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Metabolites of Phosphate Flame Retardants and Alternative Plasticizers in Urine from Intensive Care Patients

Michiel Bastiaensen[1*], Govindan Malarvannan[1], Frederic Been[1], Shanshan Yin[1], Yiming Yao[1,3], Johan Huygh[2], Katrien Clotman[2], Tom Schepens[2], Philippe G. Jorens[2], Adrian Covaci[1*]

[1] Toxicological Centre, Department of Pharmaceutical Sciences, University of Antwerp, Universiteitsplein 1, 2610 Wilrijk, Belgium

[2] Department of Critical Care Medicine, Antwerp University Hospital, University of Antwerp, Wilrijkstraat 10, 2650 Edegem, Belgium

[3] MOE Key Laboratory of Pollution Processes and Environmental Criteria, College of Environmental Science and Engineering, Nankai University, Tianjin 300071, China

*Corresponding Authors: michiel.bastiaensen@uantwerpen.be; adrian.covaci@uantwerpen.be

Abstract

Several regulatory offices called for the phase-out of di (2-ethylhexyl) phthalate (DEHP) in medical devices if safer alternatives are available. In medical devices, the occurrence of alternative plasticizers (APs) is widely variable among types of devices. However, plasticizer use is constantly evolving, as there is no reference to guide manufacturers in the choice and amount to be integrated into their products. As intensive care unit (ICU) patients need numerous indwelling plastic devices during their treatment, we hypothesized that these patients are exposed to APs and phosphate flame retardants and plasticizers (PFRs). Urinary metabolites of APs and PFRs were analyzed in the urine of adult ICU patients (n=24) over a time period of four days. Our results show that adult ICU patients are exposed to PFRs as well as to APs concentrations were much lower compared to the levels of DEHP metabolites in the same samples. However, significantly higher than in controls (n=15) this exposure resulted in detectable urinary levels in almost every patient and at every studied time point. Increasing temporal trends were observed for several metabolites from admission until day 3 at ICU. The use of specific medical devices such as continuous venovenous hemofiltration (CVVH) and extracorporeal membrane oxygenation (ECMO) was associated with an increase in urinary concentrations for several PFR metabolites, despite

the lack of information on the presence of these plasticizer chemicals in such medical devices. Further research into the possibly toxic effects of these chemicals released from medical devices is urgently needed.

Keywords

Alternative plasticizers; phosphorous flame retardants; medical devices; intensive care; biomonitoring; urine

Introduction

Medical devices are composed of polymer plastics, such as polyvinylchloride (PVC), that require plasticizers to obtain their characteristic flexibility and softness [1-3]. The phthalate di(2-ethylhexyl)phthalate (DEHP) was the most frequently used plasticizer until it was listed by the European authorities as carcinogenic, mutagenic, reprotoxic (CMR1B) in 2008 [4]. This led to its replacement in some medical devices with alternative plasticizers (APs), such as di-(2-ethylhexyl) adipate (DEHA), di(isononyl)-cyclohexane-1,2-dicarboxylic acid (DINCH), di-(2-ethylhexyl) terephthalate (DEHT), acetyl tri-nbutyl citrate (ATBC), and tris (2-ethylhexyl) trimellitate (TOTM) [5, 6]. Some of these alternative plasticizers are used in specific devices, i.e. DINCH or ATBC are mainly used in red blood cell PVC bags due to their capacity to prevent excessive haemolysis during storage [7]. However, plasticizer use is constantly evolving as there is no reference to guide manufacturers in the choice of plasticizers and the amount to be integrated into their products.

Currently, toxicity data on APs and information regarding leaching from medical devices are scarce or at most incomplete [8, 9]. It is also unclear whether other plasticizer chemicals, such as phosphate flame-retardants and plasticizers (PFRs), are applied in medical devices as an alternative to DEHP. One study found that certain whole blood collection systems contained high concentrations of 2-ethylhexyl diphenyl phosphate (EHDPHP) and triphenyl phosphate (TPHP), which might be of significance for the medical treatment of patients who have a large part of their plasma exchanged [10]. Another study has reported higher concentrations of ΣPFRs in hospital dust compared to private home environments due to the presence of tris(2-butoxyethyl) phosphate (TBOEP), tris(1,3-dichloro-2-propyl) phosphate (TDICPP), tris(chloroethyl) phosphate (TCEP) and tris(2-chloroisopropyl) phosphate (TCIPP) [11]. Although both APs and PFRs are increasingly used worldwide, data gaps still exist about the potential toxicological endpoints which indicates the need not only for robust toxicity but also exposure data [6, 12]. Similar to phthalates, APs and PFRs can gradually leach from products and migrate into the surrounding environment (dust, blood or other body fluids) since they are not covalently bound to the plastics in which there are embedded [11, 13]. Critically ill patients treated in intensive care units (ICU) are potentially exposed to high levels of these chemicals as they are connected to a large number and

