Extraction of *o*-phenylphenol from silicone tubing by a sulfobutylether cyclodextrin formulation

Jeffrey A. Zimmerman*, John M. Ballard, Hai Wang,
Allen Wu, Kimberly A. Gallagher

Pharmaceutical Research and Development, Merck Research Laboratories, P.O. Box 4, West Point, PA 19486, USA

Abstract

During a compatibility study between a formulation containing sulfobutylether cyclodextrin (SBECD) and silicone tubing, an extraneous peak was observed in the HPLC chromatograms. The extraneous material was identified by LC/MS, MS/MS and UV as a previously unreported tubing extractable, *o*-phenylphenol (*o*-PP). Several studies were conducted to assess the impact of formulation and process-related variables on the amount of the *o*-PP extracted, including contact time, sulfobutylether cyclodextrin concentration, formulation pH, and tubing sterilization and storage conditions. Results show that the concentration of the SBECD, tubing processing and storage conditions and the duration of the study influenced the levels of *o*-PP extracted. However, the largest contributor to the amount extracted was the lot of tubing tested. The extracted levels varied widely from lot to lot (0.007–3.89 μg/cm^2 of tubing surface area). A dynamic study conducted to simulate a manufacturing process also resulted in the extraction of *o*-PP by the formulation.
© 2003 Elsevier B.V. All rights reserved.

Keywords: Formulation; Cyclodextrin; Extraction; Silicone tubing; *o*-Phenylphenol

1. Introduction

The potential extraction of contaminants by liquid dosage forms from pharmaceutical packages and devices is a constant concern. While many of the extractables/leachables associated with commonly used pharmaceutical grade materials are known, unexpected contaminants can be problematic when evaluating the safety of a new product.

The leaching or extraction of a contaminant into liquid dosage forms is not uncommon. The most notable is the accumulation of the plasticizer, di-2-ethylhexyl-phthalate (DEHP), from polyvinyl chloride (PVC) components in a dosing solution during storage (Jaeger and Rubin, 1972), dialysis (Faouzi et al., 1999) and delivery of intravenous (IV) products (Jaeger and Rubin, 1970). Sterilizing filters are another familiar source of extractables. The surface modification of a polyvinylidene fluoride with hydroxypropyl acrylate (HPA) to increase hydrophilicity yields propylene glycol, unattached HPA copolymer and poly-acrylate as extractables (Kao et al., 2001).

The physicochemical nature of the dosing vehicle has also been shown to influence the amount of the contaminant extracted from the devices. Incubation of a 10% fat emulsion in a PVC infusion set at room temperature increased the extraction of DEHP more

* Corresponding author. Tel.: +1-215-652-4494;
fax: +1-215-652-4088.
E-mail address: jeff_zimmerman@merck.com
(J.A. Zimmerman).

than 100-fold relative to an aqueous vehicle (Allwood, 1985). The addition of a co-solvent or surfactant also significantly increases the extraction of DEHP relative to aqueous controls (Jenke, 2001).

Cyclodextrins (CDs) have the potential of becoming useful formulation tools as more drug candidates with poor aqueous solubility are brought forward for development. CDs are cyclomalto-oligosaccharides obtained by the enzymatic conversion of starch (Fromming and Szejtli, 1994a). In general, the hydrophobic interior of the CD molecule provides an appealing environment for lipophilic drugs, while the exterior hydrophilic surface of the CD facilitates the aqueous solubility of the molecule. Currently, there are six FDA-approved products utilizing different forms of CDs, including α-cyclodextrin (α-CD), hydroxypropyl cyclodextrin (HPCD) and sulfobutylether cyclodextrin (SBECD). (Physicians' Desk Reference, 2002).

While CDs have the capacity to solubilize lipophilic drugs, they also provide the potential for the extraction of small molecule, lipophilic contaminants from polymeric materials. The same contaminants might not otherwise be observed in aqueous vehicles. While conducting a silicone tubing compatibility study on an SBECD formulation of a new drug candidate, an extraneous peak was observed in HPLC chromatograms. This paper discusses the analytical methodology used to identify the extractable from pharmaceutical grade platinum-cured silicone tubing, and presents how process and formulation parameters such as the concentration of SBECD, formulation pH, tubing sterilization, and temperature and time of tubing storage impact the amount extracted.

