizers can  
61        migrate from the PVC matrix into the blood of patients undergoing maintenance hemodialysis or  
62        hemofiltration (6–13) and thus come into contact with the patient. Indeed, it has been demonstrated  
63        that a non-negligible amount of diethylhexyl phthalate (DEHP) leaches from PVC into patients  
64        undergoing haemodialysis (6,7,14,15).

65        DEHP and BPA have been reported to be endocrine disrupting chemicals with effects on the  
66        reproductive and thyroid systems (16–18). They have also been categorized as carcinogenic,  
67        mutagenic or toxic for reproduction (CMR) 1B under the Classification Labeling and Packaging (CLP)  
68        Regulation (19,20) due to their reprotoxicity suspected in humans. In the field of medical devices,  
69        DEHP now must not exceed 0.1% by mass of plasticized material, as defined by the European  
70        regulation (19), unless it is justified on the basis of an assessment of the risks related to potential  
71        estimated exposure. Therefore, some alternative to DEHP plasticizers have been proposed, especially  
72        for patient groups undergoing clinical procedures with high exposure, as recommended by the

73 Scenihr (20). For example, in some dialysis tubings, DEHP has been replaced by other plasticizers such  
74 as diethylhexyladipate (DEHA) (21).

75 Unfortunately, and contrary to DEHP, DEHA migration from haemodialysis systems has not been  
76 assessed and thus the level of patient exposure to this DEHP-alternative remains unknown. Even  
77 worse, due to the contact with blood, plasticizers such as DEHA will be metabolized during the CVVH  
78 procedure (22), and thus may expose patients to metabolites that are known to be cytotoxic (23)  
79 and/or may have a biological activity (24). Indeed, as described by Nehring et al. (25), DEHA is rapidly  
80 hydrolyzed into MEHA that may be further metabolized (before urinary elimination) into adipic acid  
81 and two main oxidative products, MEHHA (mono-2-ethylhydroxyhexyl adipate) and MEOHA (mono-  
82 2-ethyloxohexyl adipate) (figure 1). An evaluation of the risks posed by exposure to MEHA during  
83 CVVH is therefore necessary.

84 To perform such a task, the risk exposure assessment for hospitalized patients needs the  
85 development of models that reflect the real clinical conditions within which the MD are used. Based  
86 on our previous methodology, used to build an infusion model (26) and an ECMO model (27), we  
87 aimed to evaluate the potential migration of plasticizers by developing 2 CVVH models, allowing the  
88 determination of (1) the highest rates of DEHA which could migrate into a simulant flowing in a  
89 marketed adult CVVH circuit by disregarding any metabolisation and (2) the clinical-reflecting  
90 exposure of patients to this plasticizer and its metabolites during the clinical procedure by assessing  
91 their migration into blood.

92

## 93 **2. Materials and methods**

94 To assess the migration of DEHA during CVVH from PVC MD and the exposure to DEHA and its  
95 metabolites synthesized during the contact with blood, two types of models were implemented (see  
96 section 2.1)

97 *Medical devices used in the study:*

98 Both models were performed using the same CCRT machine and disposables (Prismaflex® system and  
99 ST150 Prismaflex set®, batch n°15J2802G, expiry date: 01/10/2017), graciously provided by Gambro  
100 Industries. In this set, the hemofilter is an AN69® ST membrane (surface treated acrylonitrile and  
101 sodium methallyl sulfonate copolymer membrane) and the tubings are made of DEHA plasticized  
102 PVC.

103 *Plasticizers and metabolites used for analytical quantification:*

104 DEHA (CAS: 103-23-1) was purchased from Sigma Aldrich, France. The primary metabolite MEHA  
105 (CAS: 4337-65-9) was synthesized and characterized by the UMR 990 team, Clermont-Ferrand,  
106 France.

107

## 108 2.1 The models

### 109 2.1.1 CVVH model with ethanolic simulant

110 The aim of this model was to estimate the total leaching of DEHA from the PVC tubings during a  
111 CVVH procedure, regardless the conversion of the plasticizer to its metabolite (MEHA), and thus to  
112 estimate its exposition dose.

113 The model is presented in figure 2.

114 The model conditions established according to the clinical practice were the following ones:

- 115 - Simulant: a mixture of absolute ethanol (Sigma Aldrich) and water (Versylène®, Fresenius)  
116 50/50 v/v was chosen to simulate the blood due to its close but higher capacity to extract  
117 DEHP from PVC matrix than that of blood and the absence of the enzymes likely to  
118 metabolize DEHA. The simulant volume was 5L in order to correspond to the mean blood  
119 volume of a normal adult. It was contained in a closed 5L glass flask.

120 - Flow rate: the flow rates were set up in accordance with the standard CVVH practice, i.e the  
121 blood flow rate was set up at 200 mL/min, the prefilter flow rate at 800 mL/h, and the  
122 replacement fluid flow rate at 1600 mL/h.

123 - Contact time: the experiment was performed during 72 hours.

124 The CVVH modelling was performed in a closed circuit without the hemofilter, because of the  
125 degradation of the filter membrane by the ethanolic simulant. The modelling was performed in  
126 triplicate.

127

### 128 2.1.2 CVVH model with blood simulant

129 The aim of this model was to reflect more closely the clinical conditions and to measure the real  
130 exposure of patients to DEHA and its metabolites. To this end, the simulant used was blood.

131 The model is presented in figure 3.

132 The model conditions established according to the clinical practice were the following ones:

133 - Simulant: 10 UI/mL heparinized sheep blood provided by Fiebig Nährstofftechnik animal  
134 blood products. The volume of blood used was 500 mL and was regularly replaced with  
135 312mL during the procedure to compensate the losses due to the samplings.

136 - Flow rate: the flow rates were set up in accordance with the standard CVVH practice , i.e the  
137 blood flow rate was set up at 200 mL/min, the prefilter flow rate at 800 mL/h, and the  
138 replacement fluid flow rate at 1600 mL/h

139 - Contact time: the experiment was performed during 72 hours.

140 The CVVH modelling was performed in a closed circuit with the hemofilter and an effluent line. The  
141 modelling was performed in triplicate.

### 142 2.2 Samples



143 In both models, ethanolic simulant and blood samples were collected at H0 (immediately after the  
144 priming of the circuit) and after 1, 2, 4 and 8 hours and then every 8 hours during 72 hours. These  
145 samples were collected in triplicate and stored in hemolysis tubes. The samples were stored at 4 ° C  
146 until plasticizer quantification by GC-MS.

147 In the first model, 5 mL samples were withdrawn from the return line (post filter sampling site). In the  
148 second model, there were 3 sampling sites: pre-filter, post-filter and effluent line. The samples were  
149 immediately centrifuged at 3000 rpm during 6 minutes then stored at 4°C until plasticizer  
150 quantification by GC-MS.

151

### 152 2.3 Analysis of DEHA and its primary metabolite

153 Different analyses were conducted:

154 - Quantification of DEHA:

155 ○ in the PVC matrix of the medical devices: to obtain the initial amount of DEHA in MD  
156 before performing the migration assay according to the model

157 ○ in the ethanol/water simulant at each contact time within the first model

158 - Quantification of DEHA and its metabolites in the sheep plasma from the blood collected at  
159 each contact time within the second model.