different types of plastic medical devices. Previous studies have shown that ICU patients, both adults and children, had extremely elevated levels of phthalate esters and bisphenol A (BPA) in serum and urine compared to healthy individuals [14, 15]. In fact, it has been reported that urinary DEHP and BPA metabolite concentrations were significantly correlated with the number of medical devices used in neonatal intensive care units [16, 17]. Increasing concerns have been raised regarding the migration of DEHP from medical devices and the consequent exposure of ICU patients, particularly for neonates [18]. Yet, no information is currently available in this regard for other plasticizer chemicals.

The goal of this work was to assess if ICU patients are exposed to PFRs and APs by analyzing specific metabolites in urine and to compare these levels to those of healthy individuals (who were not treated at ICUs). Furthermore, our study aimed at determining if an increase in the exposure levels to these chemicals could be observed upon admission and if the intensity of the exposure could be explained by the use of two frequently used types of devices consisting of large plastic tubing i.e. dialysis and extracorporeal membrane oxygenation (ECMO).

Materials and methods

Urine samples (n=84) were obtained from 24 patients on admission to the ICU of the Antwerp University Hospital and repeated samples were taken during the following 3 days of their stay in the ICU [14]. Collection always took place at the same time of the day. The group of admitted patients also included six patients who were enrolled preoperatively before a scheduled non-cardiac thoracic surgical intervention. In this group, urine samples were obtained on admission in the hospital (< 24 h pre-operatively), and repeat samples were taken after surgery and installation of indwelling medical devices during their stay in the ICU (also 4 days in total, when available). We hypothesized that the levels could be higher in patients in whom specific devices with large and long plastic tubing were used. Some patients in the study population were treated with continuous venovenous hemofiltration (CVVH) (Prismaflex® with M150® hemofilter from Gambro) or extracorporeal membrane oxygenation (ECMO) (oxygenator Quadrox ID® from Maquet; tubing and centrifugal pump Revolution® available from Sorin). The exclusion criteria were liver failure, known allergies to plastics, anuric patients for

whom renal replacement therapy is not feasible or was declined, a hospital stay of > 48 h prior to ICU admission, pregnancy and the presence of chronically implanted medical devices (ventriculoperitoneal shunt, chronic dialysis catheter and pacemaker). Information on included patients and the analyzed samples is presented in Table SI-1. Furthermore, urine samples from 15 healthy individuals were used as a control (healthy adults, no patients, not age and gender matched but from the same sampling year). Our study was approved by the Ethical Committee of the Antwerp University Hospital (EC Reference Number: 12/46/373, Belgian Registry Number: B300201215630). Written informed consent was obtained from either the patient or their closest relative. All data were processed anonymously.

Urine samples were analysed using validated analytical protocols based on solid-phase extraction (SPE) and liquid-chromatography tandem mass-spectrometry (LC-MS/MS) [19, 20]. Target compounds are listed in Table SI-2. BPA and DEHP metabolites were previously measured in the same population and data were taken from Huygh et al [14]. Urinary creatinine levels were not available for all samples. Adjustment for urinary dilution was therefore omitted from this study. Limits of quantification (LOQ) were calculated as three times the standard deviation of procedural blank concentrations. Data below the limit of quantification (LOQ) were filled up with a value LOQ x detection frequency of the batch (ICU or controls) as suggested by James et al [21]. Differences between subgroups, i.e. ICU vs controls, time trends over 4 days and associations with medical devices (CVVH and/or ECMO), were investigated with non-parametric testing (Mann-Whitney U test for binominal data, Kruskal-Wallis for multiple subgroups). To reduce the chances of obtaining false positive results, the Bonferroni correction was applied when multiple pairwise comparisons were calculated with Kruskal-Wallis test. In that case the obtained p-value was divided by the number of comparisons being tested. Associations with medical devices were calculated for the whole study population, healthy controls included. Two-sided p-values of 0.05 or less were considered to indicate statistical significance. Only metabolites with a detection frequency above 50% were included in statistical testing. Statistical analysis was carried out using SPSS Statistics version 24 (IBM Corp, Armonk, NY, US) and figures were prepared using the open-source software package R (version 3.5.0).