2. Materials and methods

2.1. Materials

The SBECD (CAPTISOL®) and phenylphenol isomers were purchased from Cydex (Overland Park, KS) and Aldrich (Milwaukee, WI), respectively. All other reagents used were of analytical grade. Silicone tubing (Tygon® 3350 Sanitary Silicone Tubing, 0.125–0.25 in. i.d.; Nalgene®, 0.125 in. i.d.) was pharmaceutical grade manufactured by St. Gobain (France).

2.2. Methods

2.2.1. Extraction

Prior to extraction studies, unless otherwise specified, silicone tubing was rinsed for 3 min with Water for Injection (WFI) at 80 °C and steam-sterilized at 121 °C for 30 min. The extraction of silicone tubing was accomplished by filling the prepared tubing with the SBECD solution of interest, sealing the ends to prevent evaporative losses and incubating the system at room temperature. Prior to analysis, the entire contents of the tubing were emptied into a glass vessel, mixed to ensure homogeneity and sampled. In studies where the extractable concentration was monitored over time, the solution was emptied from the tubing, mixed, sampled and replaced in the same tubing from which it had been removed.

2.2.2. LC/MS analysis

Aliquots of solutions that contained the extractable were injected onto an Ansys Technologies Inertsil® Phenyl column (250 mm × 4.6 mm; 5 μm particles) at ambient temperature. The mobile phase was acetonitrile/water (50:50, v/v) at a flow rate of 0.5 ml/min; the HPLC was interfaced to a Finnigan MAT TSQ 7000 mass spectrometer, via an ESI probe. Parameters were: spray voltage, 4.5 kV; heated capillary, 250 °C; sheath gas (N_2), 70 psi; auxiliary gas (N_2), 30 arbitrary units. MS/MS parameters were: collision energy, 30–45 eV; collision gas pressure (Ar) 1.3 mTorr. Flow-injection ESI/MS/MS analysis of isomers of phenylphenol was performed by injection into acetonitrile/water (50:50, v/v) at a flow rate of 0.2 ml/min.

2.2.3. UV absorbance spectra

UV spectra were recorded by injection of the extractable compound and phenylphenol standards onto an Agilent 1100 liquid chromatograph equipped with a diode array detector, with chromatography on an Ansys Technologies Inertsil® Phenyl column (250 mm × 4.6 mm; 5 μm particles) held at 40 °C. The mobile phase was aqueous 0.1 M citrate buffer, pH 4.0/acetonitrile at a flow rate of 1.0 ml/min. The following gradient program was applied, percentage organic (time, min): 45 (0), 58 (11), 58 (17), 45 (18), 45 (25).

2.2.4. Quantitation of o-phenylphenol

Analysis of the quantitative extraction study samples was performed by isocratic HPLC using an Inertsil® ODS-2 column (250 mm × 4.6 mm; 5 μm particles) at ambient temperature, with a mobile phase of water/acetonitrile (60:40, v/v) at a flow rate of 1.0 ml/min and a UV detector set at 245 nm. The limit of detection for the *o*-PP was 0. 01 μg/ml, based on a signal-to-noise ratio of 3:1.

