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Accumulation of extractables in buffer solutions from a polyolefin plastic container

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Abstract

Plastic materials are widely used in medical items, such as solution containers, transfusion sets, transfer tubing and devices. An emerging trend in the biotechnology industry is the utilization of large plastic containers to prepare, transport and store an assortment of solutions including buffers, media and in-process and finished products. The direct contact of such containers with the product at one or more points in its lifetime raises the possibility that container extractables may end up in the finished product. The interaction between a polyolefin container material and several test solutions representative of buffers and media used in biopharmaceutical applications was investigated. This manuscript summarizes the identification of the major extractables associated with the polyolefin container and documents the levels to which targeted extractables accumulate in the test solutions under several storage regimes.

Keywords: Container extractables; Material/solution compatibility; Polyolefin containers

1. Introduction

Plastic materials are widely used in medical items, such as solution containers, transfusion sets, transfer tubing and devices. The physiochemical nature of these materials provides medical products with their necessary, desirable performance characteristics. While an important performance characteristic of plastics used in medical application is chemical inertness, interactions between a plastic material and a contacted pharmaceutical product are well documented (Arbin et al., 1986; Berg et al., 1993; Danielson et al., 1983; Goydan et al., 1990; Kim-Kang and Gilbert, 1991; Kim et al., 1990; Reif et al., 1996; Sarbach et al., 1996; Snell, 1993; Ulsaker and Hoem, 1978). Such interactions may

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include sorption, the uptake of product components by the plastic material, or leaching, the release of plastic material components to the product. In the case of leaching, both the identity of the leached substances and their accumulation levels may impact the ultimate utility of the product.

An emerging trend in the biotechnology industry is the utilization of large plastic containers to prepare, transport and store an assortment of solutions including buffers, media and in-process and finished products. The direct contact of such containers with the product at one or more points in its lifetime raises the possibility that container extractables may end up in the finished product. Thus, one facet in the assessment of the product's safety and efficacy involves the determination of the extent to which container extractables accumulate in the finished product.

The interaction between a polyolefin container material and several test solution representative of buffers and media used in biopharmaceutical applications was investigated. This manuscript summarizes the identification of the major extractables associated with the polyolefin container and the determination of the levels of targeted extractables in buffer solutions stored in the polyolefin for 6 weeks at 40 °C and for 3 months at 25 or 40 °C.

2. Materials and methods

2.1. Container material

The container used was a commercially available multi-layer polyolefin laminate. The laminate contains medical grade very low density polyethylene (VLDPE) as the fluid contact layer, a barrier layer of ethyl-vinyl-alcohol (EVOH) and a polyurethane-type adhesive. The test containers were constructed in a 6 in. × 6 in. configuration, which, when filled with 250 mL of buffer, had an estimated contact surface area to solution volume ratio of 1.9 cm^2/mL. This surface area to solution volume ratio falls within the range anticipated for field-use containers with a capacity of 20–600 L. The containers were sterilized in a manner consistent with their intended biopharmaceutical application (gamma irradiation, dose range 27.2–35.8 kGy).

2.2. Extraction media

The following buffer media, chosen as being representative of solutions used in the biopharmaceutical industry, were used in this study.

I 0.06 M ammonium sulfate, 0.022 M 4-Morpholinesulfonic acid (MES), 0.0024 M Potassium phosphate, pH 5.4,
II 0.05 M Tromethamine (Tris), 0.15 M sodium chloride, pH 7.2,
III 2.0 M Tris,
IV 0.02 M Tris–hydrochloride, 0.05 M sodium chloride, pH 8.0,
V 0.02 M sodium phosphate, 0.15 M sodium chloride, 0.02% Tween-80, pH 5.5,
VI 1% Tween-80 stock solution,
VII 6.0 M guanidine-hydrochloride,
VIII 0.15 M sodium chloride, pH 5.4,
IX Binary ethanol/salt buffer mixtures (15, 30 and 45% (v/v) ethanol in 0.15 M sodium chloride, pH 5.4).

The media were prepared in pre-sterilized glass bottles using commercially available reagent grade chemicals. Adjustments to pH were made with reagent grade sodium hydroxide or hydrochloric acid as appropriate.

2.3. Test samples and controls, identification of target extractables

Identification of target extractables is facilitated if the concentration of the extractables is relatively high and the extraction matrix is analytically simple. While the conditions used to produce samples for extractables identification should meet these objectives, the conditions and the extraction medium should simulate intended product use. Otherwise, the extractables profile obtained during product use may be different from that obtained from simulated use or exaggerated conditions. Thus, unbuffered water was used as the extraction solution and the extraction conditions were 70 °C for approximately 3 days. Two bags and two blanks (glass bottles) were filled with 250 mL water and stored under such conditions.

2.4. Test samples and controls, accumulation of target extractables over time

Three extraction conditions, defined by storage duration and temperature, were established as reasonable simulations of product use. The three conditions included: storage at 15–30 °C for 3 months; storage at 40 °C for 6 weeks; and storage at 40 °C for 3 months. Such storage conditions are in excess of the expected product use scenario of ambient temperature (or lower) storage for 6 months or less.

Twelve containers and five glass bottles were filled with 250 mL each of each extraction matrix. Four of the filled containers and one of the filled glass bottles (control or blank) were stored at each of the time/temperature variations identified above. Analysis of such blanks provides the ability to distinguish between container extractables and buffer system impurities. Two of the glass bottle samples were stored at ambient temperature and used in the method development process.

