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Identification and Quantitation of Sorbitol-Based Nuclear Clarifying Agents Extracted from Common Laboratory and Consumer Plasticware Made of Polypropylene

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Abstract

Reported here is the mass spectral identification of sorbitol-based nuclear clarifying agents (NCAs) and the quantitative description of their extractability from common laboratory and household plasticware made of polypropylene. NCAs are frequently added to polypropylene to improve optical clarity, increase performance properties, and aid in the manufacturing process of this plastic. NCA addition makes polypropylene plasticware more aesthetically pleasing to the user and makes the product competitive with other plastic formulations. We show here that several NCAs are readily extracted with either ethanol or water from plastic labware during typical laboratory procedures. Observed levels ranged from a nanogram to micrograms of NCA. NCAs were also detected in extracts from plastic food storage containers; levels ranged from 1to 10 μg in two of the three brands tested. The electron ionization mass spectra for three sorbitol-based nuclear clarifying agents (1,3:2,4-bis-*O*-(benzylidene)sorbitol, 1,3:2,4-bis-*O*-(p-methylbenzylidene)sorbitol, 1,3:2,4-bis-*O*-(3,4-dimethylbenzylidene)sorbitol) are presented for the native and trimethylsilylderivatized compounds together with the collision-induced dissociation mass spectra; gas and liquid chromatographic data are also reported. These NCAs now join other well-known plasticizers such as phthalate esters and bisphenol A as common laboratory contaminants. While the potential toxicity of NCAs in mammalian systems is unknown, the current data provide scientists and consumers the opportunity to make more informed decisions regarding the use of polypropylene plastics.

The ubiquitous nature of plasticizers in the laboratory has plagued analytical chemists and other scientists for decades. Plasticizers, slip and mold release agents, antioxidants, and other compounds extractable from plastics can complicate preparation of samples, contaminate analytical instrumentation (e.g., mass spectrometers), confound data interpretation, and consume valuable time and resources during their identification. When reference information is available in the literature or databases, potential contaminants observed in analytical measurements can be rapidly identified with mass spectrometry or other analytical tools. Many laboratories that specialize in analytical chemistry, especially mass spectrometry, minimize contaminants by using only glass and other inert materials (e.g., Teflon, stainless steel, etc.) in the laboratory, by cleaning glassware with exposure to high temperatures, and by eliminating

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sources such as stretchable sealing films or plastic tubing. As scientific research becomes more interdisciplinary, adherence to such strict procedures increases in difficulty. In contrast to analytical chemists, biologists often rely heavily on plasticware to conduct their research and it may be impractical to eliminate plastic materials from biological research; therefore, a compromise must be made and the potential for artifacts introduced from plasticware must be considered.

While the potential interference in analytical measurements of plasticizers and other contaminants is appreciated, the possible effect of these chemicals on laboratory animals and humans is the subject of extensive study and strong debate. Concern over the possible adverse health effects associated with plasticizers mimicking other naturally occurring compounds present in biological systems has brought these compounds under intense scrutiny. Phthalate esters and bisphenol A (BPA) are two such examples. Phthalate esters are primarily used in the production of vinyl-based plastics, which in turn are used to manufacture a variety of products ranging from cosmetics to medical devices to toys.[1,2] Phthalate esters are considered to be among the most common contaminants in the laboratory, and their analytical measurement is the subject of numerous reports.[3-5] Extensive research on the potential health effects associated with exposure to phthalate esters has yielded conflicting data.[6-8] BPA is the monomer used in production of polycarbonate plastic and epoxy resins. Humans are routinely exposed to this compound as BPA-containing plastics are found in food and beverage containers.[9] Recent scientific panels convened by the Environmental Protection Agency and National Institutes of Health reported that while adverse health effects are observed in animal models there is insufficient data to support conclusions regarding effects in humans and that more extensive research is necessary.[9-13]

Although phthalate esters and BPA will likely continue to be used in the production of plastics, the industry is constantly evolving and producing novel compositions of these polymers. Recently, nuclear clarifying agents (NCAs) have become widely used in the production of polypropylene. Three common sorbitol-based NCAs are marketed under brand names that include Irgaclear, Irgaclear DM, and Millad. These additives increase optical clarity, improve physical properties, and aid in aspects of the production process.[14] Improved optical clarity makes the plastic more aesthetically pleasing to the consumer and therefore a more economically viable product. The enhanced physical properties make it an attractive alternative to competing plastics like polystyrene and poly(ethylene terephthalate). Although extensive literature exists on the physical properties of NCAs, limited data are available on their analytical characteristics, such as mass spectra or chromatographic behavior. The matrix-assisted laser desorption/ionization mass spectrum of Irgaclear DM was published by Kim et al. in 1998.[15] Feigenbaum et al. in 2002 mentioned the extraction of Irgaclear D from polypropylene and included some gas chromatographic (GC) data.[16] Otherwise, to our knowledge, there are no other literature reports on the mass spectral or chromatographic behavior of these compounds.

Polypropylene plastic is widely used to produce common items found in the laboratory including pipet tips, conical tubes, and microcentrifuge tubes. It is also frequently used to make reusable food storage containers found in many households. Many of these items are virtually transparent, suggesting they contain sorbitol-based NCAs. We hypothesized that such NCAs may be extracted from plasticware under routine use and, in the laboratory and home become a source of contamination in sample extractions, analytical measurements, and food. We report here the extractability of three sorbitol-based NCAs from commonly used laboratory and household polypropylene plasticware, their electron ionization mass spectra (native and trimethylsilyl derivatives) and their collision-induced dissociation (CID) mass spectra. With this information, scientists will now have analytical data to help identify these compounds in their analyses, and the scientist and consumer will be able to make more informed decisions regarding their daily use and exposure to polypropylene plastics.