3. Results

During a compatibility study between platinum-cured silicone tubing and an IV formulation containing SBECD, the drug solution was filled into a section of silicone tubing and incubated at room temperature for 24 h. Analysis of that sample by HPLC revealed an extraneous peak in the chromatogram. The same extraneous peak was subsequently also observed in the chromatographic profile of the IV placebo solution that had been stored in silicone tubing. The unknown peak in placebo solutions was investigated by LC/MS and MS/MS analysis. The extractable was not detected by LC/ESI/MS in the positive ion mode; this indicated the probable absence of nitrogen atoms from the structure. However, a strong signal (m/z 169, $[M - H]^-$) was generated in the negative ion mode, which indicated the presence of a strongly acidic group. Since the chromatographic retention of the unknown was shown to be unaffected by the pH of the mobile phase, a carboxylic acid group was thought not to be present. The $[M - H]^-$ ion at m/z 169 was very resistant to fragmentation, which suggested that the unknown had an aromatic structure. The MS/MS spectrum (Fig. 1) showed ions at m/z 141, 115, 93 and 65; the ion at m/z 93 and the losses of 28 amu (m/z 169 → 141; 93 → 65) suggested a phenolic-type structure. Overall, the MS data were consistent with the extractable being one of the three isomers of phenylphenol (MW 170). A comparison of the MS/MS and UV spectra of the unknown with those of the *o*-, *m*- and *p*-isomers of phenylphenol showed conclusively that the unknown peak was due to *o*-phenylphenol (*o*-PP). The strong signal at m/z 115 differentiated the *o*-isomer from the other isomers, where the m/z 115 signal was very weak or non-existent. The UV spectrum of *p*-phenylphenol
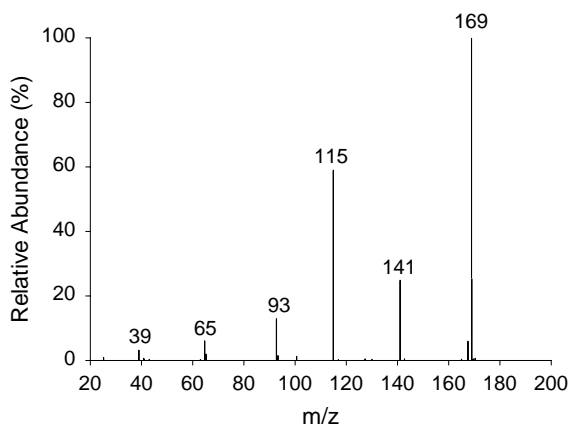

Fig. 1. MS/MS spectrum of the extractable.

(λ_{max} 260 nm) was distinctly different from that of the *o*- and *m*-substituted isomers, which were somewhat similar to each other. However, the diode array UV spectra of the unknown and *o*-PP were identical to each other, with λ_{max} at 246 and 286 nm (Fig. 2).

The identification was confirmed by chromatographic spiking experiments, when the *m*- and *p*-substituted phenylphenols were both found to elute ahead of the extraneous peak with base-line resolution, while the standard of *o*-PP co-eluted with it.

The possibility that the extractable was actually the sodium salt of *o*-PP, which would have given the same MS and MS/MS data as *o*-PP, was discounted by the results of extraction experiments. Extraction with wa-
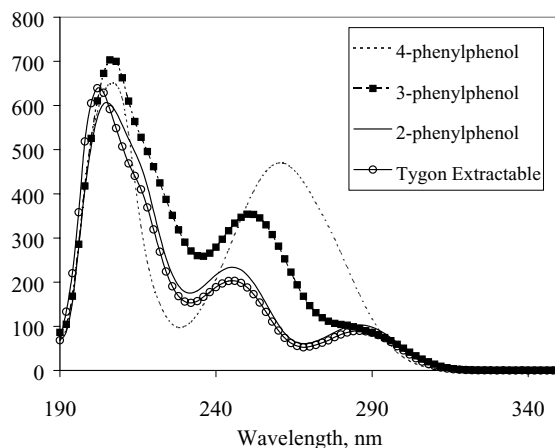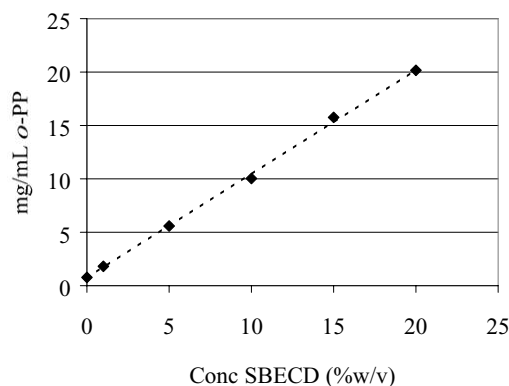

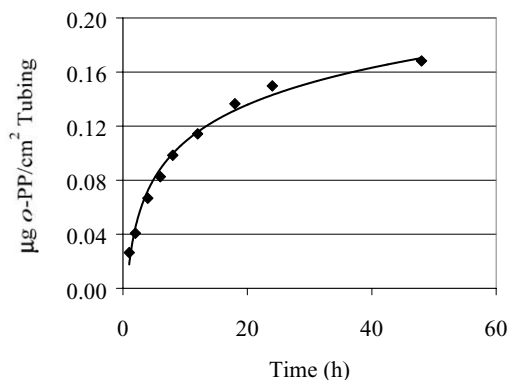Fig. 2. UV absorbance spectra of phenylphenols.

