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

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

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Abstract

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 Due to its known reprotoxicity, diethylhexyl phthalate (DEHP) has been replaced by alternatives such as diethylhexyladipate (DEHA) in some of these tubings.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

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

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

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

Keywords

Continuous venovenous hemofiltration, medical devices, plasticizer, diethylhexyladipate

Highlights

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

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

1. Introduction

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

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

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

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

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

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

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

2. Materials and methods

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

Medical devices used in the study:

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

Plasticizers and metabolites used for analytical quantification:

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

2.1 The models

2.1.1 CVVH model with ethanolic simulant

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

The model is presented in figure 2.

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

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

- Flow rate: the flow rates were set up in accordance with the standard CVVH practice, i.e the blood flow rate was set up at 200 mL/min, the prefilter flow rate at 800 mL/h, and the replacement fluid flow rate at 1600 mL/h.
- Contact time: the experiment was performed during 72 hours.

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

2.1.2 CVVH model with blood simulant

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

The model is presented in figure 3.

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

- Simulant: 10 UI/mL heparinized sheep blood provided by Fiebig Nährstofftechniik animal blood products. The volume of blood used was 500 mL and was regularly replaced with 312mL during the procedure to compensate the losses due to the samplings.
- Flow rate: the flow rates were set up in accordance with the standard CVVH practice , i.e the blood flow rate was set up at 200 mL/min, the prefilter flow rate at 800 mL/h, and the replacement fluid flow rate at 1600 mL/h
- Contact time: the experiment was performed during 72 hours.

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

2.2 Samples

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

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

2.3 Analysis of DEHA and its primary metabolite

Different analyses were conducted:

- Quantification of DEHA:
    - in the PVC matrix of the medical devices: to obtain the initial amount of DEHA in MD before performing the migration assay according to the model
    - in the ethanol/water simulant at each contact time within the first model
- Quantification of DEHA and its metabolites in the sheep plasma from the blood collected at each contact time within the second model.
-

Plasticizer quantification was carried out by gas chromatography coupled with mass spectrometry (GC-MS) in the simulant solution samples or after a chloroform extraction from the MD or from the simulant (supplementary file no1, SF no1), according to the method published by Bourdeaux *et al*. (21). Briefly, plasticizer quantification was performed using a Clarus 500 (Perkin Elmer,USA) using electronic impact ionization 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). The oven temperature curve started at 200 °C for 1 min then rose to 300 °C at a rate of 20 °C/min.

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

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

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

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

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

The validation parameters of this method were as follow :

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

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

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

3. Results

3.1 DEHA in PVC MD

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

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

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

3.2 Migration of DEHA into ethanolic simulant during CVVH

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

The release of DEHA follows a linear release kinetic during the first 8 hours of contact and reaches a plateau at about 230 µg/mL, which corresponds to a quantity higher than 1200 mg of DEHA (in 5 L of simulant) potentially received by the patient.

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

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

The figure 6 presents the concentrations of DEHA and MEHA in µg/mL at the three sites of sampling (pre-filter, post-filter and effluent line)

DEHA and MEHA were found at T0 (immediately after priming) but not at the same sites: DEHA is only present in the effluent line at a background level of 0.15±0.02 µg/mL whereas 1.17±0.05 µg/mL of MEHA is found at the post-filter site. DEHA and MEHA appear gradually into blood (pre- and postfilter).

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

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

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

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

4. Discussion

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

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

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

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

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

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

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

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

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

- the first model was done in a closed-loop, which may explain the higher concentrations of DEHA released in ethanol than those released into blood. As the extraction ability of the water/ethanol mixture is higher than that of blood as demonstrated by Luo et al (45), the conditions of this first model may have led to an overestimation of the maximum exposure dose of DEHA.
- Contrary to the *ex-vivo* models of Lewis et al (10) or Haishima et al (29), we studied the concentrations of both the plasticizer DEHA and its metabolite MEHA, which helps understand and assess the real risk of exposure linked to their respective toxicities. However, to reflect the clinical conditions, this study should be completed by an *in-vivo* evaluation (biomonitoring) of the oxidized metabolites of DEHA in urines of inpatients undergoing CVVH. In the work of Bastiaensen et al,

