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Journal of Pharmaceutical and Biomedical Analysis
35 (2004) 57–64
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Analysis by liquid chromatography and infrared spectrometry of di(2-ethylhexyl)phthalate released by multilayer infusion tubing

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Received 16 October 2003; received in revised form 19 December 2003; accepted 20 December 2003

Abstract

Di(2-ethylhexyl)phthalate (DEHP), a plasticiser present in infusion equipment, is known to be harmful to human health. Various studies have shown that DEHP is released into drug solutions from polyvinyl chloride (PVC) infusion lines. New multi-layer tubing has therefore been marketed to overcome this problem. We assessed the inertness of this tubing when placed in contact with a solution of CELLTOP®. Chromatographic assay of DEHP showed no significant difference in DEHP levels in the solution when placed in contact with PVC and with multi-layer tubing. Analysis by infrared spectrometry showed that DEHP was initially present in the polyethylene layer of the multi-layer tubing even before contact with the drug solution. Contact with the solution results in release of DEHP from the container into the contents. The substance responsible for this release is in fact an excipient of CELLTOP®, polysorbate. This release of DEHP further proves to depend on parameters such as temperature, time of contact between solution and tubing, and the concentration of polysorbate in the infused drug solution.

Keywords: DEHP; PVC; Etoposide; Polysorbate; HPLC; FT-IR

1. Introduction

Polyvinyl chloride (PVC) is widely used in the production of medical materials (infusion bags and kits, extension tubing, blood and plasma bags, catheters, enteral feeding tubes, dialysis materials, gloves, etc.). It is a relatively stiff polymer that needs added plasticisers to increase its flexibility. Di-ethylhexyl phthalate (DEHP) is the plasticiser most widely used in the manufacture of medical materials. As it is not covalently bound to the plastic matrix, it can be released from the PVC when this is placed in contact with lipophilic solutions [1].

The possibility of DEHP released from medical material being a toxic hazard for patients has been widely discussed [2–5]. Lack of clinical data on the effects of DEHP makes it impossible to demonstrate any causal relation between exposure to DEHP and its toxic effects. At present, human risk assessment can be based

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doi:10.1016/j.jpba.2003.12.018

only on extrapolation from animal data. In rodents, DEHP displays a chronic toxic effect on liver cells and spermatogenesis [5–8]. However, it has been demonstrated that the mechanism causing liver tumours in rodents does not apply in humans [3,8]. On the other hand, the mechanism of toxicity that affects testicles and growth do seem to apply to humans [3]. The Health Canada expert consultative group considers that certain subpopulations may incur an increased risk of adverse effects linked to DEHP (babies, infants, patients under extracorporeal oxygenation, patients who are to undergo blood transfusion or heart surgery, patients following certain intravenous treatments, especially total parenteral nutrition, lipophilic drugs) [4]. New-born infants are particularly concerned by these risks because situations involving contact with PVC can be frequent in neonatology wards [9,10]. To reduce the risk of release of DEHP, alternatives to using PVC are needed. For the intravenous infusion of lipophilic drug solutions, multilayer tubing has been proposed. Such tubing combines the flexibility of PVC (outside) with the inertness of a polyethylene (PE) liner. Our purpose was to test the inertness of this type of tubing. The aim of our study was to analyse the behaviour of this tubing towards a solution of CELLTOP®, a commercial form of etoposide, already known to induce release of DEHP from PVC infusion tubing [11].

Fourier transform infrared (FT-IR) spectrometry enabled us to carry out a qualitative analysis of the polymer surface in contact with the drug solution. In addition, using assay by liquid phase chromatography, we quantified the release of DEHP and identified the components responsible and the conditions that favour this process.

2. Materials and methods

2.1. Materials

2.1.1. Medical equipment and drugs

We used CAIR tubing of two types (co-extruded PVC/polyethylene batch 03A16 and PVC batch 01F07) and VYGON tubing (three-layer PVC/ethylene vinyl acetate (EVA)/polyethylene batch 261101) of length 50 cm and inside diameter 2.5 mm. ECOFLAC® bags (B.Braun Médical batch 222B09) made of pure low density polyethylene (LDPE) provided the reference spectrum of polyethylene for FT-IR analysis and served to hold the solutions of etoposide and excipients.