Four containers and one glass bottle control for each of the eight test solutions were removed from 40 °C storage after 6 weeks and 3 months and from 25 °C storage after 3 months and equilibrated to ambient temperature for analysis. These solutions were analyzed via a single injection of each sample. Additionally, a portion of the control solution was spiked to contain an additional 1 mg/L of each analyte and analyzed. The ability to recover this spike is a measure of analytical accuracy.

2.5. Analysis and analytical methods

Water extracts and controls were subjected to several screening tests to establish the general chemical properties of the extractables and to search for specific extracted compounds. The screening methods included pH, UV absorbance (measured with a 1 cm quartz cuvette with water as the reference) and total organic carbon (TOC). Several chromatographic methods were used to search for specific extracted substances. These methods included gas (GC) and liquid (LC) chromatography with mass spectrometric (MS detection) and ion exclusion chromatography (ICE) with suppressed conductivity detection.

Specific analytical methods including ICE and LC/MS were developed, validated and then utilized to analyze the stored test samples and controls. The development and validation of the analytical methods used for quantitative purposes in this study is summarized elsewhere (Jenke et al., 2004). Operational specifics associated with these methods were as follows:

IEC (for acetate and formate):
Column: Dionex (Sunnyvale, CA) HPICE-AS1,
Mobile phase: 1 mM (or mN) HCl at 0.9 mL/min,
Regenerant: 5 mM tetrabutylammonium hydroxide at ≈ 1.0 to 1.5 mL/min,
Sample size: 10 μL,
Sample preparation: none (direct injection) except for the 6 M Guanidine formulation (matrix VII), which was diluted 1 to 20 with water due to the large injection response.

LC/MS:

Column: Phenomenex (Torrance, CA) Prodigy C8, 150 by 4.6 mm, 5 μm particles.

Mobile phase gradient: components were 10 mM ammonium acetate and methanol.

Elapsed time (min)	Proportion, 10 mM ammonium acetate	Proportion, methanol
0.00	95	5
1.40	95	5
1.50	50	50
15.00	5	95
20.00	5	95
20.10	95	5
25.00	95	5

Mobile phase flow rate: 0.6 mL/min.

Detection strategies: UV at 230 nm. API-ES (positive ion): gas temp, 325 °C; fragmentor, 65; Drying gas, 11.0 L/min; nebulizer pressure, 35 psig; Vcap, 5000 V. Specific ions monitored included caprolactam, 114; Extractable A, 229; Extractable C, 296; Extractable B, 271. API-ES (negative ion): gas temp, 325 °C; fragmentor, 65; Drying gas, 11.0 L/min; nebulizer pressure, 35 psig; Vcap, 5000 V. Specific ions monitored included hexanoic acid, 115; Extractable C, 277; stearic acid, 283.

Sample size: 100 μL,

Sample preparation: none (direct injection). However, an in-line trap column was used to eliminate injection of matrix salts into the analytical system. The entire injection volume of the sample was passed through the trap column with the effluent going to

waste. After a certain flush time (1–1.5 min), the trap column was backflushed, with the effluent containing the target analytes being eluted into the analytical system. Trap columns used included an Alltech C18 precolumn, 5 μm particles (used during method evaluation) and an Alltech Altima C18, column, 30 mm × 4.6 mm, 5 μm particles (used in the analysis of the 6 week test samples). It is noted that the analysis of the ethanol-containing samples was performed with direct injection (no trap column).

Differing quantitation strategies were used depending on the level of the extractables. A level of 0.2 ppm represents a critical accumulation threshold and thus dictates the level of identification and quantitation required. For extractables that are present at levels at or below 0.2 ppm, tentative identifications (based on structural information) and approximations of concentrations (e.g., use of response factors, surrogate standards, etc.) were determined. For extractables present at levels greater than 0.2 ppm, rigorous identification and quantitation was necessary. For identification, this means that structural information was supported by confirmation of properties with a reference standard. For quantitation, this means that appropriate response surface analyses (i.e., calibration curves) with documented levels of accuracy and precision were performed.

Additionally, TOC levels in the control and test articles associated with matrix VIII were also measured using an OI Analytical (College Station, TX) Model 700 TOC Analyzer.

2.6. Reference materials

Reference materials for Extractables A1, A2, B and C were internally synthesized. Reference standards for the other targeted extractables were commercially available analytical grade reagents (for example, Aldrich Chemical, Milwaukee, WI, USA), 99% purity or greater.

3. Results and discussion

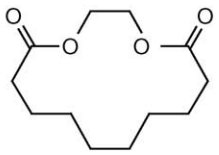
3.1. Identification of target extractables

Water solutions stored in the containers and glass controls (250 mL fill, 70 °C for 3 days) were analyzed for their general chemical properties such as pH, UV absorbance and TOC. These analyses provide qualitative information related to the amount and chemical nature of accumulated organic extractables. For example, the pH of the water stored in the containers ranged from 4.67 to 4.71, while the solutions stored in the inert glass controls had pH values of 6.03 and 6.18. Thus, one concludes that some of the extractables have acidic functional groups. The UV absorbance associated with the extracted compounds was very low over the wavelength range studied (200–300 nm) and the absorption spectra contained no significant peaks or features. Net extracted absorbances (sample − control) were 0.026 at 220 nm, 0.012 at 240 nm and 0.007 at 250 nm. This information implies that the extracted compounds do not contain chromophoric functional groups and are predominately non-aromatic. The extracted TOC associated with these samples (sample–control) was 1.72 mg/L. Thus, the total amount of extracted carbon was small.

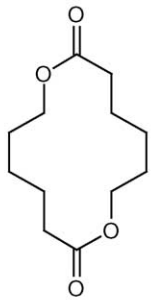
In order to identify target extractables, water extracts and controls were analyzed by several chromatographic methods whose elution and detection conditions were such that they could potentially respond to a large number of analytes. The results of each screening analysis is considered in greater detail as follows.