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

5. Conclusions

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

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Figures

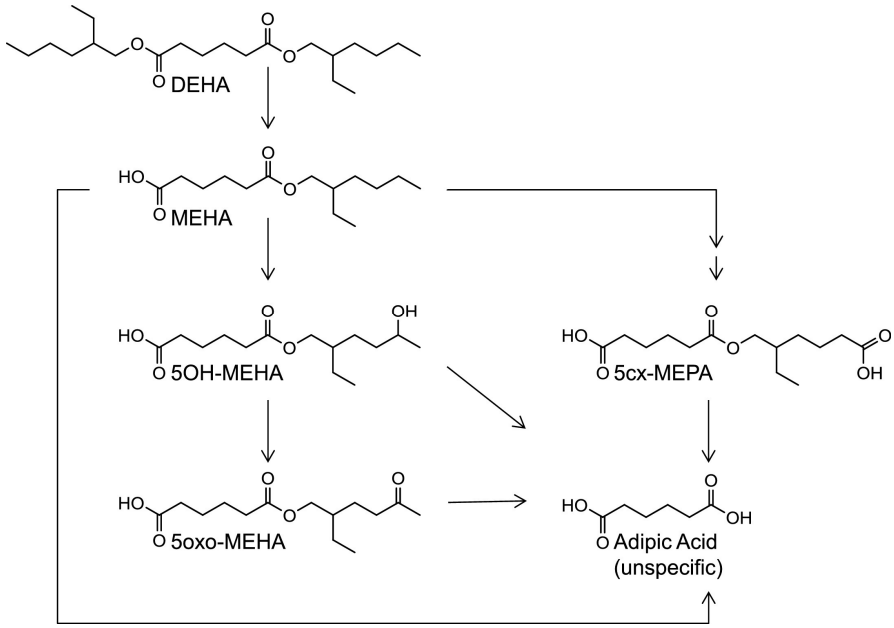
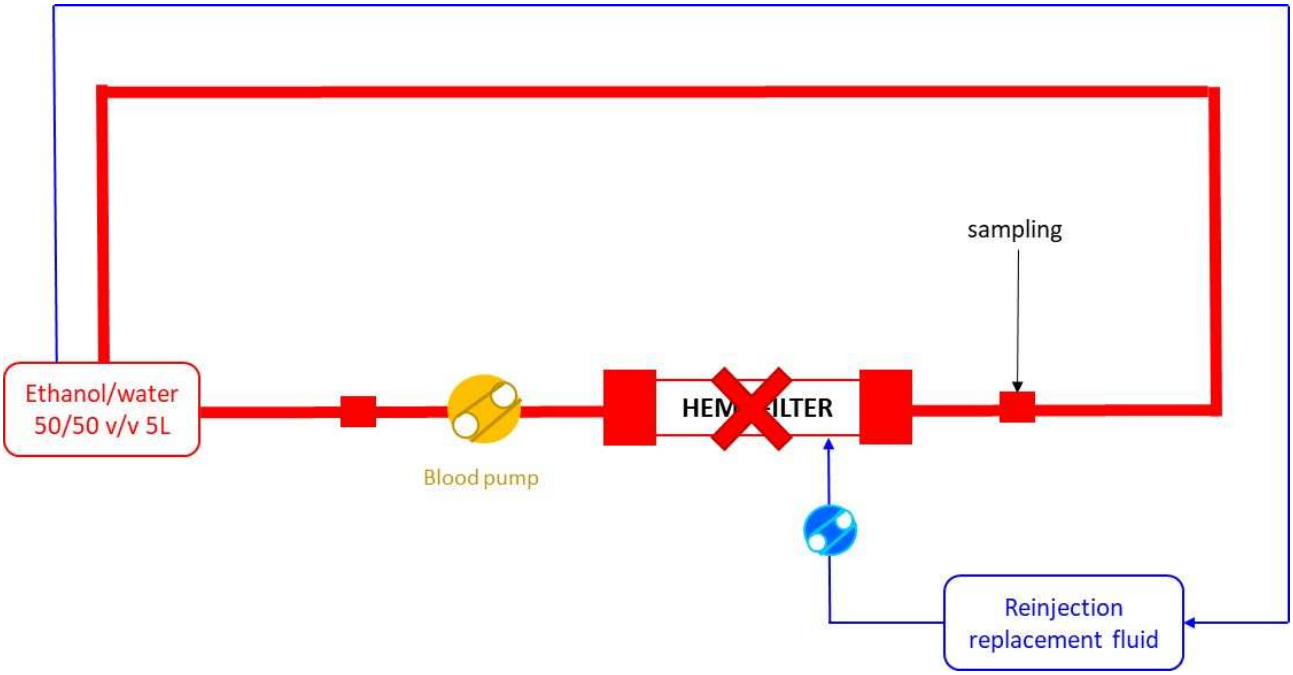