EXPERIMENTAL SECTION

Materials

Samples of neat sorbitol-based NCAs were provided by Ciba Specialty Chemical Co. (Tarrytown, NY) for use as analytical standards. The structure, systematic name, and trade name are provided for each compound in Figure 1A-C; the base structure, sorbitol, is shown for reference purposes in Figure 1D. Absolute ethanol (ACS/USP grade) and water (HPLC grade) used in this study were from Pharmco-Aaper (Shelbyville, KY) and Fisher Scientific (Fair Lawn, NJ), respectively. All other solvents were HPLC grade or better. Tri-Sil silylating reagent (TMS) was obtained from Pierce (Rockford, IL).

We tested common laboratory items made of polypropylene including 1.5-mL microcentrifuge tubes, 15-mL conical tubes, and pipet tips (200 and 1000 μL; both standard and barrier styles). Polypropylene food storage containers typical of those found in household kitchens (~120- and 240-mL size) were also tested. Manufacturer and brand information for plasticware investigated are provided in Supplementary Table 1.

Extraction of Laboratory and Household Polypropylene Plasticware

Ethanol (1 mL) was added to a 1.5-mL snap-cap microcentrifuge tube with a volumetric glass pipet. The cap was closed, and sample was mixed by vortex action for ~10 s. The vial was left overnight on the benchtop at room temperature, and the ethanol was transferred with a Pasteur pipet to an autosampler vial for analysis. These procedures were repeated in fresh tubes using water as solvent. Aliquots of the ethanol and water were pipetted directly into separate autosampler vials from stock bottles to serve as negative controls. Experiments were performed in triplicate for each tube brand, size, and solvent combination using a new tube for each experimental replicate. A similar procedure was used for evaluating 15-mL conical tubes with 5-mL aliquots of ethanol or water left in the tubes overnight; afterward, 1 mL was transferred to autosampler vials for analysis.

Into 4-mL glass vials, 1 mL of ethanol was added with a glass volumetric pipet. A plastic pipet tip was secured onto an appropriate pipet and the tip placed in the ethanol. The ethanol was drawn up to the maximum volume and expelled a total of three times. The sample was then transferred to an autosampler vial with a Pasteur pipet and analyzed. Using the same pipet tip, this procedure was repeated using a second 1 mL of ethanol and transferred to an autosampler vial. This two-step “washing” procedure was replicated using water as a solvent. Aliquots of the ethanol and water were placed directly into separate autosampler vials with a Pasteur pipet from stock bottles to serve as negative controls. All experiments were performed in triplicate for each tip brand, size, and solvent combination using a new tip for each experimental replicate.

Two sets of plastic food containers were evaluated; one set of containers was analyzed directly from the manufacturer's packaging, the other was washed in a household dishwasher on normal wash cycle with detergent and without heated drying. Water was placed in each container (10 mL) using a glass volumetric pipet. The containers were then closed and placed on the benchtop overnight. To simulate heating of food, the containers were placed in a consumer-grade microwave oven (lids partially unseated) for 1 min on the highest power setting. After cooling for several minutes, the water was transferred to a glass vial sealed with a Teflon-lined screw cap. Samples were stored at 4 °C until analyzed, which in some instances caused the solutions to become cloudy. Some samples remained cloudy even after re-equilibration to room temperature. In order to clarify the solutions, aliquots of each were transferred to glass centrifuge tubes and centrifuged at 2600 rpm for 20 min prior to analysis. A 1-mL aliquot was

placed in an autosampler vial and analyzed. An aliquot of the HPLC-grade water was used as a negative control. The experiment was performed in triplicate for each brand of container.

Preparation of Standards

Stock solutions of the sorbitol-based NCAs were prepared by placing a small amount of each compound (~100 mg) into a Teflon-lined screw cap glass test tube and adding 10 mL of ethanol. Each tube was vortexed, and the resulting slurry was centrifuged at 3500 rpm for 15 min at 25 °C to separate the undissolved compound. The supernatant was removed from each tube with a glass pipet and transferred to a fresh tube. Each sample was centrifuged again at 3500 rpm for 15 min at 25 °C, and the supernatant was transferred to a Teflonlined screw cap vial using a Pasteur pipet. The solubility of each compound in ethanol was estimated gravimetrically by placing 5 mL of each solution (considered to be saturated) into a pre-weighed vial. The solvent was evaporated under a gentle stream of nitrogen while heating at ~35 °C, and the vial was reweighed. This process was repeated in triplicate for each sample, and the solubility was calculated as the average of the three replicates. From the stock solution of each compound, 10-fold serial dilutions were prepared for use in an external calibration curve for quantitative analysis by high-performance liquid chromatographic–mass spectrometry (HPLC–MS).

Samples of NCAs were prepared for gas chromatography/mass spectrometry (GC/MS) analysis by preparing saturated solutions of each compound in acetone using the procedure described above. Saturated stock solutions were diluted 10-fold in acetone prior to analysis. Trimethylsilyl ether derivatives were prepared by taking 1-mL aliquots of the stock solutions in acetone and drying them under a gentle stream of nitrogen. Tri-Sil reagent (0.5 mL) was added to each vial, which were immediately capped and placed in an oven at 75 °C for 30 min. Samples were allowed to cool to room temperature and injected directly into the GC/MS.