160 -

161 Plasticizer quantification was carried out by gas chromatography coupled with mass spectrometry  
162 (GC-MS) in the simulant solution samples or after a chloroform extraction from the MD or from the  
163 simulant (supplementary file no1, SF no1), according to the method published by Bourdeaux *et al.*  
164 (21). Briefly, plasticizer quantification was performed using a Clarus 500 (Perkin Elmer, USA) using  
165 electronic impact ionization tuned to 70 eV and an Optima 5 Accent, 5% diphenyl 95%  
166 dimethylpolysiloxane (30 m × 0.25 μm × 0.25 mmID) capillary column (Macherey-Nagel, Germany).  
167 The oven temperature curve started at 200 °C for 1 min then rose to 300 °C at a rate of 20 °C/min.

168 The oven then remained at 300 °C for seven minutes. The total analysis time was 15 min (21 min  
169 between two injections). The gas mobile phase was N55 helium , with a flow rate through the column  
170 of 1.20 mL/min.

171

172 According to Silva *et al* (22), in blood DEHA is quickly metabolized into the hydrolytic monoester  
173 mono-2-ethylhexyl adipate (MEHA), which is further metabolized into adipic acid and other oxidative  
174 products by liver microsomes.

175 We therefore assessed the rates of DEHA released into blood by the hemofilter system and the  
176 amounts of MEHA synthetized during the CVVH procedure.

177 The analyses of DEHA and its metabolite in the blood were also performed by GC-MS after an  
178 extraction step described in SF no1. To measure the MEHA produced during the CVVH process, an  
179 additional step of derivatization was necessary. To this end, 400 µL of ethyl acetate, 200 µL of  
180 methanol and 100 µL of trimethylsilyl diazomethane (2 mol/L solution in hexane) were added to the  
181 dry residue immediately after the extraction of the metabolites from the blood. After one hour of  
182 contact, the solution was evaporated under nitrogen and the dry residue was dissolved in 1mL of a  
183 2µg/mL BBP solution. Samples were then analyzed by GC-MS, according to the method published by  
184 Bourdeaux et al (21).

185 For both DEHA and MEHA analysis, the method from Bourdeaux et al (21) was adapted in order to  
186 quantify them in a single run. To this end, the oven temperature curve started at 200°C for 1 min and  
187 rose to 300°C at a rate of 10°C/min. The oven then remained at 300°C for 2 minutes.

188 The validation parameters of this method were as follow :

- 189 - Calibration ranged from 0.1 to 7.5 µg/mL for DEHA and from 1 to 7.5 µg/mL for MEHA
- 190 - The limits of detection (LOD) and quantification (LOQ) values were respectively 0.03 µg/mL  
191 and 0.1 µg/mL for DEHA and 0.05 µg/mL and 1 µg/mL for MEHA. Estimation of the LOD was

192 performed based on ICH guidelines Q2R1 Validation of Analytical Procedures using the  
193 signal-to-noise methodology with a signal-to-noise ratio of 3:1.

- 194 - The mean inter-day precision values were all under 10% for DEHA and under 15% for MEHA.
- 195 - The accuracy of the method was comprised respectively between 2.02% and 11.31% for  
196 DEHA and 2.46% and 9.18% for MEHA.
- 197 - The extraction recovery was experimentally determined by individually spiking blank sheep  
198 blood samples with DEHA and MEHA and measuring the respective quantities of the  
199 plasticizer and its metabolite, on three different samples for three successive days. Mean  
200 recovery coefficients were of 101.13 and 102.02 % for respectively DEHA and MEHA.
- 201 - For the model 2 using sheep blood, the background levels of DEHA and MEHA were  
202 evaluated by passing samples of the sheep blood taken straight from their primary container  
203 (glass bottles), before any other use. No DEHA or MEHA was detected. The priming of the  
204 circuit was afterwards done directly from the glass bottles containing the sheep blood, so as  
205 to avoid any contaminations.

206

### 207 **3. Results**

208

#### 209 **3.1 DEHA in PVC MD**

210 The hemofilter set is composed of 9 tubings and a PVC effluent bag. The amount of DEHA in the  
211 different tubings varied from 34.2 % to 46.2% (figure 4).

212 DEHA was not present in the effluent bag. This bag was plasticized with 31.8 % of DEHP, and is not  
213 used in this study because the plasticizer released does not come into contact with the patient's  
214 blood.

215 No other plasticizer than DEHA was present in the 9 tubings. Only a few traces of DEHP were  
216 detected but they remained below the limit of quantification (LOQ)

217

### 218 **3.2 Migration of DEHA into ethanolic simulant during CVVH**

219 Figure 5 presents the concentrations of DEHA released from PVC hemofilter set in the first model.

220 The release of DEHA follows a linear release kinetic during the first 8 hours of contact and reaches a  
221 plateau at about 230  $\mu\text{g}/\text{mL}$ , which corresponds to a quantity higher than 1200 mg of DEHA (in 5 L of  
222 simulant) potentially received by the patient.

223 This quantity represents less than 1.5% of the total amount of DEHA contained in the hemofilter set.

224

### 225 **3.3 Analysis of DEHA released and MEHA produced into blood during CVVH**

226 The figure 6 presents the concentrations of DEHA and MEHA in  $\mu\text{g}/\text{mL}$  at the three sites of  
227 sampling (pre-filter, post-filter and effluent line)

228

229 DEHA and MEHA were found at T0 (immediately after priming) but not at the same sites: DEHA is  
230 only present in the effluent line at a background level of  $0.15\pm 0.02 \mu\text{g}/\text{mL}$  whereas  $1.17\pm 0.05$   
231  $\mu\text{g}/\text{mL}$  of MEHA is found at the post-filter site. DEHA and MEHA appear gradually into blood (pre-  
232 and postfilter).

233 Indeed, both concentrations of DEHA and MEHA show a constant increase during the first 24  
234 hours (figures 7 and 8). However, their kinetic profile is different: the slope of the regression line  
235 of MEHA is more than twice higher than that of DEHA, both for pre-filter and post-filter sites. So,  
236 MEHA is produced nearly as fast as DEHA is released into blood from PVC tubing during the first  
237 24 hours of the CVVH procedure. Whereas the increase in DEHA concentration slowed down  
238 during the experiment, MEHA shows a significant and constant increase over the perfusion time,

239 both before and after the filter. Concentrations of MEHA were still increasing at the end of the  
240 perfusion, reaching a mean of 370 µg/mL at T72h.

241 Moreover, the mass of MEHA quantified in the blood was between two and three times higher of  
242 magnitude than that of DEHA. In fact, the concentration of MEHA doubles after the second hour  
243 of infusion time, is six fold after 8 hours and about 12 times higher at 24h, when compared to T0  
244 concentrations.

245 No DEHA was found in the effluent line, whereas MEHA is present from the 24<sup>th</sup> hour, at a  
246 quantity of 2.6% and 2.5% of the quantity found in the postfilter and prefilter samples.

247

248

#### 249 **4. Discussion**

250 Due to the rapidly growing worldwide regulations to limit the use of DEHP, other plasticizers like  
251 DEHA are more and more used as alternatives to soften the PVC of MD (28), such as dialysis tubings.  
252 Patients in ICU undergoing hemofiltration are therefore potentially exposed to DEHA and its  
253 metabolite MEHA, during CVVH procedures which may last 72 hours or more.