Figure 1 : Metabolism of DEHA in humans (reproduced from Nehring et al, (25))



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

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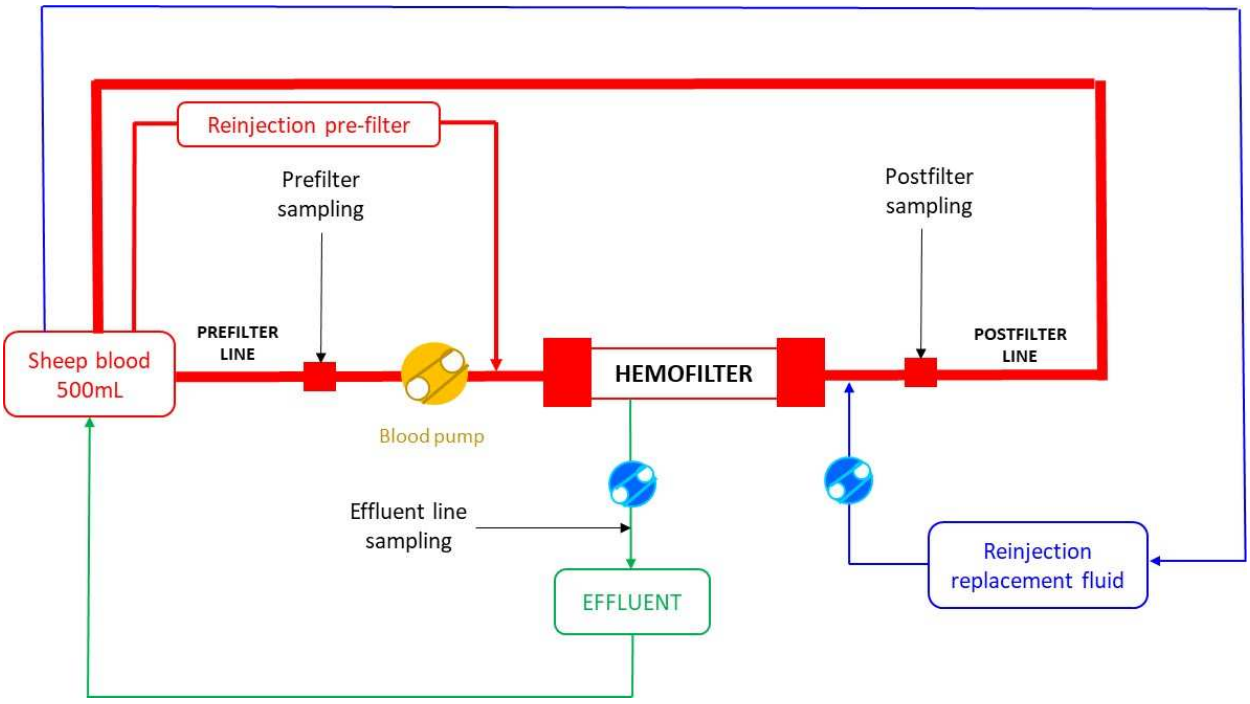

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516 *Figure 3: Scheme of Continuous Venovenous Hemofiltration (CVVH) model with blood simulant*