The drug solution of etoposide was made up from CELLTOP® 100 mg/5 ml (Asta médica batch 1E0058) for injection and 0.9% sodium chloride (Braun Ecoflac® 500 ml batch 222B09). The DEHP standards were prepared from pure DEHP supplied by Prolabo (batch 84325). All the equipment used for the preparation of solutions (syringes, containers, etc.) was DEHP-free.

2.2. Liquid phase chromatography

DEHP assay was performed using a JASCO line equipped with a PV-980 pump, an AS-950 automatic injector and a UV-975 detector. Data acquisition was carried out using a D7500 integrator (Merck Hitachi). We used a C18 LICHROSPHER 5 μm column (125 mm × 4 mm i.d.) (VWR international).

2.2.1. Infrared spectrometry

The polyethylene of the tubing was analysed using an Avatar 320 (Nicolet) Fourier transform infrared spectrometer fitted with a DTGS KBr detector. To analyse the plastic we used a flat ATR multi-reflection ZnSe crystal and a hollow crystal of the same composition for the analysis of the liquids. We processed the results using Omnic 5.1 software.

2.3. Method

We carried out two types of study on the selected tubing: (i) a quantitative chromatographic analysis of DEHP released into the solutions during contact with the tubing, and (ii) a qualitative analysis by FT-IR of the polymers composing the tubing.

2.3.1. Sample preparation

We made up two solutions of etoposide of concentrations 0.2 and 0.4 mg/l, which are those most often used in clinical practice. Dilutions were carried out in Ecoflac® low density polyethylene (LDPE) bags (B. Braun Medical) free of DEHP to avoid possible bias. The concentrations of active ingredients and excipients present in the different solutions are detailed in Table 1.

Table 1
Etoposide and excipients concentrations (mg/ml) of various dilutions of CELLTOP®

	Etoposide	Polysorbate 80	Citric acid	Macrogol 400	Alcohol
CELLTOP®, 100 mg/5 ml	20	80	2	600	303
Etoposide 0.1	0.1	0.4	0.01	3	1.515
Etoposide 0.2	0.2	0.8	0.02	6	3.03
Etoposide 0.4	0.4	1.6	0.04	12	6.06

Two chromatographic analysis groups were set up:

- First, to evaluate the specific influence of the different excipients on DEHP release and compare that release for the different types of tubing we prepared solutions of polyethylene glycol (Macrogol), polysorbate (Tween 80), citric acid and ethanol diluted in 0.9% sodium chloride (Ecoflac®) at concentrations identical to those of CELLTOP® containing 0.4 mg/ml of etoposide (Table 1). We ran these solutions into each of the three varieties of tubing selected (PVC CAIR, PVC/PE CAIR, PVC/EVA/PE VYGON), and the male and female Luer-lock connectors on each length of tubing were joined up. The solutions were left in contact with the tubing for 2 and 24 h at ambient temperature.
- Secondly, we evaluated the influence of factors such as temperature, the concentration of the solution in contact with the tubing, and the contact time, on DEHP release. To this end we limited our tests to solutions containing the chemicals implicated in DEHP release. Each solution was placed in the tubing as described previously. The tubes were placed in an oven at 37 and 27 °C and in a refrigerator at 5 °C for times of up to 97 h.

The solutions were then recovered for chromatographic analysis, and the tubing for FT-IR analysis.

2.3.2. Liquid phase chromatography

The samples analysed were composed of a mixture of 900 μl of solution taken from the tubing and 100 μl of mobile phase. The injected volume was 50 μl. The mobile phase used was a 15/70/15 mixture of water, acetonitrile and tetrahydrofuran (THF). The DEHP calibration in the range 20–80 μg/ml was carried out using a 1 mg/ml parent solution of DEHP in the mobile phase. The UV detection wavelength was set at 254 nm.

2.3.3. Fourier transform infrared (FT-IR) spectrometry

We tested for DEHP in the polyethylene of the multilayer tubing before and after treatment with the solutions. As reference we used the spectrum obtained from pure liquid DEHP using the hollow ZnSe crystal, and as a blank, that from pure LDPE from an Ecoflac® bag obtained using the flat ZnSe crystal.