Instrument Information

Analyses were performed using both HPLC-MS and GC/MS. The HPLC-MS instrument consisted of a Shimadzu binary HPLC system (Shimadzu Scientific Instruments, Columbia, MD) with a CTC LC PAL autosampler (Leap Technologies, Carrboro, NC) coupled to a 4000 Q TRAP mass spectrometer (Applied Biosystems, Foster City, CA) through a Turbo V electrospray ionization source. Samples were resolved with a binary solvent gradient using a Luna C_{18} HPLC column (150 × 3 mm, 3-μm particle size; Phenomenex, Torrance, CA). The mobile phases were (A) H_2O with 5 mM ammonium acetate and (B) methanol with 5 mM ammonium acetate. The gradient began at 50% B for 5 min, was ramped to 100% B over 5 min, and held at 100% B for 5 min. The column was then reconditioned at 50% B for 5 min. The flow rate was 0.3 mL/min, the injection volume was 10 μL, and the column temperature maintained at 30 °C. The mass spectrometer was operated in the multiple reaction monitoring (MRM) mode for quantitative analysis and enhanced product ion mode for acquisition of CID mass spectra. In both, the source was operated in positive (+) mode with the spray voltage, declustering potential, and collision energy set to 5500, 80, and 15 V, respectively. Curtain gas, gas 1, and gas 2 were set to 15, 60, and 20 psi, respectively, and the CAD gas was set to medium. MRM transitions for each compound were chosen as the ammonium adduct $[M + NH_4]^+$, and the protonated molecular ion $[M + H]^+$, generated through the loss of NH_3. CID mass spectra were acquired with instrumental conditions described above except a scan range of 100−450 Da was used.

Electron ionization mass spectra were acquired by GC/MS using an Agilent 6890 GC coupled to an Agilent 5973 mass spectrometer (Wilmington, DE). The instrument was equipped with a 30-m, DB-5MS column (250-μm i.d., 0.25-μm film thickness; J&W, Folsom, CA). The injection port was maintained at 300 °C, and 1-μL injections were performed using a pulsed splitless injection of 25 psi for 1 min. Helium was the carrier gas at an average linear velocity

of 38 cm/s. The temperature program began at 150 °C for 1 min, was then ramped at 10 °C/min to 325 °C, and held for 11.5 min. The MS transfer line was heated to 300 °C, the ion source to 230 °C, and the quadrupole to 150 °C. Ionization energy was 70 eV. Mass spectra were acquired by scanning from 75 to 600 Da.

RESULTS AND DISCUSSION

Mass Spectra

The EI mass spectra of the sorbitol-based NCAs evaluated in this study are shown in Figure 2A-C. The mass spectra share several common characteristics including the presence of a pseudomolecular ion at $[M - 1]^{+\cdot}$ resulting from the loss of a labile benzylic hydrogen atom[17] for Irgaclear, Irgaclear DM, and Millad (see Figure 1A–C, **I**, **II**, and **III**, respectively). The loss of a methyl group at $[M - 15]^{+}$ is observed for **II** and **III**, but not **I**. A high-mass ion arising from cleavage of the alkyl bond between C4 and C5 and resulting in the loss of 61 Da is a common fragment in all three spectra. Another notable fragment is due to multiple bond cleavage between C1 and C2, C2 and C3, and C3 and C4 producing ions at *m*/*z* 149, 163, and 177 (Figure 2A–C, respectively). The base peak in each spectrum results from cleavage of the acetal bonds linking the benzyl groups to the sorbitol base structures resulting in ions at *m*/*z* 105 (benzaldehyde), 119 (methylbenzaldehyde), and 133 (dimethylbenzaldehyde) for **I**, **II**,and **III**, respectively. Structures with selected fragmentations for each compound are inset with their respective mass spectra (Figure 2A-C).

The EI mass spectra of the trimethylsilyl derivatives of the NCAs are shown in Figure 3A–C. Several common features were observed between the three derivatized NCAs. The molecular ion for each silylated compound is present as $M^{+\cdot}$, and a relatively abundant ion at $[M - H]^{+\cdot}$ was present due to loss of a labile benzylic hydrogen atom. Loss of a methyl group at $[M - CH_3]^{+\cdot}$ was observed in all three mass spectra, which was likely due to a combination of methyl loss from the TMS group as well as loss of the benzyl methyl groups for **II** and **III** (Figure 3B and C, respectively). Cleavage of the C5 and C6 bonds produced a low-mass fragment at *m*/*z* 103 and the resulting high-mass fragment at $[M - 103]^{+\cdot}$ for each compound. Two additional high-mass fragments were formed from cleavage of the C4 and C5 bond yielding a common fragment at *m*/*z* 205 and the fragment at $[M - 205]^{+\cdot}$. The base peak in the mass spectra for **II** and **III** (Figures 3B and C) resulted from cleavage of the acetal bonds that link the benzyl group to the sorbitol base structure producing a methylbenzaldehyde fragment ion (*m*/*z* 119) and a dimethylbenzaldehyde fragment ion (*m*/*z* 133). A similar ion was observed for **I** (Figure 3A) from the benzaldehyde fragment (*m*/*z* 105), which was not the base peak. The base peak for **I** was an ion at *m*/*z* 179, whose origin was not understood.

CID mass spectra were acquired via electrospray ionization (Figure 4A–C). Due to the lack of an acidic or basic moiety for protonation/deprotonation, ammonium adducts were employed to increase ionization efficiency. The ammonium adducts of each NCA were selected for CID. The mass spectra for NCAs were similar in that each showed an ammonium adduct, a protonated molecular ion (resulting from the loss of NH_3 from the ammonium adduct and not from protonation of the molecular species), and the loss of one water. The $[M + H]^{+}$ ion was the base peak in all three spectra. Although the NCAs studied here have two alcohol groups, none showed a second water loss by CID. Multiple water losses have been observed for analogous compounds such as oxysterols.[18] Each compound exhibited a unique fragment ion resulting from dissociation of the acetal bonds that link the alkyl-benzyl group to the sorbitol backbone. The fragment ion from **I** differed slightly from the other two compounds in that a loss of benzaldehyde produced a *m*/*z* 251 ion. A loss of xylene produced the *m*/*z* 281 ion, while loss of trimethylbenzene produced the ion at *m*/*z* 295 for **II** and **III**, respectively. Figure 4A–C depicts abundant and unique fragments for each molecule together with mass spectra.