254 In our study, the high levels of DEHA and MEHA in the sheep blood demonstrate, for the first time,  
255 that adult ICU patients could be continuously exposed to substantial levels of DEHA released from  
256 the CVVH tubings. As shown by the first model, a patient could be exposed every day to more than 1  
257 g of DEHA. In the field of extracorporeal circulation as treatment of ICU patients, the release of  
258 plasticizers from PVC tubes has been evaluated exclusively with DEHP. In the works of Dine *et al* (15),  
259 Kambia *et al* (14) and Faouzi *et al* (6), patients with chronic renal failure underwent dialysis for a 4 h-  
260 period three times a week. The authors showed that patients are exposed to 16.40 mg, 122.95 mg  
261 and 75.26 mg respectively for 4h sessions 3 times a week, corresponding approximatively to 7.03  
262 mg ; 52.69 mg and 32.3 mg per day of DEHP. With *ex-vivo* hemodialysis models, quantities of DEHP

263 found in blood could reach 6.10 mg (10) and 7.80 mg (29) in standard conditions reflecting a 4 hours  
264 hemodialysis session. In our study, patients hospitalized in ICU may potentially be exposed to high  
265 quantities of DEHA, reaching 764 mg after 4 hours of CVVH procedure. This data has to be related to  
266 the specific nature of CVVH, which differs from intermittent hemodialysis. Some specific parameters  
267 related to CVVH are in favor of a higher release of plasticizer : the length of the tubing (30), the  
268 contact time with blood of patients (31) and the flow rate (32). In our study the calculated  
269 administered daily intake of DEHA for a normal adult of 70 kg is higher than 16 mg/kg/j, which could  
270 be compared to the reference values of DNEL. Due to the lack of data related to the IV route, it  
271 should be relevant to compare our estimated result (16 mg/kg/d) to an average DNEL of 6.23  
272 mg/kg/d as proposed by Bui et al (24), rather than the limit of 1.3 mg/kg/d fixed by European  
273 Regulation (33) for dietary exposure. However, the last review of DEHA toxicology provided by the  
274 Consumer Product Safety Commission (CPSC) reported a DNEL of 170 mg/kg/d in the general  
275 population. Despite this new data, the risk related to DEHA exposure via CVVH can not be excluded,  
276 because of the inherent cytotoxicity of DEHA, as reported by Eljezi *et al* (23). The amounts of DEHA  
277 released in the simulant exceed by far the limit of 0.1 mg/mL during all time of the CVVH procedure  
278 (23).

279 Moreover, this may be a worrying situation because patients are also exposed to the metabolite  
280 MEHA. Indeed, DEHA is converted continuously to MEHA, from the first hours of the perfusion since  
281 MEHA appears in blood immediately after priming at a level of  $1.17 \pm 0.05$   $\mu\text{g/mL}$  in the postfilter site.  
282 Thus, due to the major contact between the tubings and patients' blood, patients undergoing CVVH  
283 are exposed to DEHA but even more to MEHA, for which measured concentrations were higher than  
284 those of DEHA. In this work, both studied models are complementary and showed that patients are  
285 potentially exposed to a maximum of 1200mg of DEHA within one session of CVVH (model 1) and  
286 that all this quantity will be transformed continuously into MEHA (model 2) which which could  
287 intoxicate these same patients. Throughout our experiments, the amount of DEHA remains lower  
288 than that of MEHA, with DEHA over MEHA ratios ranging from 15.4% to 32.7% in the prefilter site

289 and from 17.7% to 31.5% in the post-filter site. This reflects an important and fast first hydrolysis  
290 happening in the blood and is of great concern because these high MEHA blood levels match the  
291 concentrations that may induce effects *ex-vivo*. Indeed, from day 1, the concentrations of MEHA are  
292 above the cytotoxicity limit of 0.1mg/mL reported by Eljezi et al (23). This result is far from that  
293 obtained by Munch et al, who showed that the ratio between DEHP and MEHP in blood is constant  
294 during the perfusion experiment with a DEHP ratio ranging between 95.8% and 97.6% and MEHP  
295 ratio ranging between 2.4% and 4.2% (34). According to Melzak et al (35), plasticizers like DEHP  
296 interact with albumin that is contained in blood. Due to their poor solubility in water, like DEHP, we  
297 can hypothesize that DEHA and MEHA also interact with albumin contained in the sheep blood in the  
298 second model and are transported by this protein. The presence of DEHA and MEHA in the postfilter  
299 line and the absence of DEHA in the effluent line suggest that they are not filtered by the hemofilter  
300 because of a higher cut-off of the hemofilter than the albumin size. Regarding the low concentrations  
301 of MEHA found in the effluent line, we can hypothesize that the albumin binding sites are saturated  
302 after many hours, providing small quantities of free MEHA (which molecular weight is lower).  
303 Moreover, the effluent line is the only one not containing blood but a crystalloid solution (filtrated  
304 through the hemofilter) that can extract less plasticizers than blood.

305 The early onset of MEHA into blood might be explained by a previous hydrolysis in the PVC matrix as  
306 demonstrated with DEHP by Münch et al (34). This effect could be attributed to impurities of the  
307 additives used or to a degradation process during storage of the tubing sets. Hamed et al also showed  
308 that the contents of DEHP detected in the solutions in contact with out of date PVC bags were 10  
309 times higher than the concentration detected in new PVC bags, confirming the origin of this  
310 compound from the plastic material (36). In our study, the presence of MEHA into the CVVH tubing  
311 before the start of the CVV procedure should not be excluded.

312 Finally, patients undergoing CVVH are not only exposed to DEHA during their entire management  
313 care. They can be exposed to BPA through dialysis tubing and BPA containing polysulfones in  
314 hemodialysers or hemofilters (4,5) which may contribute to BPA burden in patients on hemodialysis

315 (37), like the dialysate contamination of  $22.7 \pm 15.6$  ng/L on average presented by Bacle et al (38). BPA  
316 is also an endocrine disruptor, linking them to infertility, developmental changes, cancer, and  
317 changes in thyroid function (39,40). DEHA has not yet been shown to induce testicular toxicity or  
318 antiandrogenic effects (41), but has been reported to cause disturbed estrous cycles and increased  
319 atresia of ovarian follicles at high doses (42). Moreover, a recent *in silico* study revealed that DEHA  
320 showed a high binding affinity with sex hormone binding globulin (SHBG), as BPA did, which may  
321 hinder the availability of estrogens and androgens to target tissues resulting in organ dysfunction  
322 (43). Ghisari et al also showed that some plasticizers, including DEHA, elicited endocrine-disrupting  
323 potential that can be mediated via interference with the estrogen and thyroid hormone systems (44).  
324 Secondly, critically ill patients admitted in ICU are subjected to numerous medical procedures, which  
325 include many plasticized MD, and thus are major sources of exposure to plasticizers, with positive  
326 correlation between the grade of exposure and blood levels of DEHP metabolites (12). Finally, renal  
327 insufficiency may impair the excretion of plasticizers such as DEHA, causing a rise in the serum levels,  
328 as it has been demonstrated by Yamakasi et al (5).

329 The design of our study allowed us to evaluate the exposure risk to DEHA for patients undergoing  
330 CVVH but has some limitations:

331 - the first model was done in a closed-loop, which may explain the higher concentrations of  
332 DEHA released in ethanol than those released into blood. As the extraction ability of the  
333 water/ethanol mixture is higher than that of blood as demonstrated by Luo et al (45), the  
334 conditions of this first model may have led to an overestimation of the maximum exposure  
335 dose of DEHA.