We used a scalpel to separate the polyethylene layer, of thickness 200 μm for the CAIR tubing and 100 μm for the VYGON tubing, from the other plastic layers forming the tubes used for the preparation of the samples assayed by liquid chromatography. In this way, we obtained samples thin and flat enough to be introduced into the sample holder of the spectrometer. The polyethylene sample thus had two sides, an inner surface in contact with the solution, and an outer surface in contact with the PVC, which we analysed using the ATR flat ZnSe crystal.

The spectra were recorded with a resolution of 8, i.e. a digital incrementation of 3857 cm^{-1}, and scan number set at 16.

2.3.4. Statistical analysis

The statistical analysis of the results was carried out using the Excel (Microsoft) ANOVA test. The significance threshold was set at 5%.

3. Results and discussion

3.1. Quantitative analysis by liquid phase chromatography

3.1.1. Validation of the chromatographic assay method

The method used was specific to DEHP. None of the chemicals present in CELLTOP® interfered with the retention peak of DEHP at 4.9 min. The

Table 2
Precision and accuracy of the chromatographic assay method for DEHP ($n = 7$)

Concentration added (μg/ml)	Intra-day assay variability			Inter-day assay variability		
	Concentration found (μg/ml), mean ± S.D.	Coefficient of variation (%)	Accuracy (%)	Concentration found (μg/ml), mean ± S.D.	Coefficient of variation (%)	Accuracy (%)
20	20.3 ± 0.7	3.4	1.5	21.3 ± 0.9	4.2	6.5
40	40.0 ± 0.6	1.5	0	40.3 ± 2.3	5.7	0.7
80	80.1 ± 1.2	1.5	0.1	81.1 ± 3.2	3.9	1.4

calibration curve was linear in the range 0–80 μg/ml of DEHP, with a correlation coefficient $r = 0.999983$ ($r^2 = 0.999966$). The precision and accuracy of the method was satisfactory as shown by the results given in Table 2.

3.1.2. *Identification of the chemicals responsible for DEHP release from multi-layer tubing*

Release of DEHP was demonstrated when the different varieties of tubing were in contact with solutions of CELLTOP® and polysorbate (Table 3). The other excipients (ethanol, citric acid, polyethylene glycol) caused no release of DEHP (level <1 μg/ml in the solutions).

This means that the release of DEHP described by Loff et al. [12,13] from pure PVC tubing also occurs from multi-layer tubing, and moreover that this release is statistically comparable in the three kinds of tubing studied. These findings show that the polyethylene layer did not act as an efficient barrier to DEHP migration in our experimental conditions.

The commercial delivery form CELLTOP® caused a release of DEHP comparable to that obtained with the 1.6 mg/ml solution of polysorbate (Table 3), which rules out release of DEHP caused by another species including etoposide itself (Table 1). This effect of polysorbate, shown by Pearson et al. [1] and Demoré et al. [14] during contact with PVC bags, implies that there is a risk of DEHP release during the infusion of any drug preparation containing polysorbate, which is used as a solubilising surfactant in many injection delivery forms.

Table 3
DEHP release of a 0.4 mg/ml etoposide CELLTOP® solution and a 1.6 mg/ml polysorbate solution during 2 and 24 h of contact with the co-extruded infusion line at ambiant temperature

	Polysorbate 1.6 mg/ml	CELLTOP® 0.4 mg/ml
CAIR co-extruded (2 h)	26.51 ± 0.60	23.46 ± 0.54
CAIR co-extruded (24 h)	61.93 ± 0.44	59.29 ± 1.07
CAIR PVC (2 h)	30.23 ± 0.98	25.28 ± 0.44
CAIR PVC (24 h)	70.23 ± 1.14	64.17 ± 1.52
VYGON three layers (2 h)	25.60 ± 0.52	23.51 ± 1.33
VYGON three layers (2 h)	64.40 ± 0.05	61.68 ± 0.32

Comparison of the two incubation times showed a statistically significant effect ($P = 1.6 \times 10^{-9}$) of contact time on DEHP release.

3.1.2.1. Influence of contact time, temperature and polysorbate concentration on DEHP release. As polysorbate was the only chemical component that displayed an effect on DEHP release, we limited our study of the influence of concentration to that of polysorbate, by preparing three solutions of 0.4, 0.8 and 1.6 mg/ml of polysorbate. In addition, for reasons of equivalence between the two types of multi-layer tubing for DEHP release and availability we selected the co-extruded CAIR tubing as the analysis model.