Results and discussion

Concentrations of PFR metabolites

Concentrations of PFR metabolites measured in urine samples are reported in Table 1. Overall, detection frequencies and median concentrations of PFR metabolites were higher in samples from ICU patients compared to controls. Maximum concentrations were higher in the ICU subgroup for all compounds. Compounds such as 4-hydroxyphenyl phenyl phosphate (4-HO-DPHP) and 4-hydroxyphenyl diphenyl phosphate (4-HO-TPHP) could be detected in urine samples from ICU, whilst these were mostly not detected in controls. This is in agreement with other biomonitoring studies where these two TPHP metabolites are rarely detected in non-occupationally exposed people [19]. Despite the increased concentrations of hydroxylated TPHP metabolites in ICU patients, there was no statistically significant difference for diphenyl phosphate (DPHP, p=0.244), the main metabolite of TPHP. This is most likely due to the fact that DPHP is not a specific metabolite of TPHP, since it can also be formed from other PFRs such as EHDPHP and resorcinol bis-diphenyl phosphate (RDP) [22]. Other metabolite levels that were higher in ICU patients were bis(2-butoxyethyl) phosphate (BBOEP) and bis(2-butoxyethyl) 3′-hydroxy-2-butoxyethyl phosphate (3-HO-TBOEP). Interestingly enough, also here the main metabolite of TBOEP (i.e. 2-hydroxyethyl bis(2-butoxyethyl) phosphate (BBOEHEP)) was not found in higher concentrations compared to controls (p=0.527). This seems contradictory since these metabolites originated from the same parent compound (as shown in Table 1 and Table SI-2). However, the 75th percentile and maximum concentrations of BBOEHEP and DPHP were substantially higher in ICU, which is also true for other metabolites (bis(1-chloro-2-propyl) phosphate (BCIPP), di-n-butyl phosphate (DNBP), bis(1,3-dichloro-2-propyl) phosphate (BDCIPP) and tris(chloroethyl) phosphate (TCEP)).

The obtained results illustrate that substantially higher levels of PFR exposure biomarkers could be measured in urine samples from ICU patients and suggest that intensive care patients are likely exposed to higher levels of PFRs compared to healthy individuals. Currently, there is no literature available to compare with these data. We hypothesize that these chemicals also leached from plastic devices (see

below). Other potential sources of PFRs in hospitals include exposure to other sources: floor waxes, electronics, upholstered furniture, and PVC coverings [11, 23]. Hospitals also have stricter fire safety standards compared to other public buildings which might be another explanation for increased exposure to PFRs for hospitalized patients [11, 12]. Only one study from Sweden has compared PFR dust concentrations from various indoor environments. The distribution pattern of PFRs found in hospitals was similar to that of schools and offices: an overwhelming dominance of TBOEP followed by tris(1,3-dichloro-2-propyl) phosphate (TDCIPP), tris(chloroethyl) phosphate (TCEP) and tris(2-chloroisopropyl) phosphate (TCIPP), but most importantly significantly higher ΣPFRs compared to private home environments [11]. In our study, metabolites of TBOEP, TCIPP and TDCIPP were more frequently detected in higher concentrations in ICU patients compared to control individuals. However, it is unlikely that dust is the main exposure route for ICU patients because the majority of the patients were ventilated in closed extra-corporeal circuits. Furthermore, median levels of PFR metabolites were several times lower than the median levels of DEHP metabolites measured in the same samples (median concentrations of DEHP metabolites mono-(2-ethyl-5-carboxypentyl) phthalate (5Cx-MEPP), mono-(2-ethyl-5-hydroxyhexyl) phthalate (5OH-MEHP), mono-(2-ethyl-5-oxohexyl) phthalate (5oxo-MEHP) in general ICU: 65.6, 62.4 and 29.7 ng/mL respectively), which still indicates the relative importance of DEHP compared to other plasticizers that might have been present [14].

Concentrations of AP metabolites

In our study population, levels of AP metabolites too were generally higher in patients admitted to the ICU compared to controls (see Table 1). Similar to PFRs, maximum concentrations were higher in ICU for all investigated compounds. Almost all metabolites were detected in ICU samples, albeit at low detection frequencies, compared to control samples where many metabolites were not detected at all. Contradictory results were also found for AP metabolites derived from the same parent compound. Two metabolites of di-2-ethylhexyl adipate (DEHA) were found in higher concentrations in ICU (i.e. mono(2-ethylhexyl) adipate (MEHA) and mono(2-ethyl-5-hydroxyhexyl) adipate (5-HO-MEHA), while mono(2-ethyl-5-oxohexyl) adipate (5-oxo-MEHA) was not detected in ICU samples. Concentrations of AP metabolites were lower than those of PFRs metabolites in the same samples.

