Determination of Bisphenol A Released from Resin-Based Dental Composite Restoratives

Editor’s note: This is the second article on Bisphenol A (BPA) in dental materials. In Volume 8, Issue 2, “Update: Bisphenol A in Dental Materials,” we explored the question, “Why would BPA appear in ‘BPA-free’ dental materials?” In recent years, there’s been increased media attention on BPA and its potential impact on health and human development. Headlines have linked BPA to heart disease, coronary artery disease, obesity, diabetes and immune system and reproductive disorders, among other health conditions. For this report, researchers in the ADA Laboratory examined the potential release of Bisphenol A in resin-based composites.

Bisphenol A (BPA) is a common compound used to make polycarbonate plastic and epoxy resins and is found in many items such as eyeglass lenses, cellphones and plastic eating utensils.\(^1\) BPA has been used in consumer products since the 1960s and is also an important component in the manufacturing of a majority of preventive and restorative dental products currently on the market.\(^2-4\) In 1996, dental sealants were identified as a low-level source of BPA exposure.\(^5\) Even in some dental composites and sealants advertised as “BPA-free”, BPA sometimes can be detected because it is used in the synthesis of bis-GMA (bisphenol A-diglycidyl ether methacrylate). Therefore, trace amounts of BPA may be present as a byproduct of the manufacturing process. Bis-GMA, or Bowen’s resin, is one of the most common ingredients used in dental composite resin materials. Bis-GMA is an aromatic ester of dimethacrylate (Figure 1) and functions to limit the polymerization-induced volumetric changes, as well as to enhance resin reactivity. The viscous nature of bis-GMA makes it extremely difficult to incorporate polymerization initiators without the addition of modifiers to ease handling properties. These modifiers, such as bisphenol A dimethacrylate (bis-DMA) and tri(ethylene glycol)dimethacrylate (TEGDMA) (Figure 1), reduce the viscosity of bis-GMA to allow the addition of stabilizers and polymerization initiators.\(^1\)

Urethane dimethacrylate (UDMA) restorative resins are available and are not manufactured from BPA (Figure 1). However, their use as a bis-GMA resin alternative is limited, because they do not develop equivalent stiffness and hardness characteristics as bis-GMA based restoratives.
Since both bis-GMA and bis-DMA can release trace levels of BPA, the use of these monomers has been heavily scrutinized in recent years. The controversy surrounding BPA stems from its ability to mimic estradiol, a hormone produced by a variety of tissues including the ovaries and testes, and to bind to mammalian estrogen receptors in laboratory studies.\textsuperscript{5-13} There are concerns that BPA might be linked to heart disease, obesity, diabetes, immune system and reproductive disorders, breast cancer and a decrease in sperm count, among other health issues.\textsuperscript{1, 11, 14-16}

How does BPA find its way into the human body? BPA exposure comes primarily from packaging of processed foods and beverages. Dental resin-based restorative materials are another source of exposure.\textsuperscript{17-20} Resin-based materials, such as composites, adhesives, pit and fissure sealants and cements, undergo monomer-to-polymer conversion during light-activated or chemically activated polymerization. While BPA is not an added component to dental resin composite materials, it can be a contaminant or residual monomer in resin-based dental materials containing bis-GMA. BPA also might be present due to the degradation of bisphenol A-dimethacrylate (bis-DMA) through the action of salivary esterases. This may affect the composite microstructure by forming pores that can lead to weakening of the composite material,\textsuperscript{18, 19, 21-23} which in turn may facilitate BPA release.

![Image](image.png)

A study by Kingman\textsuperscript{24} conducted in 2012 revealed that exposure to BPA from dental resin composite placement is very brief and miniscule compared to the U.S. Environmental Protection Agency’s acceptable daily exposure limit.\textsuperscript{25} Using the test subjects’ salivary BPA measurements before composite placement and then at intervals post-placement, researchers reported salivary and urinary BPA levels over time. The EPA-accepted BPA exposure limits are $3.5 \times 10^6$ nanograms per day for a 70 kg man and $0.5 \times 10^6$ nanograms per day for a 10 kg child, which is
500,000 times higher than the amount Kingman found from current bis-GMA-based composites, as well as above the 5,000 ng/kg/day suggested by the European Food Safety Authority (EFSA).17,25