- Effect of contact time. This is shown by the plot of release against contact time with a solution of CELLTOP® containing 0.2 mg/ml of etoposide (and 0.8 mg/ml of polysorbate) at 37 °C (Fig. 1). The release is seen to slow down with time according to a logarithmic rate law with equation $y = 7.65 \ln(t) + 15.82$ (with y DEHP release, μg/ml and t contact time, h) with a maximum of 51 μg/ml for 97 h.

The clinical importance of the effect of contact time is that most of the DEHP release takes place during the first hours of contact. After 2 h 40% of the total DEHP from the co-extruded tubing was released into

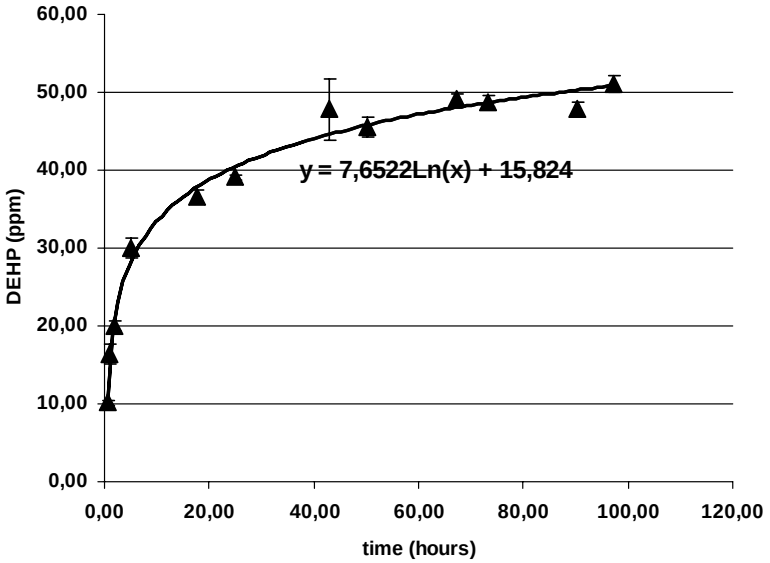

Fig. 1. DEHP realeased by CAIR co-extruded infusion line in a polysorbate solution at 0.8 mg/ml etoposide during 0.5–97 h of contact times.

the 0.2 mg/ml etoposide solution at 37 °C. Thus the level of DEHP will increase very rapidly in a solution left to stand in the tubing, a situation that can arise in clinical practice if a syringe driver breaks down or is being changed.

- Effect of polysorbate concentration. Fig. 2 shows the release of DEHP after a contact time of 20 h against different polysorbate concentrations. This release increases significantly with the concentration of polysorbate (ANOVA $P = 0.09$) in a non-linear way. This increase is described by the equation $y = 7.53x^2 + 27.21x + 4.21$ (with y DEHP release, μg/ml and x polysorbate concentration, mg/ml).

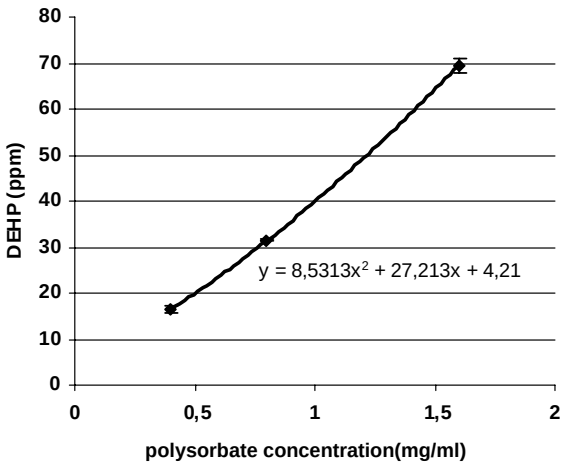

Fig. 2. Release of DEHP from the co-extruded infusion line in the 0.4, 0.8, 1.6 mg/ml polysorbate solutions after 20 h of contact.

This effect, already observed by Pearson et al. [1] with PVC bags, also plays a major role in DEHP release from multi-layer tubing.