Since the qualitative data suggested that the extractables included one or more acids, an ICE method was used to screen the extracts for low molecular weight organic acids. Consistent with the pH and UV data, such analyses indicated that both formate and acetate were present at measurable levels (greater than 0.2 mg/L) in the container extracts (but not in the glass controls). It was estimated that formate and acetate combined accounted for nearly 30% of the extracted TOC. Thus, it is logical that these analytes become targets (Fig. 1).

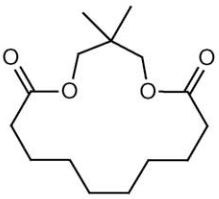
GC/MS and LC/UV/MS chromatograms obtained from the analysis of the container extracts were characterized by numerous peaks as is illustrated in Figs. 2–5. The identification of the compounds responsible for the observed chromatographic responses was ascertained by analyzing standards containing known quantities of specific compounds whose chromatographic characteristics were known. A compound identification was assigned to the peaks observed in the extract chromatogram if two criteria were met. The first criterion was a retention time match between the known peak in



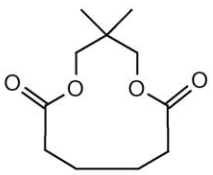
Extractable A1: $C_{12}H_{20}O_4$; formula weight, 228.28; 1,4-dioxacyclo-tetradecane-5,14-dione [5578-82-5]



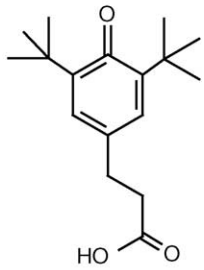
Extractable A2: $C_{12}H_{20}O_4$; formula weight, 228.28; 1,8-dioxacyclo-tetradecane-2,7-dione [13926-69-7]



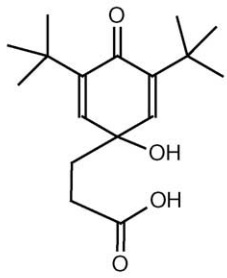
Extractable B: $C_{15}H_{26}O_4$, formula weight, 270.35; 3,3-dimethyl-1,5-dioxacyclopentadecane-6,15-dione [94113-50-5]



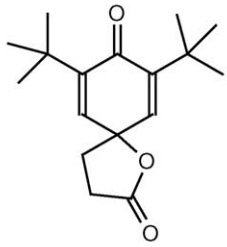
Extractable G: $C_{11}H_{24}O_4$, formula weight, 214.25; 3,3-dimethyl-1,5-dioxacycloundecane-6,11-dione [94113-47-0]



Extractable C: C17H26O3, formula weight, 278.39; 3-(3',5'-di-t-butyl-4'-hydroxyphenyl) propanoic acid[20170-32-5]



Extractable H: C17H26O4, formula weight, 294.39; 3-(3',5'-di-t-butyl-1'-hydroxy-4'-oxacyclohexa-2',5'-dienyl) propanoic acid [82237-15-4]



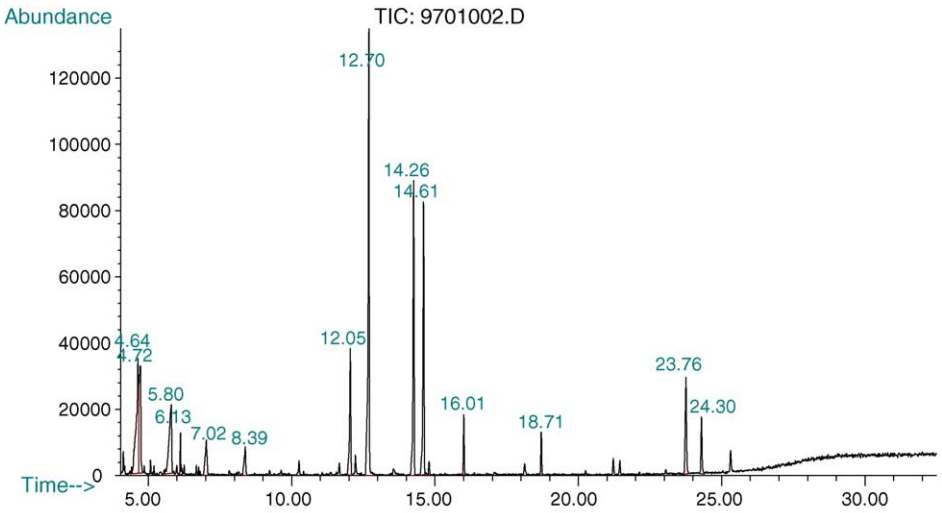
Extractable I: C17H24O3, formula weight, 276.37; 7.9-di-t-butyl-1-oxaspiro[4.5] deca-6,9-diene-2,8-dione[82304-66-3]

Fig. 1. Structures of various container extractables.

the standard and the unknown peak in the extract chromatograms. The second criterion was a match in the mass spectra between the known peak in the standard and the unknown peak in the extracts chromatograms. Typically this mass spectral match involved a comparison of the indicated molecular ions, whereas in the GC/MS applications, fragmentation patterns were also compared.

The list of definitively identified extractables is compiled in Table 1. When acetate and formate are added, this list represents the container's Aqueous Extractive Profile.