Fig. 3. Solubility of o-PP in SBECD.

Fig. 4. Extraction of o-PP as a function of contact time in silicone tubing.

ter gave extremely small amounts of the extractable compound (see Section 4), whereas the sodium salt has a very high aqueous solubility. Conversely, extraction with methanol gave a much greater amount of the extractable than did water; this result would not be expected if the o-PP were present as the sodium salt.

Once the contaminant had been identified, the investigation was widened to better understand the parameters involved in the extractability of o-PP. The formulation and process-related variables that were investigated for their impact on o-PP extraction include concentration of SBECD, formulation pH, tubing sterilization, temperature and time of tubing storage, and multiple extractions from the same sections of tubing.

Prior to evaluation of the processing variables, the solubility of o-PP was determined in solutions containing SBECD (Fig. 3). The solubilities were determined after seven days of continuous agitation and are consistent with results obtained on the second day of mixing. In the extraction studies, the maximum amount of o-PP obtained from any tubing sample was 20 times less than the aqueous solubility, and 400 times less than the solubility of o-PP in a 20% SBECD solution. Therefore, the amount of o-PP extracted was not limited by the solubilizing capacity of the vehicles.
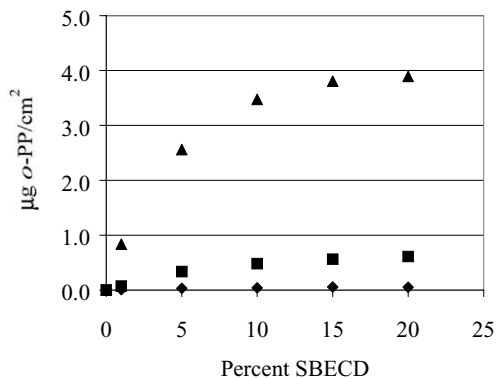
When compared on a molar ratio, a regression analysis of the solubility data shows that 1.26 mM of o-PP are solubilized by every 1 mM of SBECD. Inclusion ratios of >1 for solute-to-CD have been observed for compounds with β-CDs, including benzoic acid (2:1). The inclusion ratio, determined by the association–disassociation equilibrium between the

host and the guest, is dependent on guest properties such as shape and size (Fromming and Szejtli, 1994b).

The extraction of o-PP from the silicone tubing is dependent on the duration time of the study, as shown in Fig. 4. The data show that the process is relatively slow and the amount extracted continually increases for at least 48 h. However, as most sterile operations are generally completed in one day to minimize the potential for microbial growth, extraction studies were limited to 24 h.

The level of o-PP obtained was not only a function of the SBECD concentration, but also of the tubing lot tested. The results for three of the lots, shown in Fig. 5, are representative of almost the entire concentration range of o-PP extracted. When the data are re-plotted

Fig. 5. Extraction of o-PP as a function of SBECD concentration (lots tested: ◆ 75832, ■ 50572, ▲ 23672).
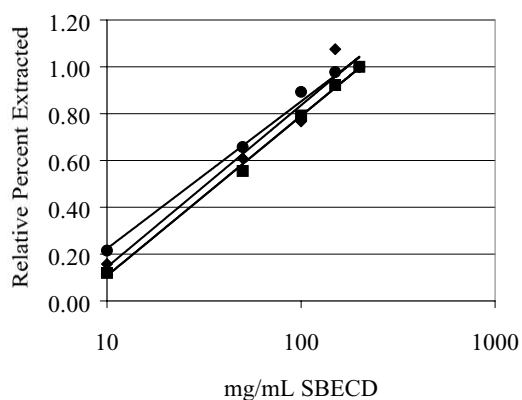
Fig. 6. Extraction of *o*-PP as a function of SBECD concentration (lots tested: ◆ 75832, ■ 50572, ● 23672).