Extraction of NCAs from Common Laboratory and Household Plastics

The analysis of two brands of 1.5-mL microcentrifuge tubes (brand "A" and brand "B") extracted with water and ethanol is shown in Figure 5A. **III** was detected in both brands when extracted with either water or ethanol. Both brands of microcentrifuge tubes had comparable levels of **III** (~0.02 μg/mL) in the water extracts; however, the ethanol extracts of brand A tubes had four times the amount of **III** (~0.25 μg/mL) when compared to the ethanol extracts of brand B tubes (~0.08 μg/mL). **II** was also detected in both brands of microcentrifuge tubes, but only in the ethanol extracts. Levels of **II** were comparable in the ethanol extractions of both brands at ~0.0007 μg/mL. Based on our data, ethanol extracts **II** and **III** more efficiently than does water. The total NCA level (**II** + **III**)was ~10-fold higher in ethanol extracts of brand A tubes and ~3-fold higher in ethanol extracts of brand B tubes compared to water extracts. The levels of **III** were 3 orders of magnitude greater than those of **II**. **I** was not detected in any of the microcentrifuge tube extracts.

Data from the extraction of conical tubes (15 mL; two brands) are shown in Figure 5B. The only NCA detected in all four samples analyzed was **II** and ranged in concentration from ~1.5 (brand B, water extract) to ~3.0 μg/mL (Brand B, ethanol extract). The levels of **II** were slightly higher in ethanol extracts from both brands of conical tubes when compared to levels in water extracts (Figure 5B). **III** was detected in every extract except the ethanol extracts of brand A tubes. Levels of **III** ranged from ~0.001 (brand B water extract) to ~0.01 μg/mL (brand B ethanol extract). The **II** levels were 2 orders of magnitude higher than the levels of **III**. **I** was not detected in any of the extracts.

Of the two styles of 200-μL pipet tips evaluated (Figure 5C–D), only **III** was detected in these experiments at levels ranging from ~0.002 to ~0.028 μg/mL. **III** was only detected in ethanol extracts of the nonbarrier tips. Additionally, the concentration of **III** increased slightly between the first and second ethanol washes (extracts A and B, respectively). These data indicate that rinsing standard pipet tips does not significantly reduce the amount of **III**. Levels of **III** in nonbarrier tips differed significantly from the barrier tips in that the highest level of **III** was detected in the first ethanol extract (extract A) of the barrier tips (Figure 5C). Levels of **III** were higher in the first wash (extract A) than the second wash (extract B) for both the water and ethanol extracts, suggesting that rinsing the barrier-style pipet tips removes a significant amount of **III**.

Similar to the 200-μL tips, **III** was the only NCA detected in any of the 1000-μL tip extractions (Figure 5D), and was only detected in the barrier tip extracts at levels ranging from ~0.005 (barrier tip, second ethanol wash) to ~0.055 μg/mL (barrier tip, first ethanol wash), indicating that rinsing barrier-style tips with ethanol eliminates a large amount of available **III** from the plastic (~10-fold difference). There was little difference in the amount of **III** detected in the first and second water extractions (Figure 5D).

The only detectable NCA in the extracts from food storage containers was **III** (Figure 5E). The levels of **III** ranged from ~0.002 (brand A, washed) to ~3.0 μg/mL (brand B, washed) and were substantially reduced by washing brand A containers (Figure 5E). Overall, the lowest levels of **III** were observed in brand A containers and the highest levels of **III** were observed in the brand B brand containers. With the exception of the brand A products, washing the containers in a dishwasher prior to extraction had little effect on the amount of **III** extracted (Figure 5E).

Discussion

In the present study, extraction procedures for all plastic labware tested were designed to represent typical applications performed in a scientific laboratory. Ethanol and water were used

as solvents as they are often stored, transferred, or otherwise manipulated with plastic labware. One could imagine many variations and permutations of these extraction procedures, including the use of different solvents; however, the intent of this study was only to demonstrate that sorbitol-based NCAs are readily extractable under normal use conditions. Food containers were extracted with water as an alternative to food items that may be stored and heated in these containers because controlling all experimental variables when extracting food was beyond the scope of this work. Also, water is required for microwave heating and is a common denominator in any food product stored in such a container. All manufacturers recommend washing the container prior to use, so one set of food storage containers was washed prior to extraction.

The total amount of sorbitol-based NCAs extracted from laboratory plasticware varied considerably between the types of plasticware analyzed. With the exception of the microcentrifuge and conical tubes, **III** was either the sole or the most abundant NCA extracted from items evaluated in this study. Approximately 10−100 ng of **III** were extracted from these items. The micro-centrifuge tubes and pipet tips were extracted with 1 mL of solvent so the actual amount of NCA extracted from these items can be determined directly from the *y*-axis units (Figure 5A, C, and D). Conical tubes were extracted with 5 mL of solvent, indicating that tens of micrograms of **II** were extracted from each tube. The food storage containers were extracted with 10 mL of water; thus, total levels of **III** were in the 10−30 μg range for brand B and brand C extracts and ~1 μg for the unwashed brand A extract.

In microcentrifuge tubes, ethanol extracted NCAs more efficiently than water. Conical tubes also had more NCAs extracted with ethanol as compared to water. Extractions of pipet tips illustrated that, for the barrier-style tips, ethanol eluted more **III** compared to water, whereas the results for the standard/nonbarrier tips showed a modest increase in extractability for **III** in ethanol when detected. With the exception of the barrier-style tips extracted with ethanol, there was no decrease in NCA level from the first wash to the second wash. Prewashing food storage containers in the dishwasher did not affect the amount of **III** extracted from the containers for brand B or C (Figure 5A–E).