178 Compared to our pilot study of 2018, levels of healthy individuals in this study were lower [20]. This is
179 most likely due to the fact that sampling for the current study took place, only shortly after the worldwide
180 increase in production volumes of APs [6]. The reported values of DINCH metabolites (cx-MINCH,
181 OH-MINCH, MINCH and oxo-MINCH) are roughly comparable to concentrations measured in US and
182 German adults [24, 25].Overall, these findings suggest that there might be specific sources of APs
183 present in the ICU environment. However, due to the low detection frequencies of the targeted
184 compounds (i.e. maximum 40% detected) these results should be considered as preliminary.

185 Table 1: Distribution of urinary concentrations of PFR and AP metabolites for both study populations (controls and ICU patients). N is the total number of
186 samples. Statistical difference between the subgroups was calculated by Mann-Whitney U test. DF: detection frequency; n.d.: not detected; n.a.: not applicable.

	Concentrations in ng/mL		Controls (n=15)						ICU (n=84)						p-value
		LOQ	DF (%)	Min	25th	Median	75th	Max	DF (%)	Min	25th	Median	75th	Max	
PFR metabolites	BCIPP	1	0					n.d.	10				n.d.	10.33	n.a.
	BCIPHIPP	0.04	93	n.d.	0.36	0.54	1.65	30.40	95	n.d.	0.41	1.90	5.29	37.18	0.114
	4-HO-DPHP	0.5	0					n.d.	23				n.d.	28.28	n.a.
	DPHP	0.048	100	0.35	0.62	0.94	1.65	5.24	99	0.05	0.58	1.29	4.30	31.27	0.244
	4-HO-TPHP	0.01	7				n.d.	0.07	30			n.d.	0.07	11.78	n.a.
	3-HO-TPHP	0.01	0				n.d.	n.d.	0				n.d.	n.d.	n.a.
	DNBP	0.17	93		n.d.	0.17	0.25	0.45	23				n.d.	3.89	n.a.
	BDCIPP	0.05	80	n.d.	0.12	0.30	0.61	9.05	80	n.d.	0.06	0.57	4.78	98.65	0.130
	TCEP	0.032	100	0.04	0.05	0.05	0.08	0.12	46			n.d.	0.05	0.29	n.a.
	5-HO-EHDPHP	0.005	100	0.02	0.03	0.03	0.12	0.30	74		n.d.	0.03	0.10	2.56	0.414
	EHPHP	0.025	100	0.59	1.20	2.84	3.75	8.00	89	n.d.	0.31	0.89	2.56	31.16	0.010
	BBOEHEP	0.005	100	0.01	0.01	0.02	0.05	0.34	79	n.d.	0.01	0.02	0.09	5.68	0.527
	BBOEP	0.05	7				n.d.	0.06	27			n.d.	0.06	0.61	n.a.
	3-HO-TBOEP	0.01	0					n.d.	19				n.d.	11.17	n.a.
AP metabolites	5-HO-MEHA	1.7	0					n.d.	5				n.d.	28.50	n.a.
	5-oxo-MEHA	1.6	27	0.43	0.43	0.43	1.62	2.82	0					n.d.	n.a.
	MEHA	0.5	7				n.d.	1.15	21				n.d.	67.38	n.a.
	MEHTP	0.3	7				n.d.	0.38	5				n.d.	0.73	n.a.
	5-HO-MEHTP	0.9	7				n.d.	1.08	9				n.d.	7.06	n.a.
	cis HO-MINCH	0.4	20				n.d.	1.66	5				n.d.	9.04	n.a.
	cis cx MINCH	0.3	40			n.d.	0.76	3.45	12				n.d.	9.53	n.a.
	cis MINCH	0.2	0					n.d.	6				n.d.	5.82	n.a.
	6-CxMPHxP	0.05	0					n.d.	4				n.d.	0.93	n.a.
	6-HO-MPHP	0.6	0					n.d.	0				n.d.	n.d.	n.a.
	6-oxo-MPHP	0.3	0					n.d.	2				n.d.	0.61	n.a.