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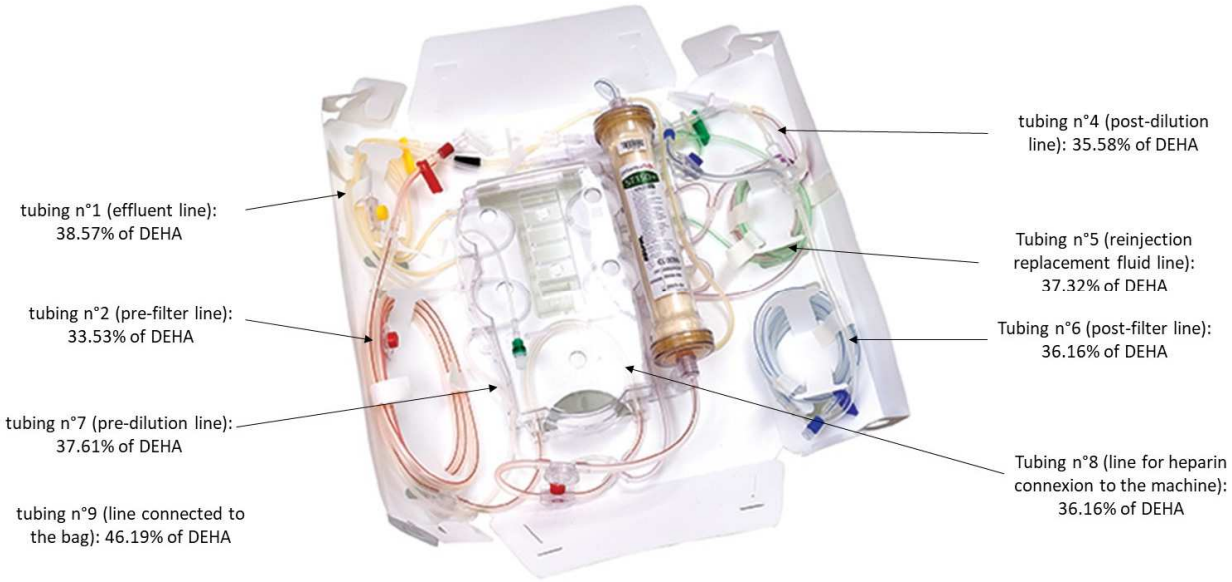

Figure 4: Composition of the Prismaflex® set used in the study (DEHA % of plasticized PVC mass) (from ecatalog.baxter.com)

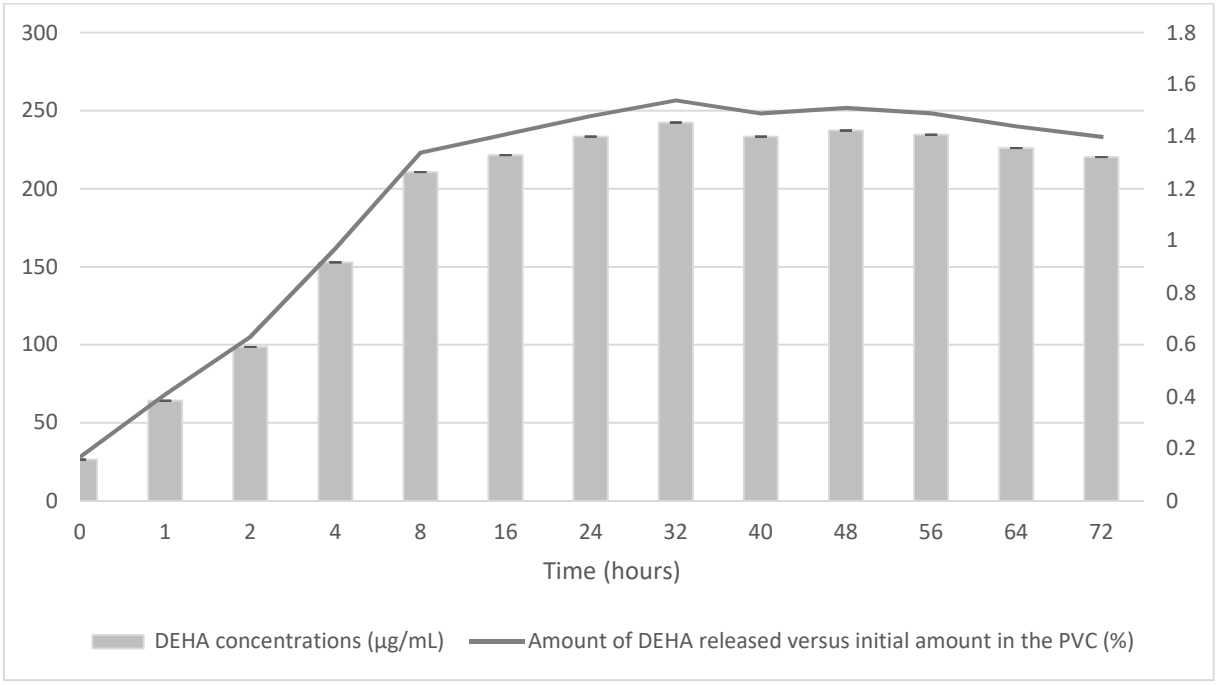

Figure 5 : Quantity of DEHA released in the CVVH circuit (first model) at each contact time (concentration in µg/mL, quantity in mg) and amount in %, g per 100 g of PVC) (mean ± standard deviation)