We believe that the levels of **III** reported in the extracts of the food containers may be underestimated. After the microwave heating step, the water appeared clear and free of precipitate; however, storage of the samples in the refrigerator caused some to become cloudy, even after re-equilibration to room temperature. Centrifugation clarified extracts enough to allow injection onto the HPLC–MS. This observation suggests that, above ambient temperatures, increased levels of **III** could be present due to increased solubility at higher temperatures. Since soup and other food items are typically consumed after heating, it is reasonable to assume additional amounts of NCAs may be leached.

When pure solvents were analyzed by HPLC–MS as negative controls, trace levels of **III** were detected in virtually all solvent injections performed. Small amounts of **III** were detected even when null injections were performed, suggesting that these compounds may be ubiquitous (data not shown). As a result, prior to quantitation the chromatographic peak areas of background levels of **III** were subtracted from the extracts analyzed in this study.

The results reported here were derived from discrete extractions of individual plastic labware performed so that a quantitative assessment of NCAs extractability could be determined; however, due to the nature of most experimental work, there is likely to be an additive affect with regard to the amount of NCAs introduced into an experiment when plastic labware is used. For example, scientists often keep small containers of solvents on the benchtop to avoid repeatedly handling large stock bottles of solvents. A survey of the departmental laboratories at the University of Texas Southwestern Medical Center shows a mixture of glass bottles and

conical plastic tubes being used for solvent storage. If solvents stored in a conical tube are pipetted with pipet tips into a plastic microcentrifuge tube (all made of polypropylene), one can clearly see how amplification in the amount of NCAs added to an experiment could occur. This accumulation would likely increase the amount of NCAs observed in extractions, delivered to cells in tissue culture, and added to any other aspect of an experiment where solvents were used. In a similar fashion, if a scientist uses the same glass bench bottle for an extended period of time and uses plastic pipet tips to remove the solvent, as the amount of solvent decreases within the container the concentration of NCAs would increase. If the same container is constantly refilled, the concentration of NCAs would continue to increase in the container over time.

The primary intent of this work is to present the mass spectral and chromatographic data on sorbitol-based NCAs, thereby providing scientists the necessary data to identify these compounds in their chromatographic and mass spectral analyses. Secondary is the desire to inform both scientists and consumers about compounds that are potentially being introduced into biological experiments in the laboratory and potentially ingested by users of these food storage containers. A review of the literature offers no data on the biological or health effects of these compounds, and we are not aware of any biological effects of these compounds on living systems, whether they are cultured cells, laboratory animals, or humans.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENT

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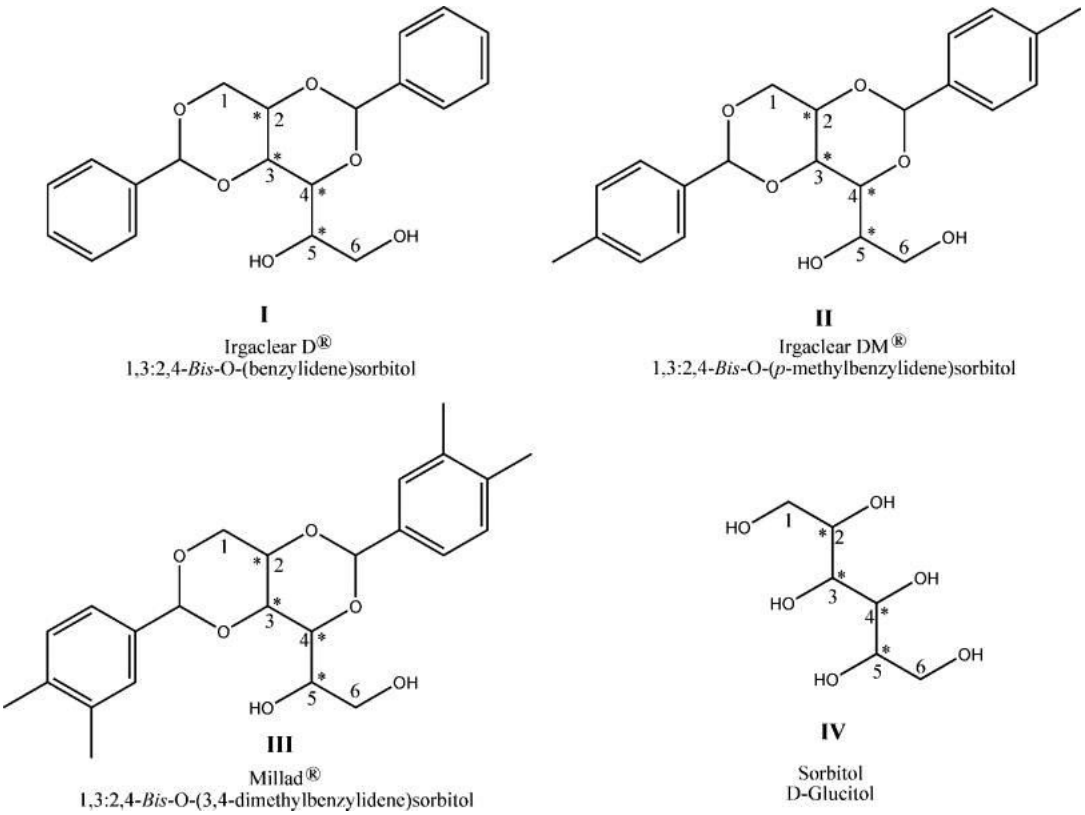

Figure 1.
Structures and trade names of sorbitol-based nuclear clarifying agents and sorbitol. Stereocenters are indicated with an asterisk (*).