336 - Contrary to the *ex-vivo* models of Lewis et al (10) or Haishima et al (29), we studied the  
337 concentrations of both the plasticizer DEHA and its metabolite MEHA, which helps understand and  
338 assess the real risk of exposure linked to their respective toxicities. However, to reflect the clinical  
339 conditions, this study should be completed by an *in-vivo* evaluation (biomonitoring) of the oxidized  
340 metabolites of DEHA in urines of inpatients undergoing CVVH. In the work of Bastiaensen et al,



341 metabolites of DEHA (5-OH-MEHA and 5-oxo-MEHA) were found with higher levels in the urines of  
342 patients undergoing CVVH (or ECMO) than those measured in control patients (46). However, it is  
343 difficult to assess the correlation of those levels with the intensity of their exposure to DEHA  
344 because no information is available on the number and the composition of the medical devices used  
345 for the two procedures (CVVH and ECMO). A global risk consideration is needed for such patients,  
346 including the potential sources and factors influencing inside the ICU environment.

347

348

## 349 **5. Conclusions**

350 Due to the current concern about the replacement of DEHP leading to the use of «safer» MD, our  
351 study showed that DEHA might not be the best alternative as MD plasticizers for CVVH tubings. It has  
352 demonstrated that DEHA migration ability is higher compared to that of other plasticizers like DEHT  
353 or TOTM (47). Considering these issues, further research into the assessment of tubings made with  
354 plasticizers with less migration potential and into the clinical effects of the leaching from MD in ICU  
355 patients undergoing CVVH should be performed.

356

357

## 358 **Acknowledgments**

359 The authors wish to thank Baxter for the loan of the dialysis machine used for both studied models.

360

361

## 362 **References**

- 363 1. Rachoïn J-S, Weisberg LS. Renal Replacement Therapy in the ICU. *Critical Care Medicine* 2019;  
364 47: 715–721.

- 365 2. Krieter DH, Canaud B, Lemke H-D et al. Bisphenol A in chronic kidney disease. *Artificial Organs* 2013; 37: 283–290.  
366
- 367 3. Shintani H. Determination of the endocrine disrupter bisphenol—A in the blood of uremia  
368 patients treated by dialysis. *Chromatographia* 2001; 53: 331–333.
- 369 4. Murakami K, Ohashi A, Hori H et al. Accumulation of bisphenol A in hemodialysis patients.  
370 *Blood Purification* 2007; 25: 290–294.
- 371 5. Yamasaki H, Nagake Y, Makino H. Determination of bisphenol a in effluents of hemodialyzers.  
372 *Nephron* 2001; 88: 376–378.
- 373 6. Faouzi MA, Dine T, Gressier B et al. Exposure of hemodialysis patients to di-2-ethylhexyl  
374 phthalate. *International Journal of Pharmaceutics* 1999; 180: 113–121.
- 375 7. Pollack GM, Buchanan JF, Slaughter RL, Kohli RK, Shen DD. Circulating concentrations of di(2-  
376 ethylhexyl) phthalate and its de-esterified phthalic acid products following plasticizer exposure  
377 in patients receiving hemodialysis. *Toxicology and Applied Pharmacology* 1985; 79: 257–267.
- 378 8. Gibson TP, Briggs WA, Boone BJ. Delivery of di-2-ethylhexyl phthalate to patients during  
379 hemodialysis. *The Journal of Laboratory and Clinical Medicine* 1976; 87: 519–524.
- 380 9. Flaminio LM, Bergia R, De Angelis L et al. The fate of leached di-(2-ethylhexyl)-phthalate (DEHP)  
381 in patients on chronic haemodialysis. *The International Journal of Artificial Organs* 1988; 11:  
382 428–434.
- 383 10. Lewis LM, Flechtner TW, Kerkay J, Pearson KH, Nakamoto S. Bis(2-ethylhexyl)phthalate  
384 concentrations in the serum of hemodialysis patients. *Clinical Chemistry* 1978; 24: 741–746.
- 385 11. Fayz S, Herbert R, Martin AM. The release of plasticizer from polyvinyl chloride haemodialysis  
386 tubing. *The Journal of Pharmacy and Pharmacology* 1977; 29: 407–410.
- 387 12. Huygh J, Clotman K, Malarvannan G et al. Considerable exposure to the endocrine disrupting  
388 chemicals phthalates and bisphenol-A in intensive care unit (ICU) patients. *Environment*  
389 *International* 2015; 81: 64–72.
- 390 13. Yakubovich M, Vienken J. Is there a need for plasticizer-free biomaterials in dialysis therapy?  
391 *Medical Device Technology* 2000; 11: 12–13, 18–21.
- 392 14. Kambia K, Dine T, Azar R, Gressier B, Luyckx M, Brunet C. Comparative study of the leachability  
393 of di(2-ethylhexyl) phthalate and tri(2-ethylhexyl) trimellitate from haemodialysis tubing.  
394 *International Journal of Pharmaceutics* 2001; 229: 139–146.
- 395 15. Dine T, Luyckx M, Gressier B et al. A pharmacokinetic interpretation of increasing  
396 concentrations of DEHP in haemodialysed patients. *Medical Engineering & Physics* 2000; 22:  
397 157–165.
- 398 16. Huang H-B, Chuang C-J, Su P-H et al. Prenatal and Childhood Exposure to Phthalate Diesters and  
399 Thyroid Function in a 9-Year Follow-up Birth Cohort Study: Taiwan Maternal and Infant Cohort  
400 Study. *Epidemiology (Cambridge, Mass.)* 2017; 28 Suppl 1: S10–S18.
- 401 17. Andrianou XD, Gängler S, Piciu A et al. Human Exposures to Bisphenol A, Bisphenol F and  
402 Chlorinated Bisphenol A Derivatives and Thyroid Function. *PloS One* 2016; 11: e0155237.

- 403 18. Andra SS, Makris KC. Association between urinary levels of bisphenol A and its  
404 monochlorinated derivative and obesity. *Journal of Environmental Science and Health. Part A,*  
405 *Toxic/Hazardous Substances & Environmental Engineering* 2015; 50: 1169–1179.
- 406 19. Regulation (EU) 2017/745 of the European Parliament and of the Council of 5 April 2017 on  
407 medical devices, amending Directive 2001/83/EC, Regulation (EC) No 178/2002 and Regulation  
408 (EC) No 1223/2009 and repealing Council Directives 90/385/EEC and 93/42/EEC (Text with EEA  
409 relevance. ) [Internet]. 2017 [cited 2018 Aug 23] .Available from:  
410 <http://data.europa.eu/eli/reg/2017/745/oj/eng>
- 411 20. Testai E, Hartemann P, Rastogi SC et al. The safety of medical devices containing DEHP  
412 plasticized PVC or other plasticizers on neonates and other groups possibly at risk (2015  
413 update). *Regulatory Toxicology and Pharmacology* 2016; 76: 209–210.
- 414 21. Bourdeaux D, Yessaad M, Chennell P et al. Analysis of PVC plasticizers in medical devices and  
415 infused solutions by GC-MS. *Journal of Pharmaceutical and Biomedical Analysis* 2016; 118: 206–  
416 213.
- 417 22. Silva MJ, Samandar E, Ye X, Calafat AM. In vitro metabolites of di-2-ethylhexyl adipate (DEHA)  
418 as biomarkers of exposure in human biomonitoring applications. *Chemical Research in*  
419 *Toxicology* 2013; 26: 1498–1502.
- 420 23. Eljezi T, Pinta P, Richard D et al. In vitro cytotoxic effects of DEHP-alternative plasticizers and  
421 their primary metabolites on a L929 cell line. *Chemosphere* 2017; 173: 452–459.
- 422 24. Bui TT, Giovanoulis G, Cousins AP, Magnér J, Cousins IT, de Wit CA. Human exposure, hazard  
423 and risk of alternative plasticizers to phthalate esters. *The Science of the Total Environment*  
424 2016; 541: 451–467.
- 425 25. Nehring A, Bury D, Ringbeck B et al. Metabolism and urinary excretion kinetics of di(2-  
426 ethylhexyl) adipate (DEHA) in four human volunteers after a single oral dose. *Toxicology Letters*  
427 2019;
- 428 26. Bernard L, Cueff R, Chagnon M et al. Migration of plasticizers from PVC medical devices:  
429 Development of an infusion model. *International Journal of Pharmaceutics* 2015; 494: 136–145.
- 430 27. Fernandez-Canal C, Pinta P-G, Eljezi T et al. Patients' exposure to PVC plasticizers from ECMO  
431 circuits. *Expert Review of Medical Devices* 2018; 15: 377–383.
- 432 28. Gimeno P, Thomas S, Bousquet C et al. Identification and quantification of 14 phthalates and 5  
433 non-phthalate plasticizers in PVC medical devices by GC-MS. *Journal of Chromatography. B,*  
434 *Analytical Technologies in the Biomedical and Life Sciences* 2014; 949–950: 99–108.
- 435 29. Haishima Y, Matsuda R, Hayashi Y, Hasegawa C, Yagami T, Tsuchiya T. Risk assessment of di(2-  
436 ethylhexyl)phthalate released from PVC blood circuits during hemodialysis and pump-  
437 oxygenation therapy. *International Journal of Pharmaceutics* 2004; 274: 119–129.
- 438 30. Bagel-Boithias S, Sautou-Miranda V, Bourdeaux D, Tramier V, Boyer A, Chopineau J. Leaching of  
439 diethylhexyl phthalate from multilayer tubing into etoposide infusion solutions. *American*  
440 *journal of health-system pharmacy: AJHP: official journal of the American Society of Health-*  
441 *System Pharmacists* 2005; 62: 182–188.