For this study, ADA Laboratory researchers investigated the amount of BPA present in 11 bis-GMA-based composite restorative resins (Table 1): Heliomolar HB (Ivoclar Vivadent, Amherst, N.Y.), Filtek Supreme Ultra Universal (3M ESPE, St. Paul, Minn.), Grandio (Voco, Briardiff Manor, N.Y.), SonicFill (Kerr, Orange, Calif.), Tetric EvoCeram Bulk Fill (Ivoclar Vivadent), X-tra fill (Voco), Filtek Bulk Fill Flowable Restorative (3M ESPE), SureFill SDR Flow (Dentsply Caulk, Milford, Del.), Clearfil Majesty Flow (Kuraray America, New York, N.Y.), Filtek Supreme Ultra Flowable (3M ESPE), Esthet-X Flow (Dentsply, York, Penn.), and two products marketed as bis-GMA-free, Embrace WetBond Class V (Pulpdent, Watertown, Mass.) and Venus Bulk Fill (Heraeus Kulzer, South Bend, Ind.)

In addition, researchers determined the amount of BPA eluted from the fully cured composites. The researchers also compared the experimentally observed levels of BPA to the acceptable exposure limits specified by the Environmental Protection Agency (EPA) and the European Food Safety Authority (EFSA).

Methods

Four different resin-based restorative materials (universal, bulk-fill, bulk-fill flowable and flowable) were selected for this study (Table 2). Two bis-GMA-free materials, raw bis-DMA, and raw bis-GMA from three
manufacturers also were evaluated.

Each of the materials evaluated contained bis-GMA or bis-GMA-based resin, with the exception of Venus Bulk Fill composite, which contains urethane dimethacrylate (UDMA) and bis-EDMA, and Embrace WetBond Class V, which also uses UDMA.
Researchers extracted one set of un polymerized specimens with acetonitrile to simulate a "worst-case scenario"—and the maximum possible BPA elution at 37°C for 24 hours. They polymerized the second set of specimens by using molds of 10 millimeters in diameter and 2 millimeters in depth and a mass of about 350 milligrams. They used an Optilux 501(Kerr, Orange, CA) polymerization unit with a light power density of greater than 600 milliwatts per square centimeter to cure the resin-based composites according to the manufacturer's instructions. They exposed specimens to air during light curing to allow for the formation of an oxygen-inhibited layer of under-cured resin, as would develop during clinical conditions. They then extracted polymerized discs with artificial saliva containing 0.3 units per milliliter of porcine esterase to simulate the BPA elution into the oral environment at 37°C for 24 hours. They also extracted one polymerized specimen of each product using similar artificial saliva conditions for 48 hours to assess any extended BPA release.

Once the scientists extracted the dental composites with acetonitrile or artificial saliva, they sent liquid extracts to a pharmaceutical development and analysis company (Intertek Pharmaceutical Services, El Dorado Hills, Calif.) for analysis by means of liquid chromatography tandem mass spectrometry (LC-MS/MS). Prior to analysis, extracts were further prepared, along with a calibration curve, using a liquid-liquid extraction procedure (in artificial saliva lacking porcine esterase or acetonitrile, as appropriate), evaporated to dryness, and reconstituted in a solution of methanol and ammonium acetate. The lower limit of BPA quantitation was 0.2 nanograms per mL (0.2 parts per billion) in the initial extracts of polymerized composites in artificial saliva, and 0.5 nanograms per mL (0.5 parts per billion) in the acetonitrile extracts of un polymerized composites. Quality control samples and a stable isotope labeled "internal standard" (d16-BPA) were used throughout the analyses to ensure accurate quantification of BPA.
Results

BPA was detected in unpolymerized dental composite materials in acetonitrile after 24 hours and the amount detected represented the maximum amount of BPA that could be released from all specimens tested. The amount of BPA released from unpolymerized composites dissolved in acetonitrile ranged from less than 5 ng per gram to about 12,000 ng per gram for bis-GMA-based composites (Table 3).