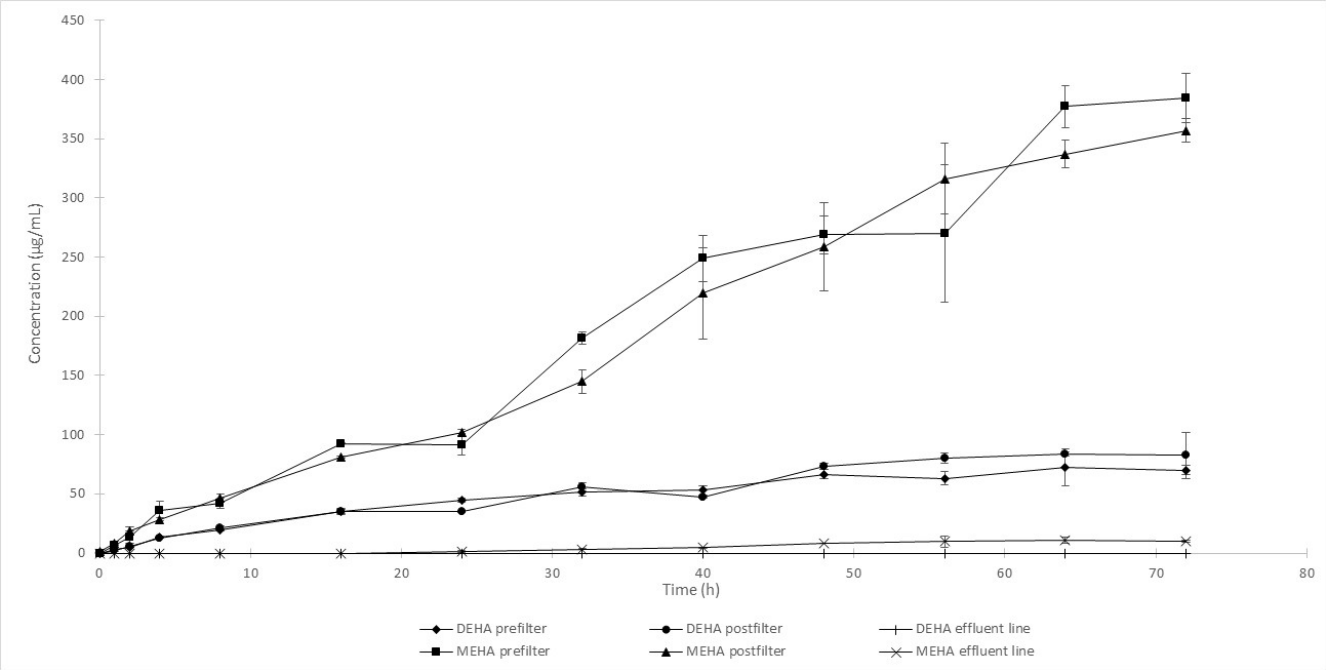

Figure 6: Concentrations of DEHA and MEHA (mean with confidence intervals) in blood during the assay with the second model