- 442 31. Loff S, Kabs F, Subotic U, Schaible T, Reinecke F, Langbein M. Kinetics of diethylhexyl-phthalate  
443 extraction From polyvinylchloride-infusion lines. JPEN. Journal of parenteral and enteral  
444 nutrition 2002; 26: 305–309.
- 445 32. Bernard L, Eljezi T, Clauson H et al. Effects of flow rate on the migration of different plasticizers  
446 from PVC infusion medical devices. PloS One 2018; 13: e0192369.
- 447 33. The Danish Environmental Protection Agency. Alternatives to classified phthalates in medical  
448 devices. 2014;
- 449 34. Münch F, Höllerer C, Klapproth A et al. Effect of phospholipid coating on the migration of  
450 plasticizers from PVC tubes. Chemosphere 2018; 202: 742–749.
- 451 35. Melzak KA, Uhlig S, Kirschhöfer F, Brenner-Weiss G, Bieback K. The Blood Bag Plasticizer Di-2-  
452 Ethylhexylphthalate Causes Red Blood Cells to Form Stomatocytes, Possibly by Inducing Lipid  
453 Flip-Flop. Transfusion Medicine and Hemotherapy: Offizielles Organ Der Deutschen Gesellschaft  
454 Fur Transfusionsmedizin Und Immunhamatologie 2018; 45: 413–422.
- 455 36. Haned Z, Moulay S, Lacorte S. Migration of plasticizers from poly(vinyl chloride) and multilayer  
456 infusion bags using selective extraction and GC-MS. Journal of Pharmaceutical and Biomedical  
457 Analysis 2018; 156: 80–87.
- 458 37. Bosch-Panadero E, Mas S, Sanchez-Ospina D et al. The Choice of Hemodialysis Membrane  
459 Affects Bisphenol A Levels in Blood. Journal of the American Society of Nephrology: JASN 2016;  
460 27: 1566–1574.
- 461 38. Bacle A, Thevenot S, Grignon C et al. Determination of bisphenol A in water and the medical  
462 devices used in hemodialysis treatment. International Journal of Pharmaceutics 2016; 505:  
463 115–121.
- 464 39. Alonso-Magdalena P, Morimoto S, Ripoll C, Fuentes E, Nadal A. The estrogenic effect of  
465 bisphenol A disrupts pancreatic beta-cell function in vivo and induces insulin resistance.  
466 Environmental Health Perspectives 2006; 114: 106–112.
- 467 40. Lang IA, Galloway TS, Scarlett A et al. Association of urinary bisphenol A concentration with  
468 medical disorders and laboratory abnormalities in adults. JAMA 2008; 300: 1303–1310.
- 469 41. Borch J, Ladefoged O, Hass U, Vinggaard AM. Steroidogenesis in fetal male rats is reduced by  
470 DEHP and DINP, but endocrine effects of DEHP are not modulated by DEHA in fetal, prepubertal  
471 and adult male rats. Reproductive Toxicology (Elmsford, N.Y.) 2004; 18: 53–61.
- 472 42. Miyata K, Shiraishi K, Houshuyama S et al. Subacute oral toxicity study of di(2-  
473 ethylhexyl)adipate based on the draft protocol for the “Enhanced OECD Test Guideline no.  
474 407.” Archives of Toxicology 2006; 80: 181–186.
- 475 43. Sheikh IA, Beg MA. Structural characterization of potential endocrine disrupting activity of  
476 alternate plasticizers di-(2-ethylhexyl) adipate (DEHA), acetyl tributyl citrate (ATBC) and 2,2,4-  
477 trimethyl 1,3-pentanediol diisobutyrate (TPIB) with human sex hormone-binding globulin.  
478 Reproductive Toxicology (Elmsford, N.Y.) 2019; 83: 46–53.
- 479 44. Ghisari M, Bonefeld-Jorgensen EC. Effects of plasticizers and their mixtures on estrogen  
480 receptor and thyroid hormone functions. Toxicology Letters 2009; 189: 67–77.

- 481 45. Luo H, Sun G, Shi Y, Shen Y, Xu K. Evaluation of the Di(2-ethylhexyl)phthalate released from  
482 polyvinyl chloride medical devices that contact blood. SpringerPlus 2014; 3: 58.
- 483 46. Bastiaensen M, Malarvannan G, Been F et al. Metabolites of phosphate flame retardants and  
484 alternative plasticizers in urine from intensive care patients. Chemosphere 2019; 233: 590–596.
- 485 47. Bernard L, Décaudin B, Lecoeur M et al. Analytical methods for the determination of DEHP  
486 plasticizer alternatives present in medical devices: a review. Talanta 2014; 129: 39–54.
- 487
- 488

489 **Figures**

490 Figure 1: Metabolism of DEHA in humans (reproduced from Nehring et al, (25))

491 Figure 2: Scheme of Continuous Venovenous Hemofiltration (CVVH) model with ethanolic simulant

492 Figure 3: Scheme of Continuous Venovenous Hemofiltration (CVVH) model with blood simulant

493 Figure 4: Composition of the Prismaflex® set used in the study (DEHA % of plasticized PVC mass)  
494 (from ecatalog.baxter.com)