In addition to the compounds listed in Table 1, the chromatographic methods suggested that several other compounds were responsible for small peaks observed. Such compounds included higher molecular



Retention Time Legend, GC/MS (underivatized)	
Retention Time (min)	**Responsible Compound**
4.64, 4.72	Hexanoic acid
5.80	Heptanoic acid
7.02	Octanoic acid
8.39	Nonanoic acid
12.05	Extractable G
12.70	Internal standard
14.26	Extractable A1
14.61	Extractable A2
16.01	Extractable B
18.71	Extractable D
24.30	Extractable E
≈ 25.5	Extractable F

Fig. 2. Total ion current (TIC) GC/MS chromatogram of the underivatized water extract (70 °C for ≈ 3 days).

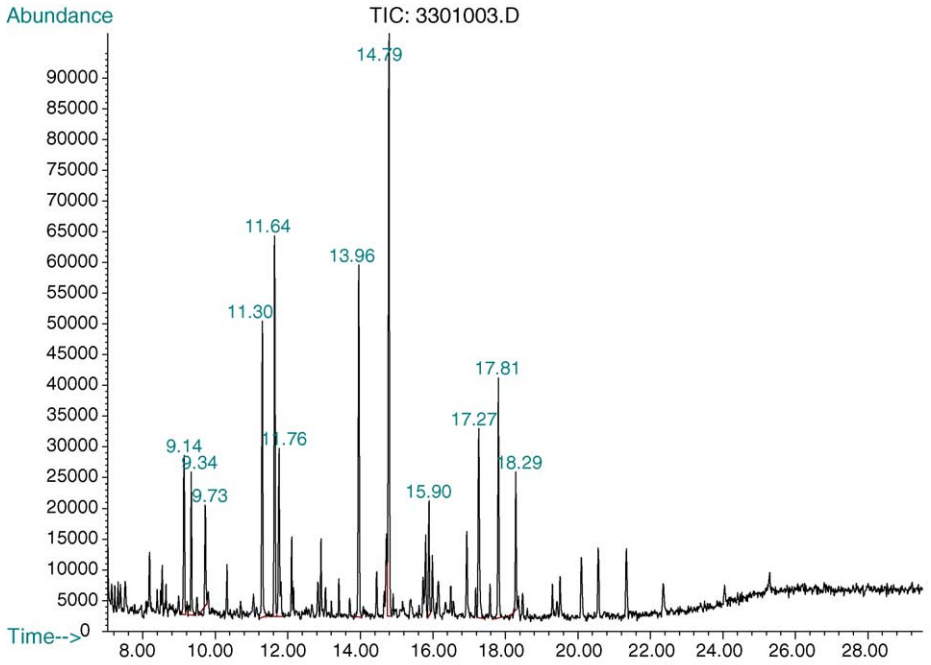
weight esters (e.g., Extractables D through F) and additional organic acids (range from C6 to C18). Since the peaks associated with these compounds were small and in many cases authentic reference standards were not available for the proposed compounds, these identifications could not be unilaterally confirmed.

Finally, there were several peaks in the chromatograms, especially for LC/MS, whose corresponding compound could not be identified. In all cases, however, such peaks were small relative to the major identified peaks.

The chromatographic methods used are a valuable tool for the identification of compounds that produce chromatographic responses. Alternatively, the same methods can also establish the absence of compounds from a given sample. Commonly encountered extractables that were not detected in the container extracts included diethylene glycol, ortho and *n*-ethyl toluenesulfonamides, sebacic acid and several phthalates [di-ethylhexyl-phthalate (DEHP), dibutyl phthalate, mono-ethylhexyl-phthalate (MEHP)].

3.2. Delineation of the targeted extractables

There are several reasons why it is appropriate to monitor the levels of target extractables as opposed to monitoring each individual member of the Extractables Profile. The first reason is that many of the identified members of the extractables profile accumulate at only very low levels. While such low levels may be measurable in the water solution used in the identification experiments, they may not be measurable (or even detectable) in the more complex buffer solutions. The second reason is strictly practical and reflects the



Retention Time Legend, GC/MS (derivatized)	
Retention Time (min)	**Responsible Compound**
9.14	Extractable G
11.30	Extractable A1
11.64	Extractable A2
≈ 13.0	Extractable B
15.90	Extractable D
≈21.4	Extractable E
≈ 22.3	Extractable F

Fig. 3. Total ion current (TIC) GC/MS chromatogram of the TMS-derivatized water extract (70 °C for ≈ 3 days).

level of effort required to quantitate each member of the profile.

Thus, the set of target extractables was identified based on four characteristics of the compounds. The first consideration is absolute concentration; thus, the list of target extractables should include those compounds that make up a large portion of the total extracted carbon. The second consideration is chemical nature of the compounds. Thus, targets should be chosen such that they effectively represent the types of functionalities present in the entire profile. Thirdly, the targets should be chosen so that they represent all the container components that are suspected to contribute extractables. Finally, any member of the extractables profile which may have a known or suspected impact on product safety or efficacy should be a target extractable. Additionally, the list of target extractables must be consistent with the ability to effectively analyze the test samples. To this end, the following extractables were chosen as targets.

3.2.1. Acetate and formate

These targets were chosen for three reasons. Firstly, they are major members of the extractables profile. Secondly, they are markers for the effect of irradiation on the product. Lastly, they are representative of those entities that may influence the pH of solutions stored in the polyolefin containers.