as shown in Fig. 6, Eq. (1) is obtained empirically,

$$\frac{C_{o\text{-PP}}}{C_{\max}} = 0.29 \ln [\text{SBECD}] - 0.51 \tag{1}$$

where $C_{\max}$ is the concentration of *o*-PP extracted into a 20% SBECD solution over 24 h, $C_{o\text{-PP}}$ is the concentration of *o*-PP extracted at the concentration of SBECD of interest and [SBECD] is the concentration of SBECD in the formulation. The linear dependence of the amount of *o*-PP extracted as a function of the SBECD concentration suggests that the equivalent of a partition coefficient exists for the system, rather than a complete removal of the *o*-PP from the tubing surface. In addition, when the data in Figs. 4 and 8 are plotted as a function of the square root of time (not shown), a linear relationship is also obtained. This further suggests that the extraction of the *o*-PP is, in part, diffusion-controlled, and the mechanism of extraction will be the subject of future studies.

Extraction studies were conducted on several lots of silicone tubing, and yielded *o*-PP levels between 0.007 and 3.89 μg/cm^2 of tubing surface area. For reference, results are also reported as μg *o*-PP/g of tubing weight. A summary of the data is presented in Table 1. For some lots, more than one extraction study was conducted, and subsequently more than one value is reported. While the amount of *o*-PP extracted from an individual lot of tubing is relatively consistent, the tubing lot-to-lot variation has a significant influence on the extracted *o*-PP levels.

Table 1
Extraction of *o*-PP by tubing lot

Tubing lot no.	i.d. × o.d. (in.)	Extracted *o*-PP	
		Tubing SA (μg/cm^2)	Tubing weight (μg/g)
Tygon 3350			
75832	1/8 × 3/16	0.05	0.19
		0.012	0.04
		0.09	0.33
		0.008	0.03
50572	1/8 × 3/16	0.61	2.25
		0.33	1.22
23672	1/8 × 3/16	3.89	14.4
		2.94	10.9
72592	1/4 × 7/16	0.31	0.84
		0.08	0.28
		0.15	0.40
48169	1/4 × 7/16	0.36	1.32
188931	1/8 × 3/16	0.01	0.02
27035	3/16 × 7/16	1.33	0.22
Nalgene			
14176332B	1/8 × 3/16	0.011	0.04
C09715	1/8 × 3/16	0.12	0.40

Nominal tubing weight per cm: Tygon: 1/8 in. × 1/4 in., 0.27 g; 3/16 in. × 7/16 in.; 0.90 g and 1/4 in. × 7/16 in., 0.74 g. Nalgene: 1/8 in. × 1/4 in., 0.29 g.

Table 2
Summary of formulation and process studies

Study conditions	μg o-PP/cm^2 tubing
Sterilization	
No sterilization	1.54
1 Cycle	1.33
2 Cycles	1.29
3 Cycles	1.22
Extraction interval (h)	
0–24	0.0496
24–48	0.0103
48–72	0.0033
72–96	0.0010
0–24 Water	0.0010

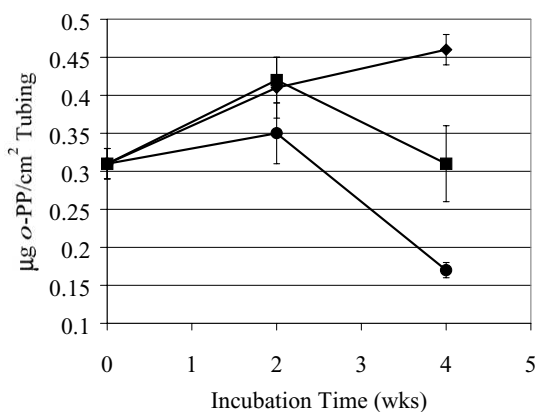Fig. 7. Effect of tubing storage temperature on the extraction of o-PP (temperatures tested: ◆ room temperature, ■ 40 °C, ● 60 °C).

The effects of sterilization and multiple extractions of the same tubing were also examined, with results summarized in Table 2. Repeated sterilization decreases the amount of the o-PP available for extraction, presumably due to volatilization of the o-PP (m.p. 58–60 °C) with increasing exposure to high temperatures. However, even after three cycles, 79% of the o-PP obtained from untreated tubing is still available to the vehicle. During repeated extraction studies, 77% of the total amount of o-PP detected was obtained in the first 24 h. In the majority of the studies, water controls were included that yielded below detectable limits of o-PP. However, in the multiple extraction study, small amounts of o-PP were detected in the controls. The maximum amount of o-PP extracted into a water control was 0.001 μg/cm^2 of tubing.