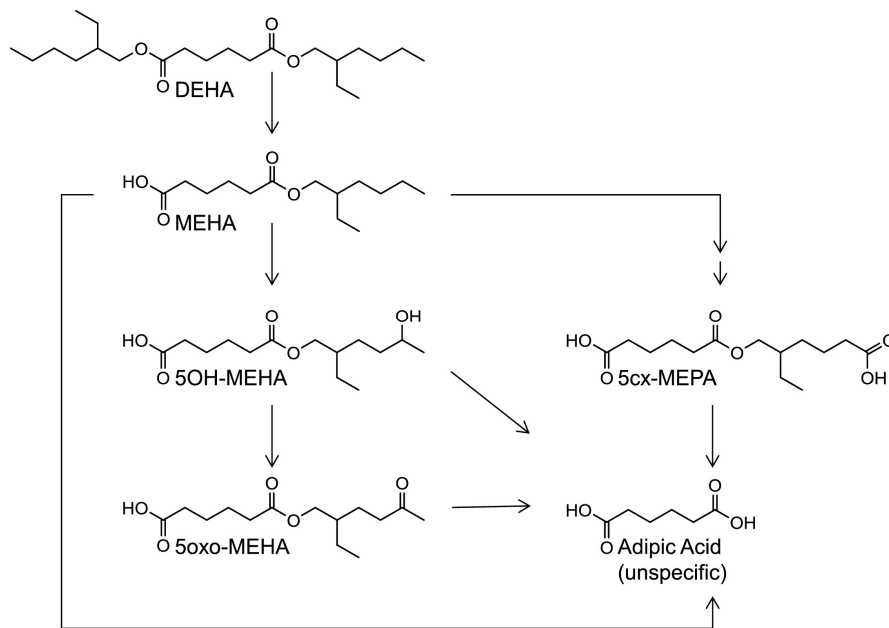
495 Figure 5: Quantity of DEHA released in the CVVH circuit (first model) at each contact time  
496 (concentration in µg/mL, quantity in mg) and amount in %, g per 100 g of PVC (mean ± standard  
497 deviation)

498 Figure 6: Concentrations of DEHA and MEHA (mean with confidence intervals) in blood during the  
499 assay with the second model

500 Figure 7: Pre- and postfilter concentrations of DEHA (mean with 95% confidence intervals) released  
501 from Prismaflex® tubings versus the square root of the time for the first 24 hours of the Continuous  
502 Venovenous Hemofiltration (CVVH) procedure

503 Figure 8: Pre- and postfilter concentrations of MEHA (mean with 95% confidence intervals) released  
504 from Prismaflex® tubings versus the square root of the time for the first 24 hours of the Continuous  
505 Venovenous Hemofiltration (CVVH) procedure

506

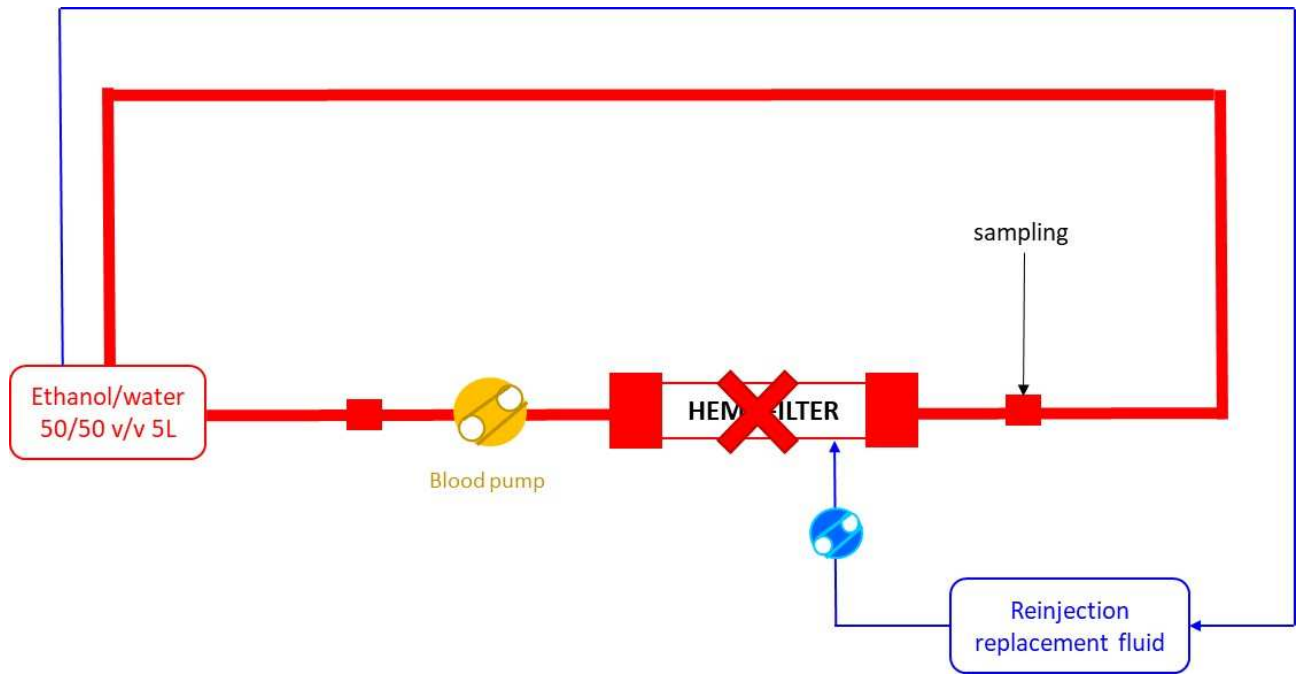


507

508 *Figure 1 : Metabolism of DEHA in humans (reproduced from Nehring et al, (25))*

509

510

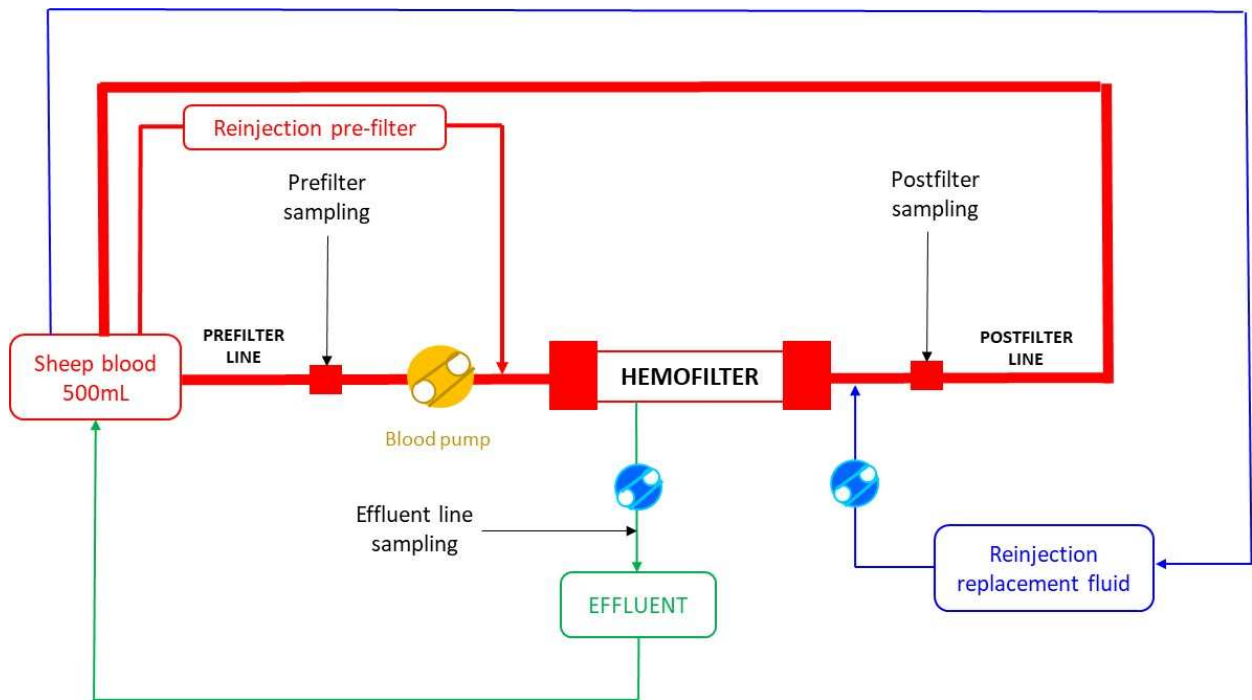


511

512 *Figure 2: Scheme of Continuous Venovenous Hemofiltration (CVVH) model with ethanolic simulant*