3.2.2. Extractables A2 and B

Extractable A2 was chosen as a target because it was present at the high levels. Extractable B was chosen as

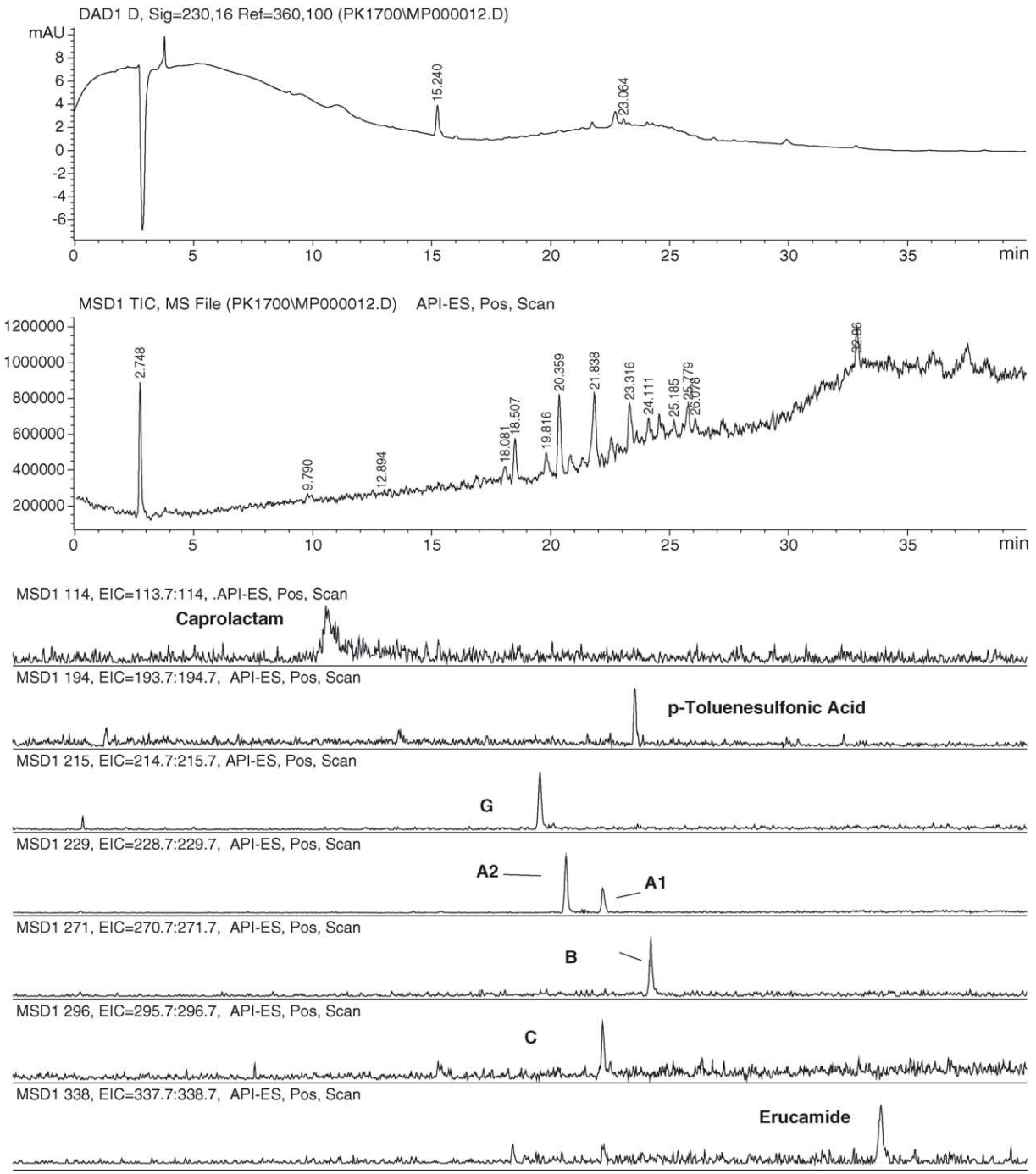


Fig. 4. LC/MS (positive ion mode) chromatograms of a water extract (70 °C for ≈ 3 days). The presence of caprolactam, *p*-toluenesulfonamide, several additional identified extractables and potentially erucamide in the extract is confirmed.