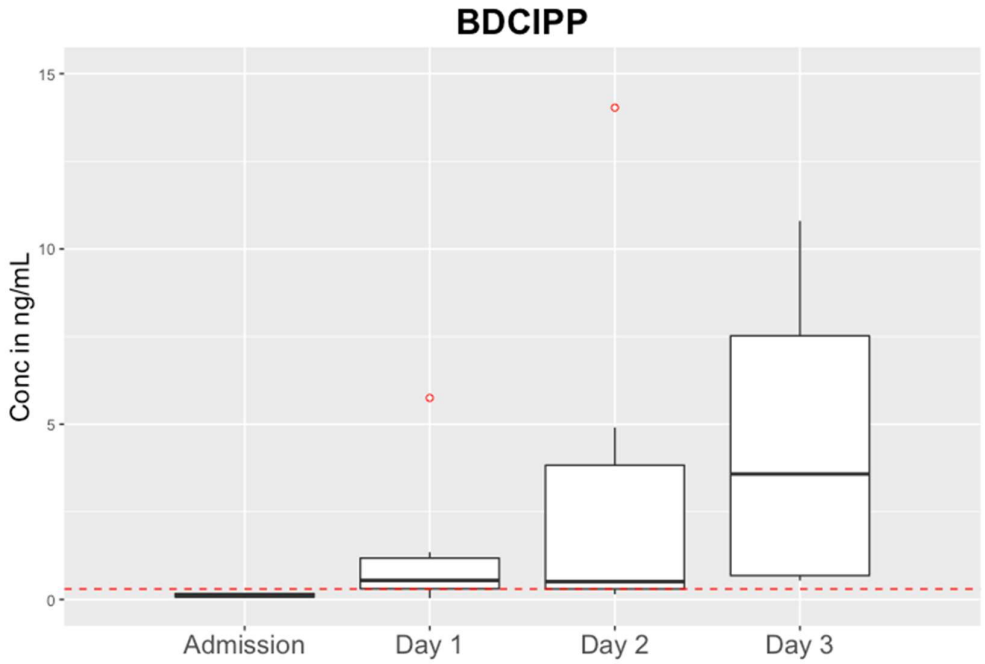
187

Association with medical devices

We hypothesized that patients treated with certain medical devices could have higher metabolite levels due to the use of specific plasticizer chemicals. Associations with two frequently used medical devices were investigated for urinary metabolite concentrations. As mentioned earlier, some but not all patients were treated with continuous venovenous hemofiltration (CVVH), or extracorporeal membrane oxygenation (ECMO), all consisting of long and large tubings. The Mann-Whitney U test was used to observe differences in metabolite concentrations between patients treated with one or both specific medical devices and participants that did not (i.e. remaining ICU patients and health controls). Results are summarized in Table 2. Concentrations of BCIPHIPP and BDCIPP were significantly higher in patients treated with CVVH ($p<0.001$ and $p=0.01$, respectively). However, no further increase was observed when both devices were used in the same patient. DPHP levels did not increase when CVVH or ECMO was applied separately ($p=0.98$ and $p=0.69$, respectively), but they were significantly higher in patients that required both devices ($p=0.01$). A similar trend was observed for 4-HO-TPHP, 4-HO-DPHP, and DNBP, however this was not statistically tested due to the lower detection frequency of these metabolites. Furthermore, urinary concentrations of BDCIPP of the six patients who were enrolled at the ICU preoperatively were much lower on admission compared to the days that followed ($p=0.01$). Figure 1 seems to indicate that transfusion from certain medical devices is a potential exposure pathway for TCIPP since the exposure took place after installation during surgery (i.e. between admission and day 1). BCIPHIPP and DPHP concentrations showed a similar trend albeit less pronounced ($p=0.02$ and $p=0.6$, respectively). Despite the significant association with CVVH, the BDCIPP time trend became less pronounced when all ICU samples were included as shown in Figure 2. This underlines the possibility that a specific point source of TDCIPP other than CVVH and/ECMO was used during the non-cardiac thoracic surgical intervention, since none of six patients who underwent the intervention had any of the two investigated medical devices installed (see Table SI-1). Unfortunately, there are no studies available on the presence of PFRs in medical devices. However, the literature shows that PFRs are used in a wide range of commercial products, mostly in combination with other flame retardants or plasticizing chemicals [23]. Non-halogenated PFRs are primarily applied as plasticizers, whereas halogenated PFRs are mostly used as flame retardants [26]. TCIPP, TDCIPP and TCEP are found in

polyurethane foam, among other applications [12]. TBOEP is frequently applied in floor wax and vinyl plastics [11]. TPHP and TNBP are also widely used for their plasticizing properties in PVC, resins, lacquers, paints and glues [12, 23]. Although these are only preliminary results, the findings of this study suggest that the major and specific source to PFRs in the ICU is related to the use of indwelling medical devices in these patients, as the highest levels were found in patients undergoing CVVH and ECMO consisting of long and large plastic tubing.