513

514

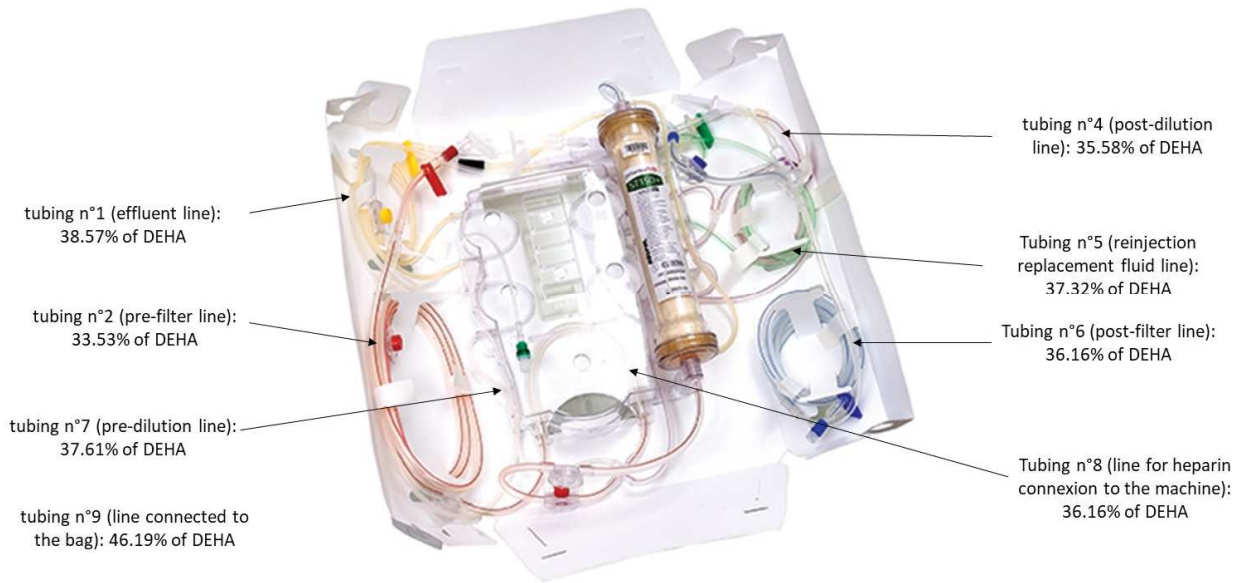


515

516 *Figure 3: Scheme of Continuous Venovenous Hemofiltration (CVVH) model with blood simulant*

517

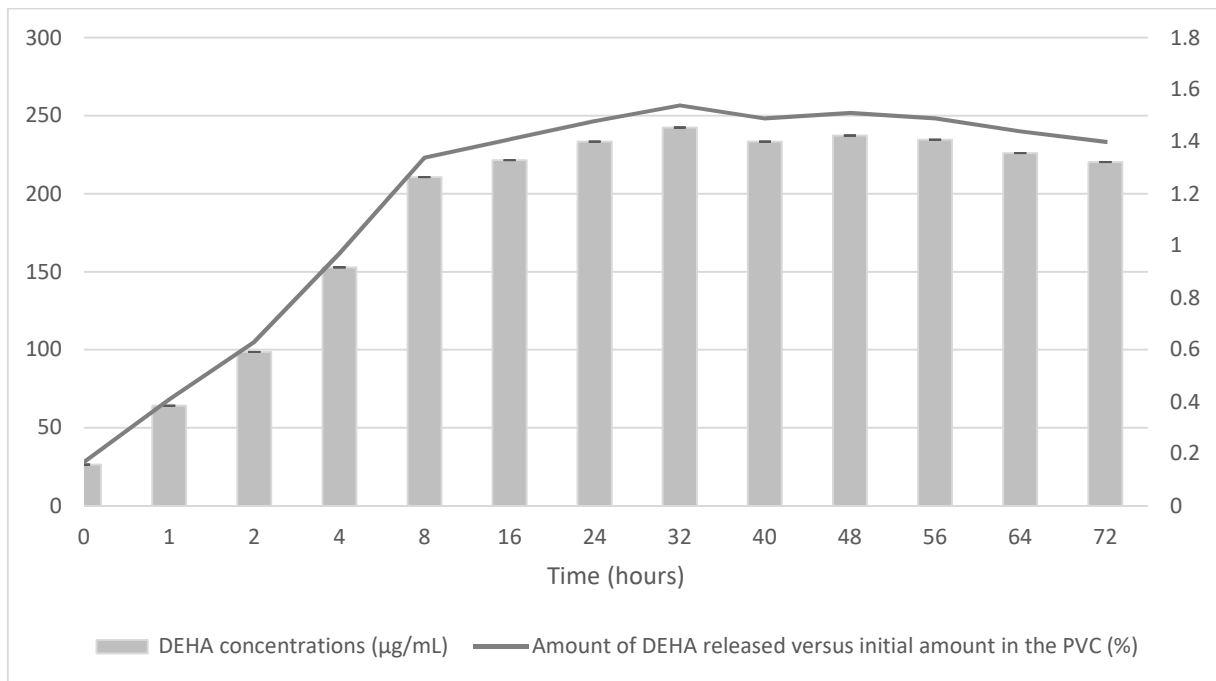
518



519

520 *Figure 4: Composition of the Prismaflex® set used in the study (DEHA % of plasticized PVC mass) (from ecatalog.baxter.com)*

521



522

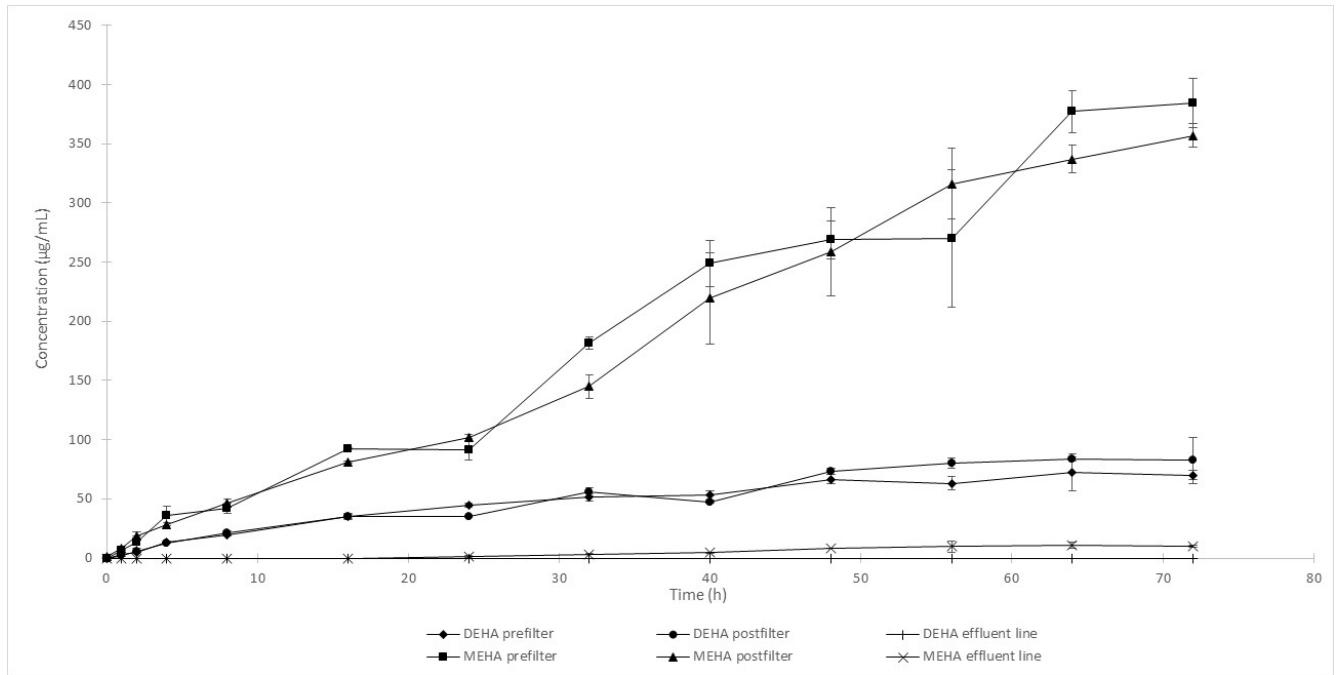
523 *Figure 5 : Quantity of DEHA released in the CVVH circuit (first model) at each contact time (concentration in µg/mL, quantity*  
 524 *in mg) and amount in %, g per 100 g of PVC) (mean ± standard deviation)*