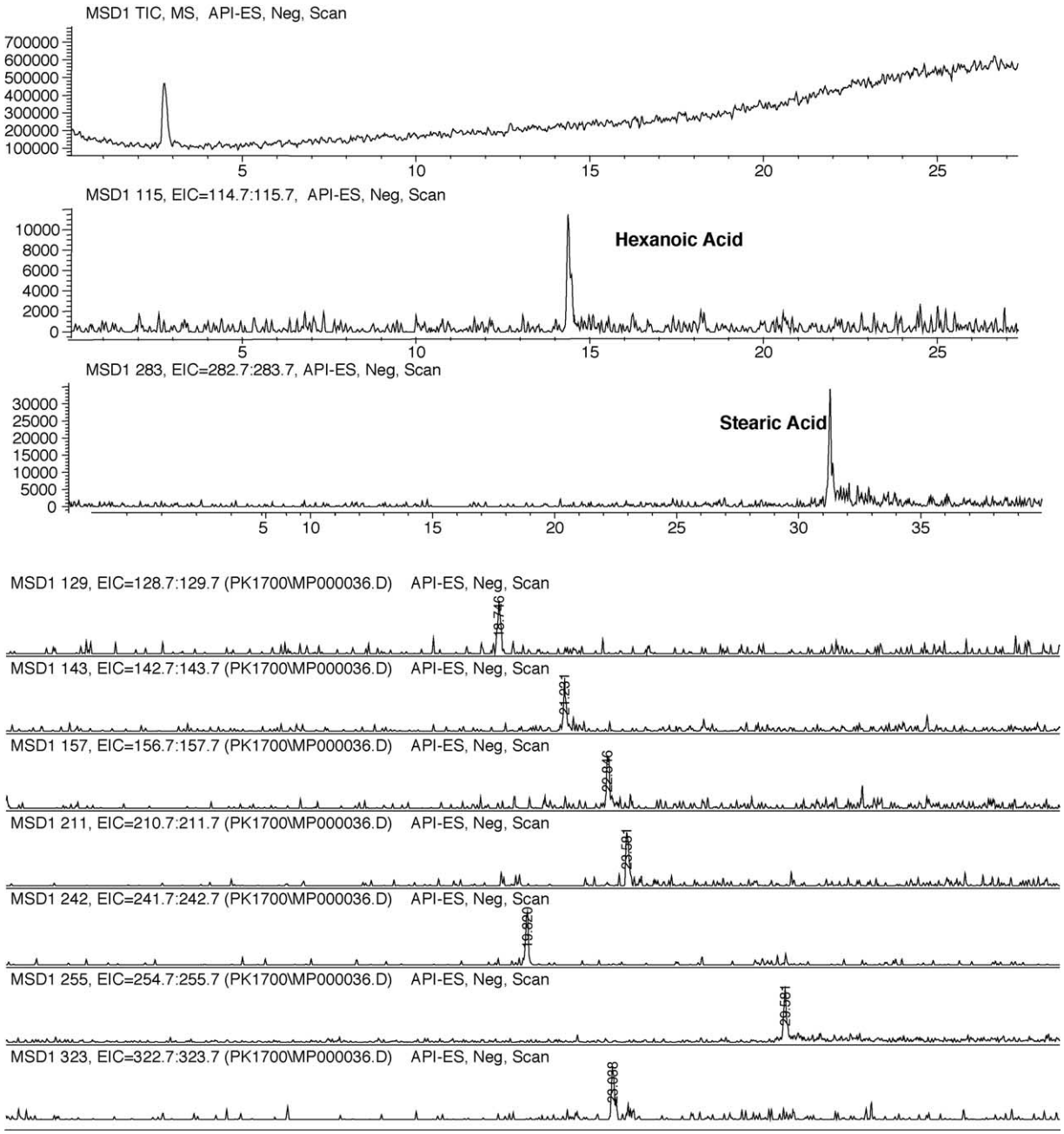


Fig. 5. LC/MS (negative ion mode) chromatograms of a water extract (70 °C for ≈ 3 days). The presence of hexanoic and stearic acids in the extract is confirmed. Additional peaks which can be associated with a series of organic acids (e.g., heptanoic through palmitic, chromatograms for ions 129, 143, 157, 211 and 255) and toluenesulfonamides (chromatograms for ions 242 and 323) were observed in the selected ion chromatograms shown. However, these tentative identifications were not confirmed via analysis of authentic standards.

Table 1
Aqueous extractive profile[f]

Compound identification information			Estimated extracted level (mg/L)[a]	
Name	Chemical name	CAS RN	By GC/MS	By LC/MS
A1[b]	1,4-dioxacyclotetradecane-5,14-dione	5578-82-5	<0.2	<0.2
A2[b]	1,8-dioxacyclotetradecane-2,7-dione	13926-69-7	<0.2	<0.2
B[b]	3,3-dimethyl-1,5-dioxacyclopentadecane-6,15-dione	94113-50-5	<0.2	<0.2
G[b]	3,3-dimethyl-1,5-dioxacycloundecane-6,11-dione	94113-47-0	<0.2	<0.2
Caprolactam	2-oxohexamethyleneimine	105-60-2	NA[c]	<0.2
Erucamide	Cis-13-docosenoamide	112-84-5	NA[c]	<0.2
I[a]	7,9-di-t-butyl-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione	82304-66-3	NQ[d]	<0.2
C[b]	3-(3′,5′-di-t-butyl-4′-hydroxyphenyl) propanoic acid	20170-32-5	NP[e]	<0.2
H[b]	3-(3′,5′-di-t-butyl-1′-hydroxy-4′-oxacyclohexa-2′,5′-dienyl) propanoic acid)	83237-15-4	NP[e]	<0.2
PTS	p-toluenesulfonamide	70-55-3	NA[c]	0.2
Caproic Acid	Hexanoic acid	142-62-1	>0.2	<0.2
CaprylicAcid	Octanoic acid	124-07-2	NQ[d]	<0.2

[a] These concentrations are specific for the extraction geometry used (250-mL extraction volume, 6 in. × 6 in. container).
[b] See Fig. 1 for structure.
[c] NA, not applicable. The method as implemented in this study is not suited for this compound.
[d] NQ, not quantitated.
[e] NP, not present at detectable levels.
[f] In addition to the compounds found in this list, formic acid (CAS RN 64-18-6) and acetic acid (CAS RN 64-19-7) were measured in the extracts at levels greater than 0.2 mg/L each.

a target since it is the highest mass compound of its class for which a reference material was available. Such compounds are representative of the container's interlayer adhesives and have been previously documented as being leached from polyolefin materials (Cruz et al., 1990).

3.2.3. Hexanoic and stearic acids

These targets were chosen because they are the end-members of the homologous series of higher molecular weight extracted organic acids. Thus, they are representative of this entire class of extracted compounds (which may be related to a particular container additive and may have some limited pH effect).

3.2.4. Extractable C

Several decomposition products of Irganox-type anti-oxidants were identified as extractables. The presence of such compounds in material extracts has been previously documented (Yu et al., 2000; Haider and Karlsson, 2002). Extractable C was chosen as a target to be representative of this group of extractables. This choice was made because this compound was generally present at higher levels than the other Irganox-related extractables and because of its analytical viability.

3.2.5. Caprolactam

Caprolactam was chosen as a target to represent extractables from the nylon layer of the container.

It is noted in passing that most of these targeted leachables have previously been identified as extractable substances related to polyolefin materials (Jenke, 2002).