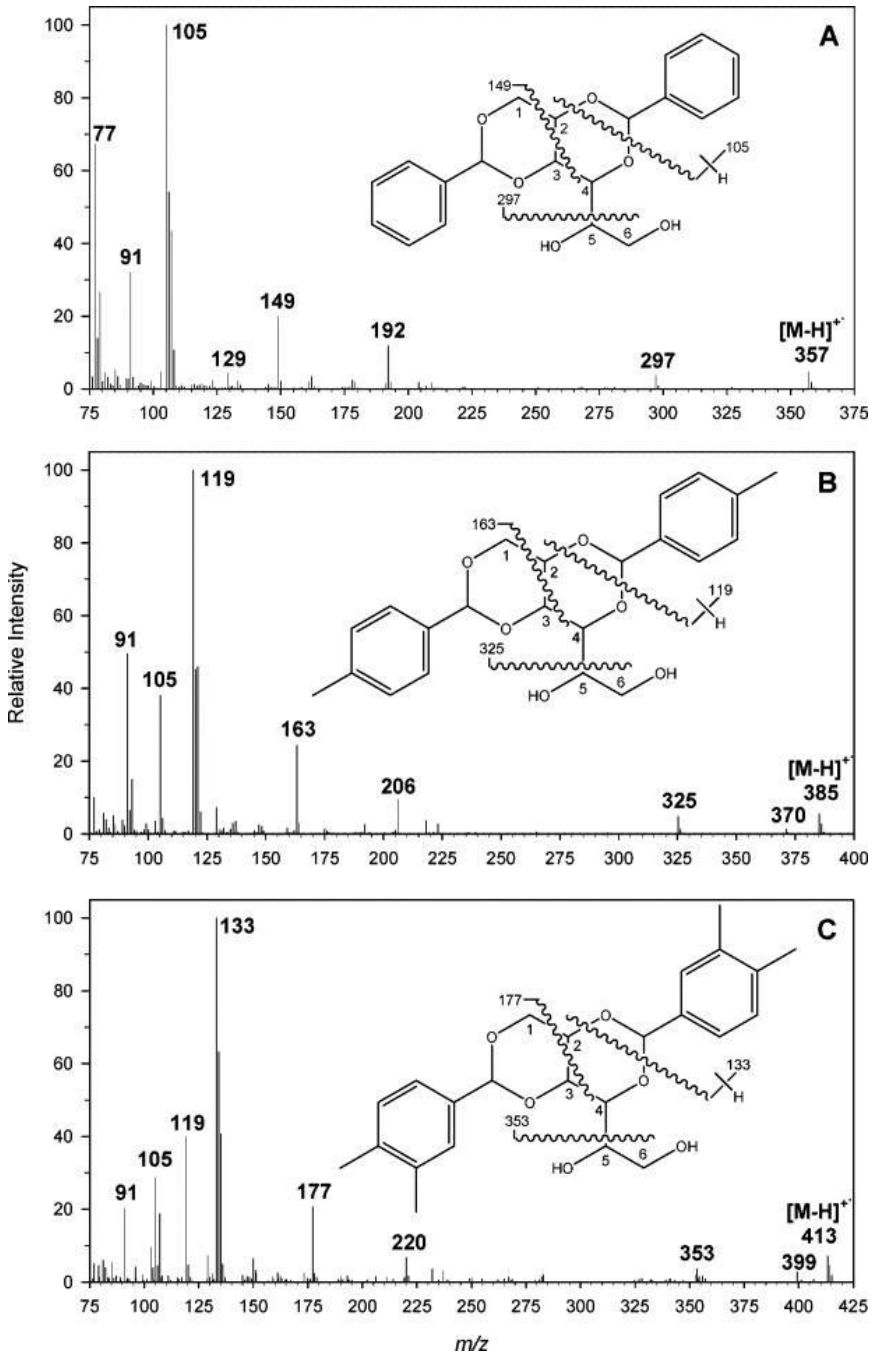

Figure 2.
Electron ionization mass spectra of sorbitol-based nuclear clarifying agents (A–C). Characteristic fragments are shown with the structure in each panel.