Figure 2: Temporal trends from admission until day 3 at ICU for BDCIPP for six patients who were pre-operatively enrolled at ICU. Indwelling medical devices were installed during surgery after the collection of the admission day sample. Red dotted line indicates the median concentration of control population (n=15). Red dots are statistical outliers.



Associations were only observed in a qualitative manner for AP metabolites. For most of the investigated AP metabolites no meaningful associations could be observed due to their low detection frequencies. MEHA and 5-HO-MEHA were only detected in patients treated with one or both devices, which indicates that the use of either device could influence these metabolite's levels. DEHA has

recently been applied in medical devices as replacement of DEHP [5, 6]. However, the impact of DEHA and other alternative plasticizers through medical devices appeared to be relatively small. Apart from medical devices, other potential sources of exposure to APs in the hospital environment include vinyl flooring, wires and cables, coatings, food packaging, and PVC materials [6].

A major limitation of this study is the lack of information on the presence of the parent chemicals of interest in medical devices. One of the factors that could explain the lack of associations with medical devices is the sampling year of our study. Urine samples were collected in 2013, only shortly after the worldwide increase in production volumes of APs [6]. Most probably, the investigated medical devices still contained mainly DEHP, as reflected by very high concentrations of its metabolites reported in our previous study measured in the same samples [14]. However, we cannot rule out that the parent chemicals were not present in the applied medical devices. Compared to DEHP, median concentrations of PFR and AP metabolites in this study were much lower. In the previous study, it was shown that patients necessitating specialized CVVH, ECMO or both had the highest levels of phthalate metabolites, sometimes with an increase of 100–1000 compared with the general adult population [14]. Thus, the effect of the application of medical devices on PFR and AP metabolite levels is clearly less pronounced compared to the phthalate DEHP. Another limitation is that the number of samples per investigated medical device are relatively small, making it difficult to draw robust conclusions. Furthermore, urinary metabolite concentrations were not adjusted for creatinine or specific gravity. The investigated trends and associations might be influenced by the degree of urine dilution and the obtained results might therefore be slightly underestimated. Finally, we were unable to investigate all potential sources and influencing factors inside the ICU environment. For example, diet has been shown to be an important exposure pathway for certain PFRs [27]. Although the composition of the diet was most likely highly similar for all bedridden patients and no data on food intake was available, we did not find a significant increase compared to a healthy control population with diverse dietary habits. Overall, ICU is a relatively controlled environment in terms of dietary and dust exposure which leads us to believe that transfusion and/or inhalation of plasticizer chemicals leaching from medical devices could be considered as the dominant pathway in this exposure scenario.

Table 2: Associations between urinary metabolite concentrations and the use of specific medical devices (CVVH and/or ECMO). N is the number of samples for the group 'device: yes'. The total number of samples included was 99, controls were added to the group 'device: no'. Mann-Whitney U test was performed using raw urinary concentrations. The level of statistical significance was set at 0.05. n.d.: not detected n.a.: not applicable.