- Effect of polysorbate concentration. Fig. 2 shows the release of DEHP after a contact time of 20 h against different polysorbate concentrations. This release increases significantly with the concentration of polysorbate (ANOVA $P = 0.09$) in a non-linear way. This increase is described by the equation $y = 7.53x^2 + 27.21x + 4.21$ (with y DEHP release, μg/ml and x polysorbate concentration, mg/ml).

This effect, already observed by Pearson et al. [6] with PVC bags, also plays a major role in DEHP release from multi-layer tubing. It would thus be desirable to use DEHP-free materials for the infusion of injectable drugs containing polysorbate 80. Polysorbate 80 is an excipient present in many pharmaceuticals administered by routes other than intravenous

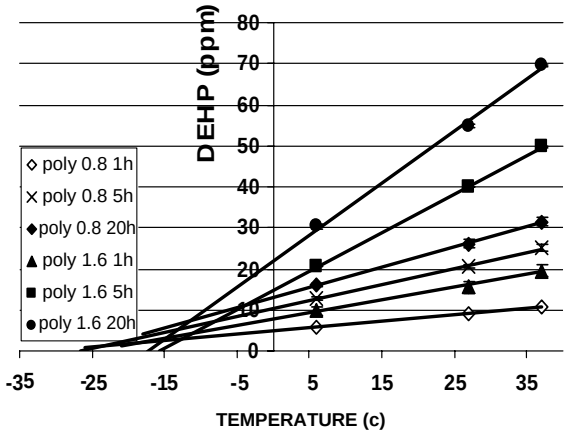

Fig. 3. Leaching of DEHP in the 1.6 and 0.8 mg/ml polysorbate solutions for 5, 27 and 37 °C after 15 and 20 h of contact with the CAIR© co-extruded infusion lines.

infusion: drugs in the form of tablets, ophthalmic solutions, injectable solutions in pre-filled syringes, etc.). However, in these conditions, polysorbate 80 raises no problems of compatibility because it does not come into contact with any material liable to contain DEHP.

- Effect of temperature. Fig. 3 shows that the release of DEHP increases linearly with temperature. Extrapolation of the plots of release against time shows a theoretical temperature at which release is nil of between −15 and −35 °C, according to concentration and contact time, which is never attained in practice during medical infusion. Also, at 27 °C, a temperature often attained in summer, the release of DEHP is high, reaching some 15 μg/ml in the first hour of contact and exceeding 50 μg/ml after 20 h. There is currently no official standard that sets an acceptable ceiling for DEHP levels in solutions for infusion, but some authors have proposed an upper limit of 5 μg/ml [15]. This level is largely exceeded, in particular at 37 °C, which can create a problem of toxicity in new-born infants infused in an incubator.

3.2. Qualitative study of the polyethylene surface by FT-IR spectrometry

We focused our attention particularly on the stretching vibrations of the carbonyl group (1747 cm^{-1}), as these are specific to DEHP, PE and PVC being devoid of such functions.

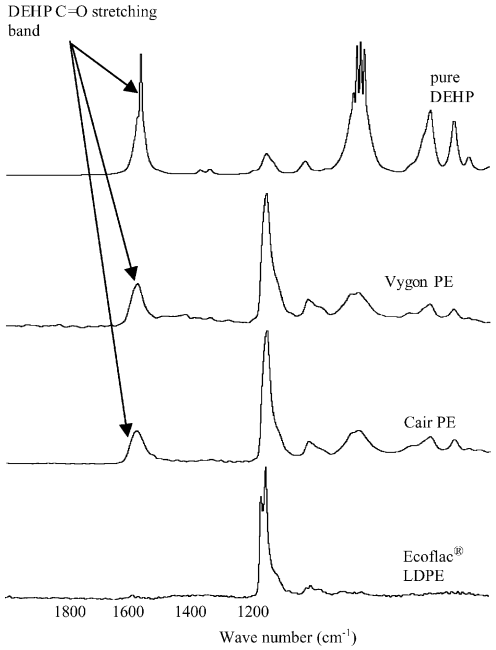

Fig. 4. DEHP, ECOFLAC LDPE and Vygon or Cair polyethylene FT-IR spectra.