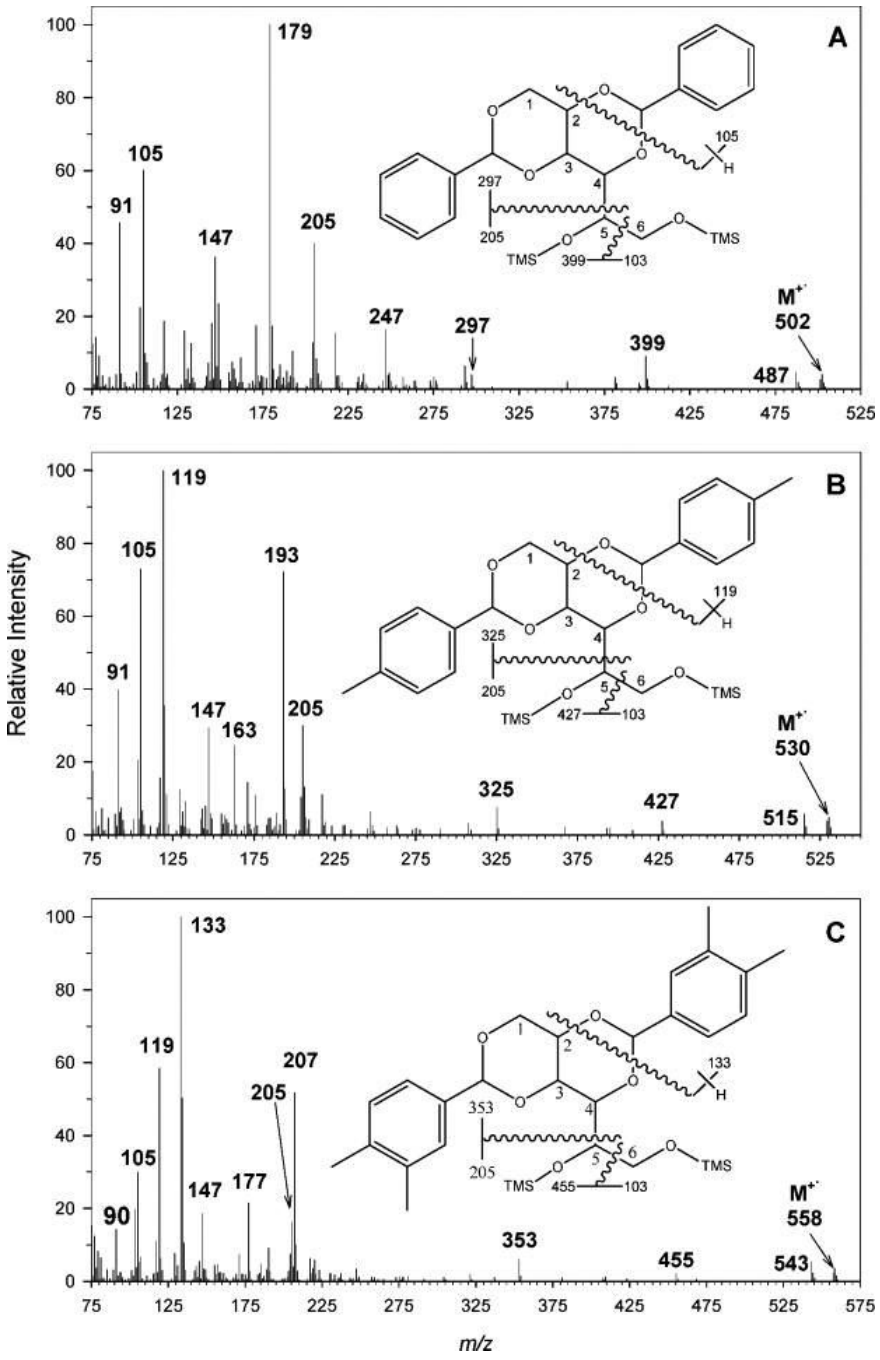

Figure 3.
Electron ionization mass spectra of trimethylsilyl derivatives of sorbitol-based nuclear clarifying agents (A–C). Characteristic fragments are shown with the structure in each panel.

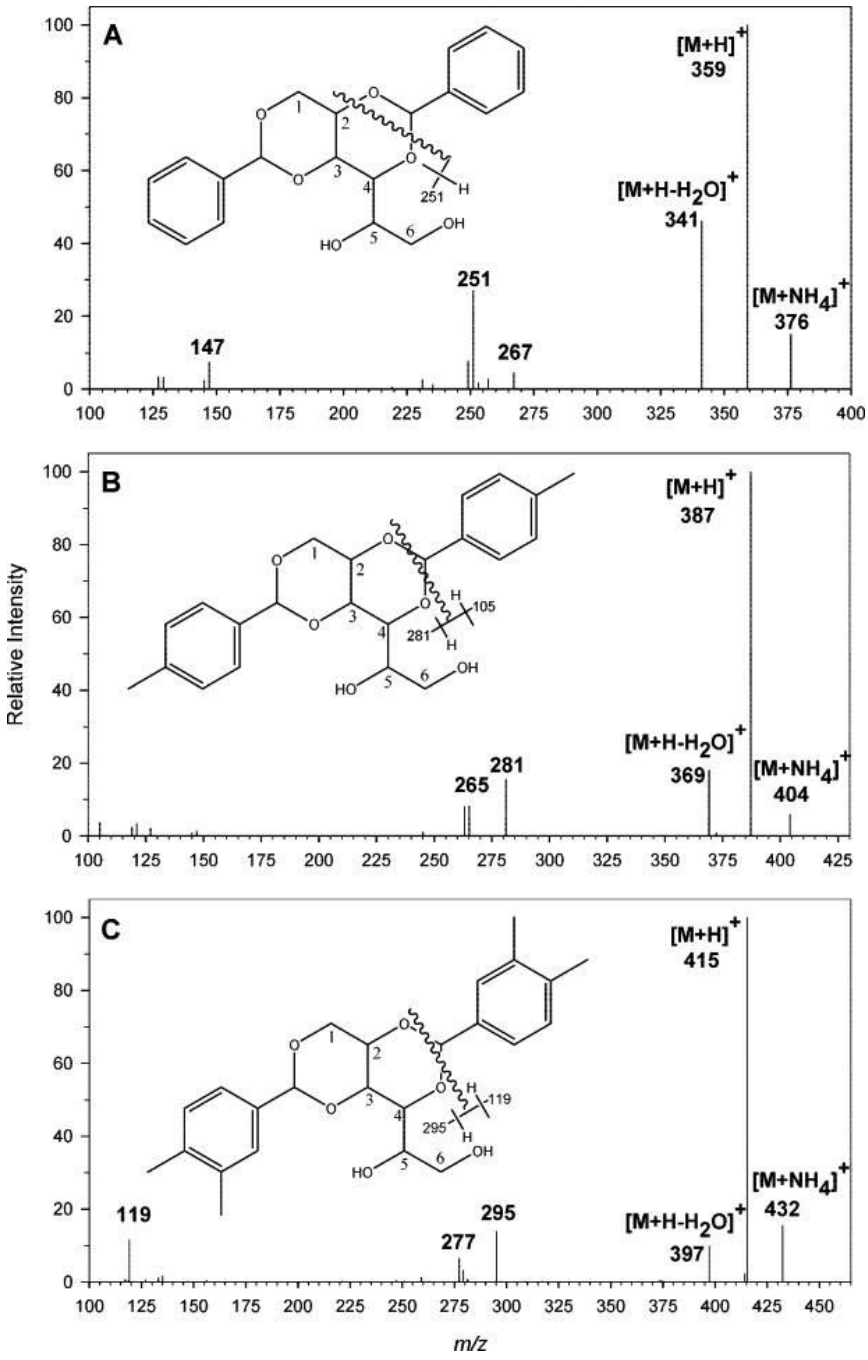

Figure 4.
CID mass spectra of sorbitol-based nuclear clarifying agents. Characteristic fragment ions are shown with the structure in each panel.