Post-sterilization data (Fig. 7) show that the tubing storage conditions have a moderate impact on the amount of o-PP extracted. Sections of tubing were prepared as described in Section 2 and stored up to 4 weeks at room temperature, at 40 and 60 °C. At designated time intervals, sections of tubing were removed from station, filled with a 20% SBECD solution, incubated for 24 h and the extracting solution assayed for o-PP. Examination of the data suggests that the o-PP blooms to the surface, followed by a volatilization which reduces the amount available to the formulation. The process appears to be accelerated with increasing temperature, as would be anticipated.

The effect of the solution pH on the extraction of o-PP was also examined. The solubility of o-PP and the corresponding sodium salt (Na o-PP, pK_a =

10.01, Albert and Serjeant, 1984) in water is 0.8 and 1200 mg/ml, respectively (Merck Index, 2001). Therefore, at higher pH values, the extraction of o-PP from silicone tubing could conceivably be more efficient via ionization. However, o-PP levels were comparable in extraction solutions with pH values ranging between 2 and 12. This demonstrates that the SBECD efficiently extracts all of the available o-PP from the tubing, and ionization does not play a significant role in the extraction process. The solubility of o-PP as a function of pH was not determined.

The results presented here demonstrate the ability of the SBECD to extract o-PP in a static silicone tubing compatibility study. However, the data are not directly applicable to product formulation and filtration processes. Therefore, a processing study was conducted to put the extraction results into perspective, relative to manufacture of a new formulation. An SBECD solution (20%; 150 ml) was recirculated through a 348 cm section of tubing (0.1875 in. i.d., 520.7 cm^2 surface area) using a peristaltic pump set at a flow rate of 93.4 ml/min. Fig. 8 shows the cumulative amount of o-PP extracted during the 9 h study and the amount of o-PP extracted per cm^2 of tubing per liter processed. The concentration of o-PP in the first 0.5 liters is relatively high. However, factors such as the concentration of SBECD in the formulation, residence time in the tubing, the lot of tubing and the analytical methodology will dictate whether the o-PP is detected in the final product.
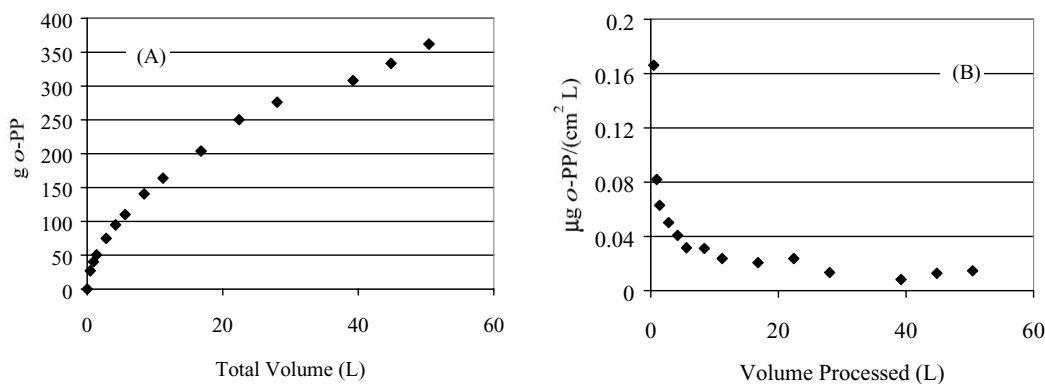
Fig. 8. Total *o*-PP extracted during a dynamic extraction study (A) and its distribution in the processed volume (B).

4. Discussion

All of the studies discussed in this paper were conducted with SBECD. However, while the extractive potential of the only other β-CD used in commercial products (HPCD) was not tested, it is reasonable to assume that it too would successfully complex with *o*-PP from the silicone tubing. It has been shown that the solubility of the lipophilic drug artemisinin is relatively comparable for five of six β-CD derivatives tested, including SBECD. Only (2,6-di-*O*-methyl)-β-CD exhibited improved solubility relative to the other five CDs, which was attributed to the unique physicochemical properties of (2,6-di-*O*-methyl)-β-CD relative to the other five β-CDs (Usuda et al., 2000).