	Concentrations per metabolite in ng/mL	Device	**CVVH** (n=14) 25th	Median	75th	p-value	**ECMO** (n=4) 25th	Median	75th	p-value	**Both devices** (n=7) 25th	Median	75th	p-value
PFR metabolites	BCIPHIPP	no	0.34	1.09	4.06	**<0.001**	0.37	1.52	5.31	0.48	0.34	1.41	5.60	0.99
		yes	2.60	8.35	20.79		0.16	1.06	4.26		0.62	1.62	3.22	
	4-HO-DPHP	no			n.d.	n.a.			n.d.	n.a.			n.d.	n.a.
		yes		n.d.	2.98				n.d.			n.d.	10.39	
	DPHP	no	0.55	1.14	3.53	0.98	0.59	1.04	3.19	0.69	0.55	0.96	2.71	**0.01**
		yes	0.79	0.98	2.77		0.35	2.67	7.40		3.09	4.77	10.56	
	4-HO-TPHP	no		n.d.	0.07	n.a.		n.d.	0.02	n.a.		n.d.	0.03	n.a.
		yes			n.d.		0.11	0.49	0.73			n.d.	7.96	
	DNBP	no		n.d.	0.19	0.08		n.d.	0.18	0.24		n.d.	0.16	n.a.
		yes			n.d.				n.d.		n.d.	0.88	3.30	
	BDCIPP	no	0.06	0.43	0.96	**0.01**	0.08	0.46	2.33	0.09	0.07	0.43	1.56	0.86
		yes	1.55	11.48	27.09			n.d.	0.93		n.d.	0.86	2.59	
	TCEP	no	n.d.	0.04	0.05	0.51	n.d.	0.04	0.05	0.68	n.d.	0.04	0.05	n.a.
		yes		n.d.	0.07		n.d.	0.02	0.05				n.d.	
	5-HO-EHDPHP	no	0.01	0.03	0.09	0.58	0.01	0.03	0.08	**0.01**	0.01	0.03	0.09	0.86
		yes	n.d.	0.02	0.18		0.18	0.47	0.51			n.d.	1.97	
	EHPHP	no	0.32	0.98	2.89	0.92	0.35	1.02	2.87	**0.04**	0.33	0.98	2.40	0.78
		yes	0.57	1.04	1.57		n.d.	0.13	1.21		n.d.	4.70	9.81	
	BBOEHEP	no	0.01	0.02	0.06	0.21	0.01	0.03	0.07	**<0.001**	0.01	0.02	0.07	**0.03**
		yes	n.d.	0.01	0.09				n.d.			n.d.	0.04	
	BBOEP	no			n.d.	n.a.		n.d.	0.06	0.76		n.d.	0.06	0.93
		yes	n.d.	0.10	0.21				n.d.				n.d.	
	3-HO-TBOEP	no			n.d.	n.a.			n.d.	n.a.			n.d.	n.a.
		yes			n.d.				n.d.		2.29	5.02	7.10	
APs	5-HO-MEHA	no			n.d.	n.a.			n.d.	n.a.			n.d.	n.a.
		yes			n.d.				n.d.			n.d.	8.10	
	MEHA	no			n.d.	n.a.			n.d.	n.a.			n.d.	n.a.
		yes	n.d.	14.70	31.52		0.73	3.20	6.23			n.d.	2.85	

Temporal trends from admission until day 3 at the ICU

Time trends of urinary concentrations from admission until day 3 were investigated for four frequently detected PFR metabolites, namely DPHP, bis(1,3-dichloro-2-propyl) phosphate (BDCIPP), 1-hydroxy-2-propyl bis(1-chloro-2-propyl) phosphate (BCIPHIPP), and 2-ethylhexyl phenyl phosphate (EHPHP) (see Figure 2). A significant increase between admission and day 3 was observed only for BCIPHIPP ($p=0.01$). Other pairwise comparisons were not statistically significant. BDCIPP ($p=0.348$) and DPHP ($p=0.775$) also followed an increasing trend albeit not as pronounced as BCIPHIPP. For EHPHP, the median concentration in controls samples was clearly higher than in ICU samples and no trend was observed over the investigated time period ($p=0.892$).

For APs, detection frequencies were usually too low to observe meaningful trends. Only for mono(2-ethyl-5-hydroxyhexyl) terephthalate (5-HO-MEHTP) and cyclohexane-1,2-dicarboxylic mono isononyl ester (MINCH), a small positive trend in concentrations could be highlighted between admission and day 3. Exploratory analysis of other less frequently detected metabolites showed that some patients did exhibit similar increasing temporal trends across multiple metabolites indicating that exposure to some chemicals might have originated from the same products.

These preliminary results seem to suggest that the presence of medical devices and spending time in the hospital environment could be explanations for higher levels of the measured metabolites. However, the excretion profiles of PFR and AP metabolites seem to be quite different from those observed for DEHP. In our previous study, metabolites of DEHP peaked after admission to the ICU and steadily declined towards day 3 [14]. A rapid decreasing trend was also observed in serum for DEHP metabolites [15], but this could also be explained by the exposure to large tubings (i.e. extracorporeal circuit) prior to ICU admissions. In our study however, a steady increase was found for most of the PFR and AP metabolites. Available data on the toxicokinetics of PFRs and APs are still relatively scarce, but are thought to be similar to the kinetics of phthalates [28]. PFRs and APs are rapidly eliminated (i.e. half-lives in the range of hours) by biotransformation to more hydrophilic metabolites through Phase-I and Phase-II reactions [29, 30]. However, it has also been suggested that some metabolites of PFRs are comparatively more

stable in the body than their parent compounds [31]. This could mean that these metabolites are excreted more slowly compared to DEHP metabolites. Perhaps a clearer pattern would have been observed if more samples were collected during the study period or past day 3 at ICU. Next to that, it is also still unclear in which proportion certain PFR and AP metabolites are excreted in urine [32]. For some chemicals such as DINCH and TBOEP urinary excretion factors were recently estimated, while for others such as DEHTP and EHDPHP no data are available yet [33, 34]. Urine, feces and expired gas are assumed to be the main excretion pathways for these chemicals, so it is possible that we have underestimated the total exposure to these chemicals by only measuring urine.