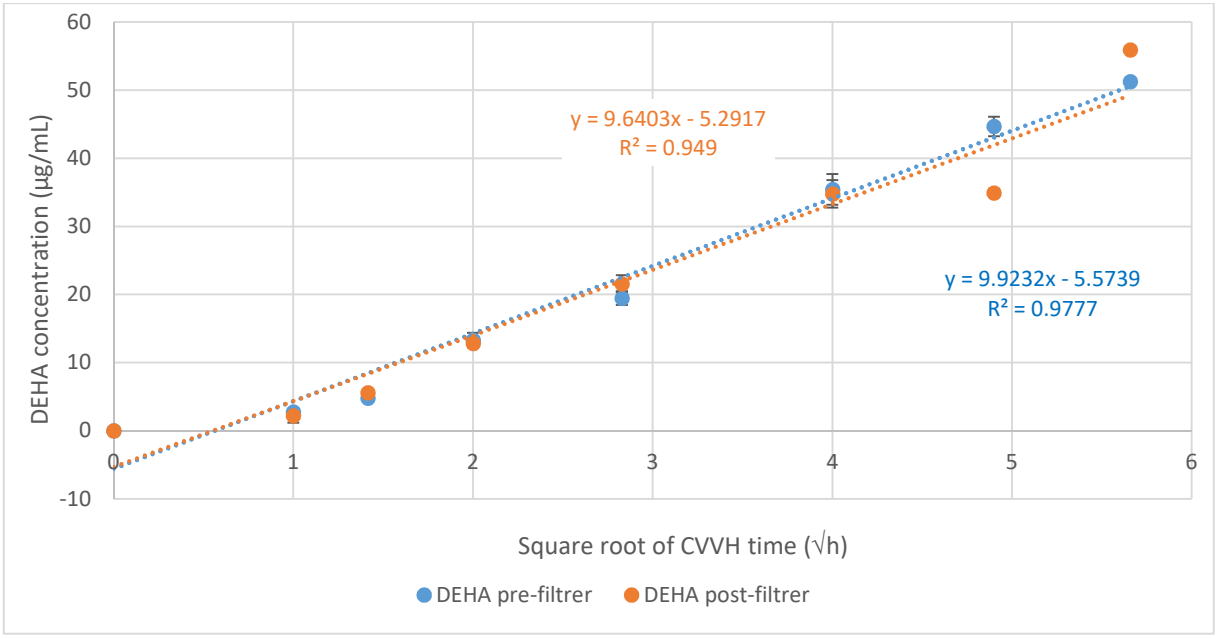

Figure 7: Pre- and postfilter concentrations of DEHA (mean with 95% confidence intervals) released from Prismaflex® tubings versus the square root of the time for the first 24 hours of the Continuous Venovenous Hemofiltration (CVVH) procedure

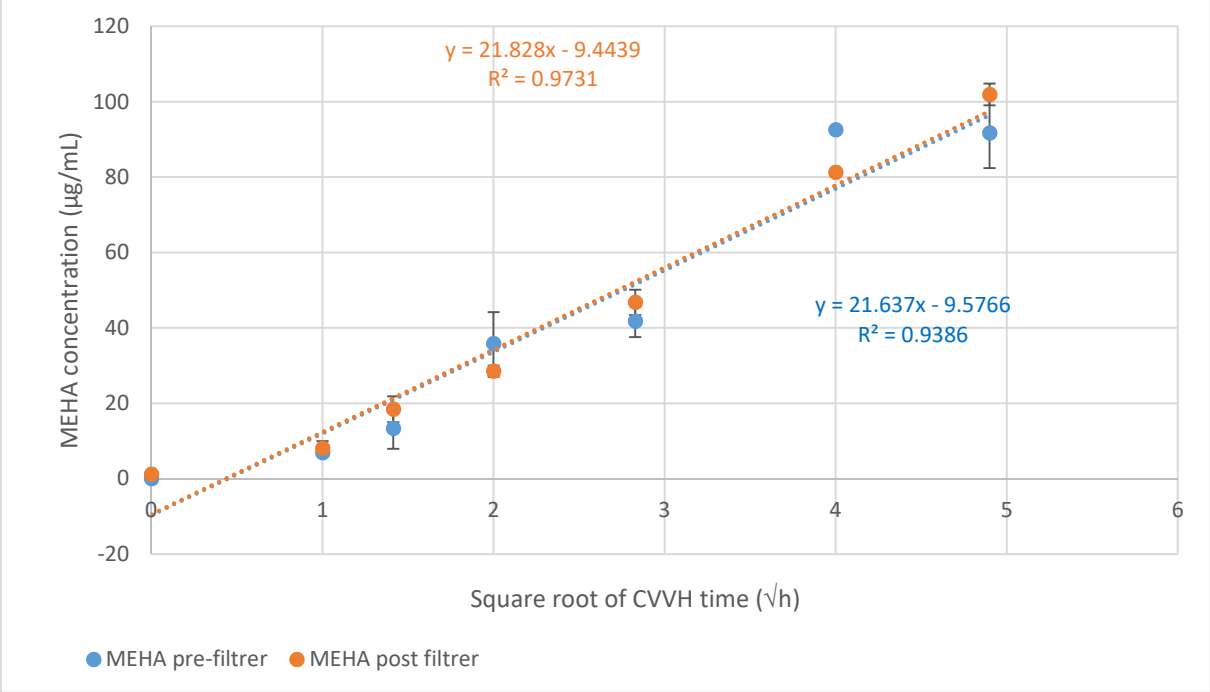

Figure 8: Pre- and postfilter concentrations of MEHA (mean with 95% confidence intervals) released from Priimaflex® tubings versus the square root of the time for the first 24 hours of the Continuous Venovenous Hemofiltration (CVVH) procedure