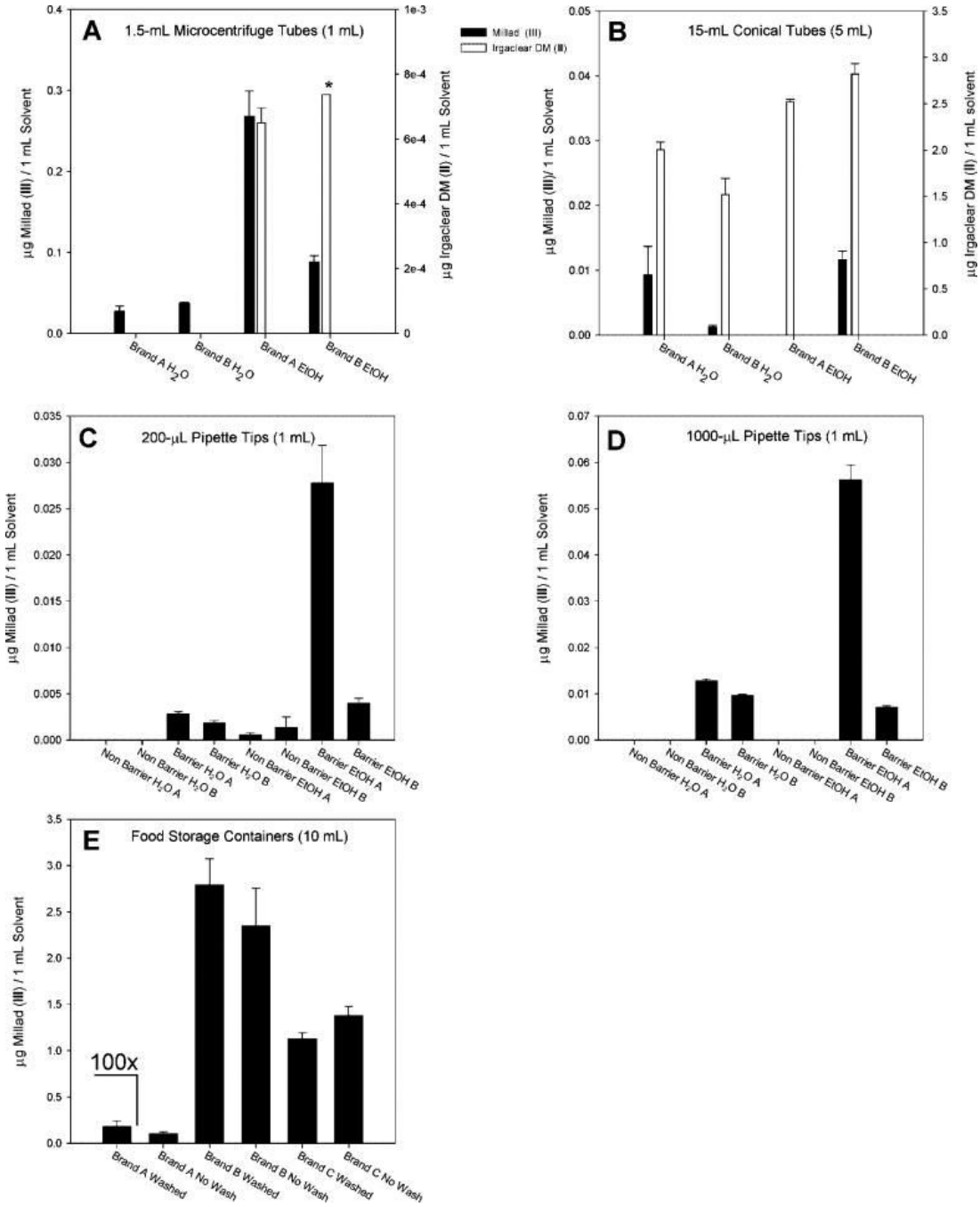

Figure 5.

Quantitative data for sorbitol-based nuclear clarifying agents extracted from common laboratory plasticware and household food containers. Error bars represent the standard error (n = 3). Note the use of left and right axes in panels A and B. The absence of data for a specific compound indicates it was not detected at the detection limits given in Table 1. For panels C and D, an "A" in the label indicates the first extraction and the "B" label indicates the second extraction. The data are reported in μg/mL and the total volume extracted in each experiment is given in parentheses following the title in each figure. The data for brand A washed is shown

at a 100-fold increase to place it on a similar scale with other data. Actual concentration is ~0.002 μg/mL. * n = 2.

Table 1

Summary of Useful Information for Sorbitol-Based Nuclear Clarifying Agents

	Irgaclear D (I)	**Irgaclear DM (II)**	**Millad (III)**
systematic name	1,3:2,4-bis-*O*-(benzylidene) sorbitol	1,3:2,4-bis-*O*-(*p*-methylbenzylidene) sorbitol	1,3:2,4-bis-*O*-(3,4-dimethylbenzylidene) sorbitol
CAS number	32647−67−9	81541−12−0	135861−56−2
molecular weight	358.14	386.44	414.2
solubility in EtOH[a]	~1.2 mg/mL	~0.25 mg/mL	~0.14 mg/mL
LOD (LC/MS)[b]	10 pg	20 pg	2 pg
retention time[c]			
LC/MS	10 min	11 min	11.5 min
GC/MS[d]	16 min/15.5 min	17.5 min/16.5 min	19 min/18 min

[a] Determined gravimetrically using a saturated solution in EtOH.

[b] Mass on column, defined as at least 2 × s/n for **I** and **II**, and 2 × average background level for **III**.

[c] Retention times are based on the instrumental methods described in the Experimental Section. Approximations are provided because a defined retention index system (e.g., methylene retention index) was not used here. Retention times are provided as an aid to the analyst wishing to analyze for these compounds.

[d] Underivatized/trimethyl silyl ether derivative.