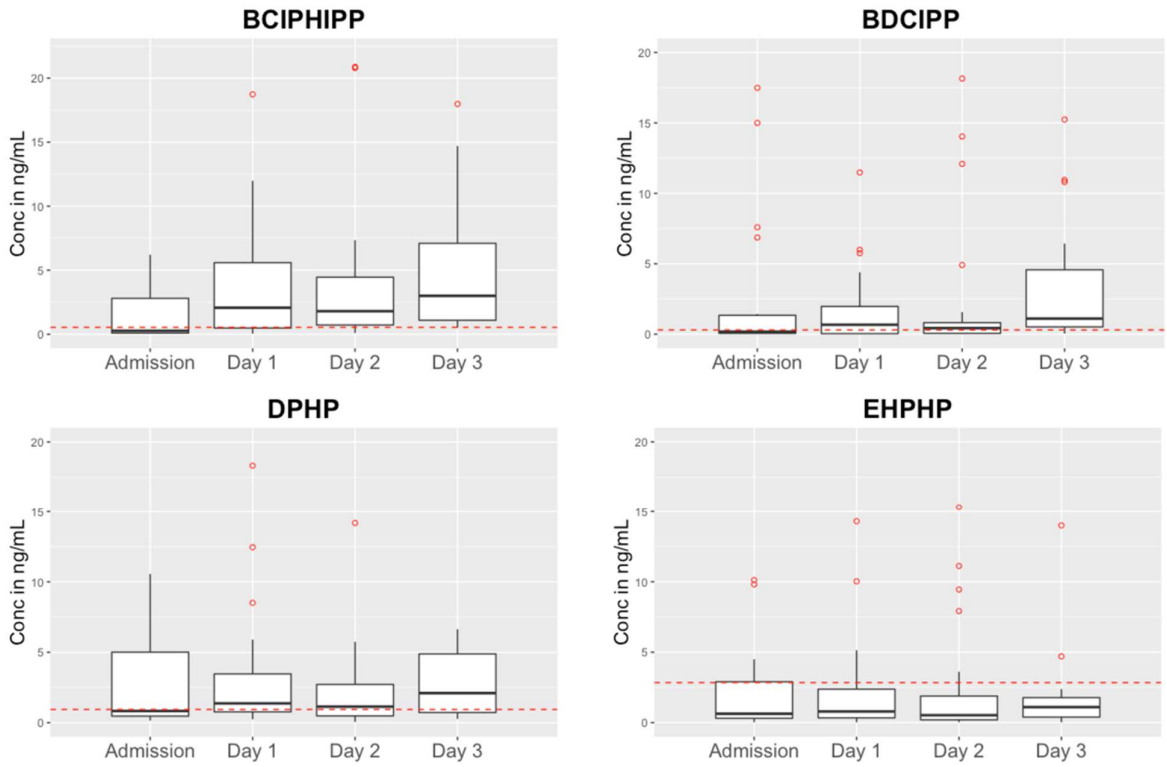


Figure 2: Temporal trends from admission until day 3 at ICU for four urinary PFR metabolites (BCIPHIPP, BDCIPP, DPHP and EHPHP). All ICU samples were included in the boxplots (n=84). Red dotted line indicates the median concentration of control population (n=15). Red dots are statistical outliers. Y-axis was set to a maximum concentration of 20 ng/mL for clarity, therefore some outliers might have been left out the figure.

Conclusions

This is the first report on PFR and AP metabolites in adult ICU patients. Urinary concentrations of metabolites of both classes of chemicals were higher in samples from patients admitted to the ICU compared to controls, which shows that the "hospital environment" can be considered as an important exposure source to these compounds. Overall, the excretion profile was shown to be different from DEHP, although the literature shows that the excretion rates of PFRs and APs are comparable to phthalates. Although the results of this study are very preliminary, they clearly show that ICU patients can be exposed to higher concentrations of plasticizer chemicals through medical devices and the hospital environment. As significantly higher concentrations were found in patients treated with indwelling devices with large tubings, we believe that ICU patients are mainly exposed through leaching from plastic. Nevertheless, further research into the possibly toxic effects of chemicals released from medical devices and hospital environments should be undertaken.

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Conflict of interest

The authors have no conflict of interest to declare.

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