525

526

527

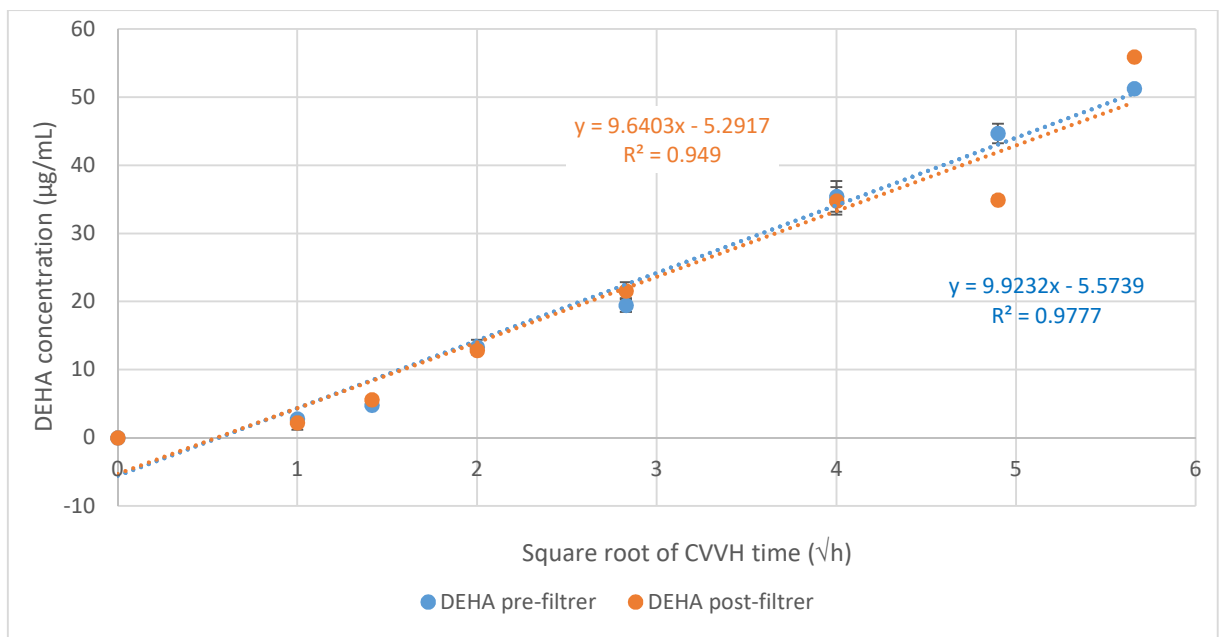




528  
529  
530

Figure 6: Concentrations of DEHA and MEHA (mean with confidence intervals) in blood during the assay with the second model

531



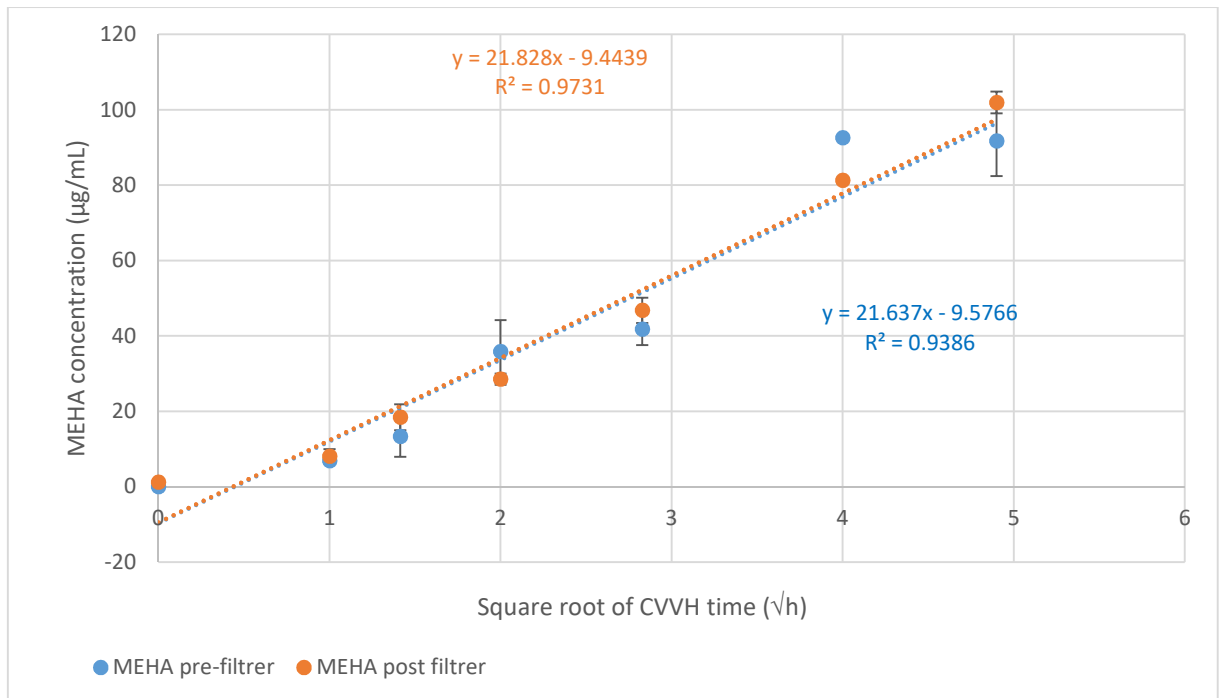
532

533  
534

Figure 7: Pre- and postfilter concentrations of DEHA (mean with 95% confidence intervals) released from Prismaflex® tubings versus the square root of the time for the first 24 hours of the Continuous Venovenous Hemofiltration (CVVH) procedure

535

536



537

538 *Figure 8: Pre- and postfilter concentrations of MEHA (mean with 95% confidence intervals) released from Priimaflex®*  
539 *tubings versus the square root of the time for the first 24 hours of the Continuous Venovenous Hemofiltration (CVVH)*  
540 *procedure*

541