The results obtained from the analysis of the PE tubing surface in contact with the solution (Fig. 4) were similar for the two models of multi-layer tubing studied: the peaks present between 1000 and 2000 cm^{-1} correspond to those of DEHP and pure PE. Analysis of the different samples cut from different locations along the tubing always showed the carbonyl stretching vibration, indicative of DEHP, but the absorption values obtained ranged very widely and were not reproducible (Fig. 5). In addition, the analysis of the PE

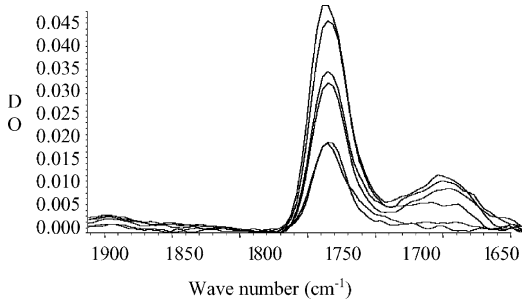

Fig. 5. FT-IR analysis of various sections of the LDPE of the co-extruded infusion line with the 1735 cm^{-1} C=O stretching band.

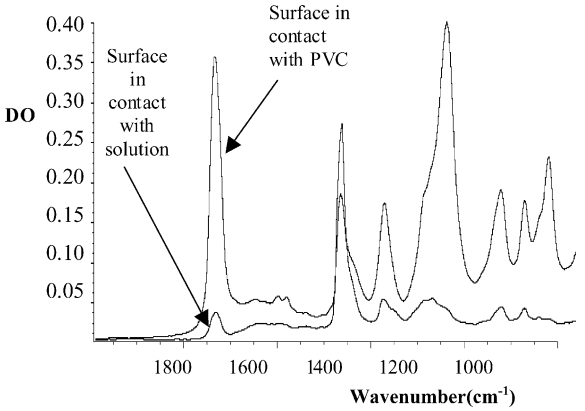

Fig. 6. Comparison of FT-IR spectra of the two surfaces of the CAIR polyethylene.

tubing surface before contact with the drug solutions also showed these peaks characteristic of DEHP.

Because of the wide variability in the levels found among the different samples, we were unable to quantify by FT-IR any difference in DEHP level on the PE before and after contact with the drug solutions. We can only assert that the surface layer of the PE lining in contact with the solution contained DEHP both before and after incubation.

In addition, comparison of the spectra from the analysis of the two surfaces of the PE layer shows a large difference in DEHP level between them (Fig. 6).

3.3. Possible explanations for DEHP release

The study of the LDPE surface by FT-IR spectroscopy shows that DEHP present in this layer is in contact with the drug solution. There are two possible explanations for this finding:

- Owing to its plasticising properties, DEHP is mobile in the polymer and diffuses from the PVC layer into the LDPE and then into the solution along a concentration gradient from the PVC through the PE and into the solution.
- The LDPE layer is not an impervious barrier to the migration of DEHP into the solution, e.g. because of pores formed during the manufacturing process.

However, the large difference in DEHP level between the two sides of the LDPE (Fig. 6) and the DEHP concentration in the solutions after incubation, and the fact that there was no difference in release from the VYGON and CAIR tubes (which have different thicknesses of PE), suggest that the DEHP travels directly from the PVC into the solution through pores.

4. Conclusion

The multi-layer infusion tubing studied in this work, though claimed to be inert towards drug solutions, does not seem to provide an efficient barrier to the diffusion of DEHP from its outer PVC layers.

Certain factors favour this release, such as temperature, time of contact between the solution and the tubing, and the concentration of polysorbate in the infusion solution. Awareness of these factors invites caution and argues for using PE-only tubing in critical conditions such as the infusion of solutions containing polysorbate, especially in new-born infants in incubators (temperature 37 °C). However, PE-only tubing is stiff and ill-suited to clinical practice. It is, therefore, desirable that new materials be developed that are free from DEHP and other toxic plasticisers. The possibility of adding a plasticiser such as octyl trimellitate could be envisaged, because its extraction rate would be considerably lower than that of DEHP [16]. However, to date, this plasticiser is not used in infusion applications, and the information available concerning its biological effects is still limited. Other work, e.g. that of Lakshmi et al. [17] point to other research approaches, such as coating PVC with polyethylene glycol to reduce DEHP release.

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