3.3. Accumulation of target leachables after storage

The trends in accumulation levels as a function of test medium and storage conditions (mean concentration results, $n = 4$ containers) are summarized in Tables 2 and 3. The following general observations are pertinent.

3.3.1. Acetate and formate

These analytes accumulate in all matrices (including ethanol) to roughly the same level with perhaps a slightly higher accumulation in the high pH solutions (e.g., 2 M Tris). The accumulation levels are roughly the same at all test intervals. These results imply that these extractables readily accumulate in solution to the level of their total available pool.

Table 2
Summary, mean levels of target extractables at each test interval

Code	Matrix	Mean concentration (ppb)		
		6 weeks, 40 °C	3 months, 25°C	3 months, 40 °C
Caprolactam				
I	Ammonium sulfate, MES, potassium phosphate, pH 5.4	42	8	13
II	0.05 M Tris, 0.15 M NaCl, pH 7.2	40	8	10
III	2.0 M Tris	28	5	6
IV	0.02 M Tris–HCl, 0.05 M NaCl, pH 8.0	44	8	13
V	Sodium phosphate, sodium chloride, 0.02% Tween, pH 5.5	17	50	53
VI	0.1% Tween	7	97	98
VII	6 M Guanidine	7	ND	ND
VIII	0.15 M NaCl, pH 5.4	41	8	8
	Maximal accumulation, ethanol model solutions	–	Not applicable	–
Extractable A2				
I	Ammonium sulfate, MES, potassium phosphate, pH 5.4	73	78	80
II	0.05 M Tris, 0.15 M NaCl, pH 7.2	64	67	58
III	2.0 M Tris	ND	ND	ND
IV	0.02 M Tris–HCl, 0.05 M NaCl, pH 8.0	51	47	27
V	Sodium phosphate, sodium chloride, 0.02% Tween, pH 5.5	75	75	72
VI	0.1% Tween	96	71	68
VII	6 M Guanidine	54	51	48
VIII	0.15 M NaCl, pH 5.4	82	74	67
	Maximal accumulation, ethanol model solutions	–	164	–
Extractable C				
I	Ammonium sulfate, MES, potassium phosphate, pH 5.4	91	7	11
II	0.05 M Tris, 0.15 M NaCl, pH 7.2	78	9	20
III	2.0 M Tris	ND	2	1
IV	0.02 M Tris–HCl, 0.05 M NaCl, pH 8.0	42	8	19
V	Sodium phosphate, sodium chloride, 0.02% Tween, pH 5.5	87	4	1
VI	0.1% Tween	118	4	4
VII	6 M Guanidine	98	1	1
VIII	0.15 M NaCl, pH 5.4	103	3	4
	Maximal accumulation, ethanol model solutions	–	240	–
Extractable B				
I	Ammonium sulfate, MES, potassium phosphate, pH 5.4	12	15	13
II	0.05 M Tris, 0.15 M NaCl, pH 7.2	12	14	12
III	2.0 M Tris	ND	ND	ND
IV	0.02 M Tris–HCl, 0.05 M NaCl, pH 8.0	13	14	11
V	Sodium phosphate, sodium chloride, 0.02% Tween, pH 5.5	14	18	15
VI	0.1% Tween	21	23	21
VII	6 M Guanidine	44	47	43
VIII	0.15 M NaCl, pH 5.4	14	14	12
	Maximal accumulation, ethanol model solutions	–	169	–

ND = not detected.

3.3.2. Extractables A2 and B

There is no marked difference in the accumulation levels of these extractables as a function of either sample matrix or storage condition. However, the maximum level of these analytes in the ethanol matrices (which can be construed as the total available pool of these analytes) is higher than the levels observed in any of the buffer matrices. Thus, the accumulation of these extractables in the buffer matrices is constrained by a mechanism other than total available pool. Since the levels are roughly the same at the three storage conditions used, the constraining mechanism does not appear to be kinetic in nature. The constraining mechanism also does not appear to be partitioning since the