Solutions of *o*-PP have been used extensively for more than 40 years to control post-harvest diseases of citrus fruits. In the United States, the whole fruit tolerance for *o*-PP is 10 ppm (Johnson et al., 2001). *o*-PP is also commonly used in the rubber industry as an antimicrobial and in hospitals as an active component of the disinfectant, Lyorthol®. It is a known irritant to the skin and eyes (Stouten, 1998), and ingestion by male rats of a 1.25% solution for 10 weeks resulted in a cytotoxic and regenerative proliferative effect on the bladder urothelium (St. John et al., 2001). There are no toxicology references that address the IV dosing safety of *o*-PP. Therefore, the toxicological implications of IV products containing *o*-PP are uncertain. A review of the pharmaceutical literature shows that *o*-PP has not been previously identified as a process contaminant, and it is not referenced in a recent article that summarizes pharmaceutical package and dosing contaminants (Jenke, 2002).

Our identification of the extractable from pharmaceutical grade silicone tubing as *o*-PP is unambiguous. A review of our records reveals that the *o*-PP has been observed independently in three different laboratories, with extractants that include methanol, SBECD-containing placebo formulations (this work) and hexane (Wu, personal communication). The *o*-PP is clearly not a contaminant of either instrumentation or solvents. Initial discussions with the manufacturer of the tubing examined in this study indicate no obvious source for the *o*-PP, and the origin of this compound has yet to be determined.

References

Albert, A., Serjeant, E.P., 1984. The Determination of Ionization Constants, A Laboratory Manual, 3rd ed. Chapman and Hall, New York, p. 143.

Allwood, M.C., 1985. Release of DEHP plasticizer into fat emulsion from IV administration sets. Pharm. J. 235, 600.

Faouzi, M.A., Dine, T., Gressier, B., Kambia, K., Luyckx, M., Pagniez, D., Brunet, C., Cazin, M., Belabed, A., Cazin, J.C., 1999. Exposure of hemodialysis patients to di-2-ethylhexyl phthalate. Int. J. Pharm. 180, 113–121.

Fromming, K.H., Szejtli, J., 1994a. Cyclodextrins in Pharmacy, Kluwer Academic Publishers, Dordrecht, p. 1.

Fromming, K.H., Szejtli, J., 1994b. Cyclodextrins in Pharmacy, Kluwer Academic, Dordrecht, pp. 49, 71.

Jaeger, R.J., Rubin, R.J., 1970. Plasticizers from plastic devices: extraction, metabolism and accumulation by biological systems. Science 170, 460–462.

Jaeger, R.J., Rubin, R.J., 1972. Migration of a phthalate ester plasticizer from polyvinyl chloride blood bags into stored human blood and its localization in human tissues. N. Engl. J. Med. 287, 1114–1118.

Jenke, D.R., 2001. Evaluation of model solvent systems for assessing the accumulation of container extractables in drug formulations. Int. J. Pharm. 224, 51–60.

Jenke, D.R., 2002. Extractable/leachable substances from plastic materials used as pharmaceutical product containers/devices. PDA J. Pharm. Sci. Tech. 56, 332–371.

Johnson, G.D., Harsy, S.G., Geronimo, J., Wise, J.M., 2001. Orthophenylphenol and phenylhydroquinone residues in citrus fruit and processed citrus products after postharvest fungicidal treatments with sodium orthophenylphenate in California and Florida. J. Agric. Food Chem. 49, 2497–2502.

Kao, Y.-H., Bender, J., Hagewiesche, A., Wong, P., Huang, Y., Vanderlaan, M., 2001. Characterization of filter extractables by proton NMR spectroscopy: studies on intact filters with process buffers. PDA J. Pharm. Sci. Tech. 55, 268–277.

O'Neil, M. (Ed.), 2001. The Merck Index, 13th ed. Merck Research Laboratories, Rahway, NJ, pp. 1309–1310.

Physicians' Desk Reference, 2002. 56th ed. Medical Economics Company, Montvale, NJ.