Table 3
Summary, mean levels of target extractables at each test interval

Code	Matrix	Mean concentration		
		6 weeks, 40 °C	3 months, 25°C	3 months, 40 °C
Acetate [net concentration reported = sample − control] (ppm)				
I	Ammonium sulfate, MES, potassium phosphate, pH 5.4	0.94	0.56	0.89
II	0.05 M Tris, 0.15 M NaCl, pH 7.2	0.78	0.66	1.01
III	2.0 M Tris	1.41	0.78	1.34
IV	0.02 M Tris–HCl, 0.05 M NaCl, pH 8.0	0.89	0.64	0.95
V	Sodium phosphate, sodium chloride, 0.02% Tween, pH 5.5	0.74	0.41	0.84
VI	0.1% Tween	0.94	0.50	0.73
VII	6 M Guanidine	ND	ND	ND
VIII	0.15 M NaCl, pH 5.4	0.87	0.58	0.89
	Maximal accumulation, ethanol model solutions	–	1.06	–
Formate [net concentration reported = sample − control] (ppm)				
I	Ammonium sulfate, MES, potassium phosphate, pH 5.4	0.63	0.41	0.70
II	0.05 M Tris, 0.15 M NaCl, pH 7.2	0.65	0.54	0.81
III	2.0 M Tris	0.37	0.26	–
IV	0.02 M Tris–HCl, 0.05 M NaCl, pH 8.0	0.63	0.49	0.73
V	Sodium phosphate, sodium chloride, 0.02% Tween, pH 5.5	0.58	0.46	0.93
VI	0.1% Tween	0.97	–	0.89
VII	6 M Guanidine	ND	–	–
VIII	0.15 M NaCl, pH 5.4	0.68	0.43	0.66
	Maximal accumulation, ethanol model solutions	–	0.75	–
Extractable C [negative ion] (ppb)				
I	Ammonium sulfate, MES, potassium phosphate, pH 5.4	9	5	8
II	0.05 M Tris, 0.15 M NaCl, pH 7.2	21	6	16
III	2.0 M Tris	6	ND	2
IV	0.02 M Tris–HCl, 0.05 M NaCl, pH 8.0	21	7	16
V	Sodium phosphate, sodium chloride, 0.02% Tween, pH 5.5	2	4	ND
VI	0.1% Tween	6	2	2
VII	6 M Guanidine	2	2	2
VIII	0.15 M NaCl, pH 5.4	5	3	4
	Maximal accumulation, ethanol model solutions	–	8	–
Hexanoic acid (ppb)				
I	Ammonium sulfate, MES, potassium phosphate, pH 5.4	277	175	215
II	0.05 M Tris, 0.15 M NaCl, pH 7.2	247	156	177
III	2.0 M Tris	298	169	175
IV	0.02 M Tris–HCl, 0.05 M NaCl, pH 8.0	319	147	182
V	Sodium phosphate, sodium chloride, 0.02% Tween, pH 5.5	276	185	217
VI	0.1% Tween	320	167	156
VII	6 M Guanidine	203	94	126
VIII	0.15 M NaCl, pH 5.4	308	174	189
	Maximal accumulation, ethanol model solutions	–	255	–
Stearic acid (ppb)				
I	Ammonium sulfate, MES, potassium phosphate, pH 5.4	5	<1	<1
II	0.05 M Tris, 0.15 M NaCl, pH 7.2	5	3	2
III	2.0 M Tris	884	558	598
IV	0.02 M Tris–HCl, 0.05 M NaCl, pH 8.0	6	11	6
V	Sodium phosphate, sodium chloride, 0.02% Tween, pH 5.5	84	67	109
VI	0.1% Tween	271	133	237
VII	6 M Guanidine	5	2	2
VIII	0.15 M NaCl, pH 5.4	4	<1	2
	Maximal accumulation, ethanol model solutions	–	122	–

ND = not detected.

Table 4
TOC reconciliation, sample matrix = 0.15 M NaCl, pH 5.4

Storage information		TOC (mg/L)				TOC reconciliation (%)
Temperature (°C)	Duration (month)	Measured[a]	Control[b]	Extracted[c]	Calculated[d]	
25	3	2.48	1.44	1.04	0.52	50.3
40	1.5	3.52	1.90	1.61	0.89	55.2
40	3	3.21	1.97	1.24	0.72	58.1

[a] Measured = mean ($n = 4$) of TOC results for four replicate extractions samples.
[b] Control = TOC of an extraction blank.
[c] Extracted TOC = measured TOC − Control TOC.
[d] Calculated TOC = summation of the TOC associated with all measured extractables.

buffers studied encompass a varying affinity for organic solutes (aqueous matrices versus Tween-containing matrices).

3.3.3. Extractable C

The 6 week, 40 °C positive ion data appears to be inconsistent with the negative ion data at all intervals and the positive ion data at the 3-month test interval and thus considered to be anomalous. This extractable accumulates to only very low levels in all matrices examined and any accumulation trends which may exist are masked by the analytical variation inherent at such low levels.

3.3.4. Hexanoic acid

The accumulation of this extractable is unaffected by sample matrix or storage conditions. Thus, it is concluded that this extractable readily accumulates to its total available pool level.

3.3.5. Stearic acid

Stearic acid accumulation is greatly influenced by buffer polarity and pH. The accumulation levels in the Tween and high pH matrices are much higher than in the other matrices examined. The accumulation does not appear to be affected by the storage duration or temperature. Thus, while the equilibrium level of this extractable is readily achieved (i.e., the accumulation is not migration constrained), the equilibrium levels noted in this study may not reflect the total available pool of this analyte (i.e., solutions with a pH higher than 2 M Tris or more "lipophilic" than 0.1% Tween may achieve stearic acid levels higher than those observed in this study).

3.4. TOC reconciliation

One of the reasons for studying matrix VIII (0.15 M NaCl, pH 5.4) was to assess the completeness with which the container's Extractable Profile had been delineated. One means of accomplishing this objective is through the TOC reconciliation. In this process, an extract's TOC level is directly measured. Additionally, the apparent TOC is calculated based on the levels of the individually measured organic extractables. The apparent TOC is the summation, for all measured organic extractables, of the extractables' measured concentration and their carbon fraction. In the ideal situation where all the members of a material's extractable profile have been identified and accurately measured, the TOC reconciliation (measured/apparent × 100%) will be 100% (within the accumulated analytical error).

TOC reconciliation data obtained at three test intervals is summarized in Table 4. The levels of extracted TOC are fairly consistent for the three storage conditions used. In general, the total amount of TOC extracted from the container by the aqueous medium used is less than 1.7 mg/L as carbon. The TOC reconciliation results are consistent for the three test intervals, with a typical reconciliation of 50–58%. These values suggest that the organic extractables profile for the container has been effectively delineated in this study.

4. Conclusions

Extractables associated with a polyolefin container material have been identified and the accumulation levels of certain of the identified substances have been determined under well-defined conditions of contact.

Such information provides a general understanding of this particular material and the behavior of its associated extractable substances. However, the information's relevance in terms of assessing material/solution compatibility in a specific product use situation is limited since it is appropriate and necessary to generate application-specific leachables information in order to establish the product safety and/or efficacy impact of material to product contact.

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