<table>
<thead>
<tr>
<th>Unpolymerized Composite Material</th>
<th>BPA in Acetonitrile (n=3) ng/g composite ± s.d.</th>
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<tbody>
<tr>
<td>Heliomolar HB</td>
<td>496 ± 0.5</td>
</tr>
<tr>
<td>Filtek Supreme Ultra</td>
<td>2231 ± 7.9</td>
</tr>
<tr>
<td>Grandio</td>
<td>1874 ± 0.6</td>
</tr>
<tr>
<td>SonicFill</td>
<td>1556 ± 3.8</td>
</tr>
<tr>
<td>Tetric EVO Ceram Bulk Fill</td>
<td>11889 ± 92.1</td>
</tr>
<tr>
<td>X-tra fill</td>
<td>651 ± 3.6</td>
</tr>
<tr>
<td>Filtek Bulk Fill</td>
<td>147 ± 1.4</td>
</tr>
<tr>
<td>SureFil SDR</td>
<td>377 ± 2.1</td>
</tr>
<tr>
<td>Clearfil Majesty Flow</td>
<td>&lt; 100*</td>
</tr>
<tr>
<td>Filtek Supreme Plus Flowable</td>
<td>343 ± 1.6</td>
</tr>
<tr>
<td>Esthet-X Flow</td>
<td>192 ± 1.7</td>
</tr>
<tr>
<td>Embrace WetBond Class V</td>
<td>&lt; 5.00*</td>
</tr>
<tr>
<td>Venus Bulk Fill</td>
<td>12,225.0 ± 706.9</td>
</tr>
</tbody>
</table>

* These values were below the limit of quantitation of 1.0 ng BPA/g and 5.0 ng BPA/g composite for these products. Interference prevented the measurement of BPA, and a lower limit of quantitation was used for the Clearfil Majesty Flow.

Table 3. Maximum potential BPA release from unpolymerized composites after 24 hours.

However, as seen in Table 4, when polymerized composites were extracted with artificial saliva for 24 hours, the BPA values dropped to a range of 1.4 to 80.4 ng of BPA per g of composite. Extraction for 48 hours resulted in similar levels and demonstrated minor, if any, additional leaching of BPA in all products.

As shown in Table 5, BPA was released from three different sources of raw bis-GMA dissolved in acetonitrile for 24 hours. These three sources of raw bis-GMA contained residual levels of BPA ranging from 25.7 to 475.9 ng/g of compound. This is an 18-fold difference from low to high BPA levels, indicating a wide range of residual BPA among different
suppliers. Furthermore, the commercial sample of bis-DMA demonstrated a very high level of residual BPA at almost 113,000 ng/g without hydrolysis by esterase. Therefore, it is easy to understand how BPA could be detected in a product that uses bis-DMA as an ingredient.

BPA in uncured composite is not proportionally extracted from the same mass as after the composite is cured. Venus Bulk Fill and Tetric EvoCeram Bulk Fill had the highest amounts of BPA in uncured material, yet proportionally had the least amount of extractable BPA in cured polymer. This demonstrates that other factors come into play that lock BPA into the polymer, making it more difficult to extract.

![Table 5: BPA levels in raw materials dissolved in acetonitrile.](image)

<table>
<thead>
<tr>
<th>Raw Material (Manufacturer)</th>
<th>BPA release (mg/g) ± s.d.</th>
</tr>
</thead>
<tbody>
<tr>
<td>bis-GMA (Estech, Inc.)</td>
<td>104.8 ± 4.4</td>
</tr>
<tr>
<td>bis-GMA (Polysciences, Inc.)</td>
<td>475.9 ± 8.6</td>
</tr>
<tr>
<td>bis-GMA (Sigma-Aldrich)</td>
<td>25.7 ± 1.4</td>
</tr>
<tr>
<td>bis-DMA (Sigma-Aldrich)</td>
<td>112.796.9 ± 21.5672</td>
</tr>
</tbody>
</table>

**Discussion/Conclusions**

Although manufacturers of dental restorative resin materials no longer add BPA to their products, there are several ways that composites may contribute to BPA exposure, such as:

- from residual unreacted BPA present in raw materials used to produce the resin restoratives;
- after enzymatic degradation of polymerized materials by salivary esterases;
- in the formation of an oxygen-inhibited layer of incompletely polymerized resin on the restoration surface;
- in the overall reduction in the degree of conversion of monomer to polymer in composites as a result of inadequate light-curing.

Tables 3 and 4 show that only a fraction of available BPA in resins is released after polymerization. It is apparent from this study that most residual BPA is locked inside the polymer, thus limiting BPA bioavailability. Both the 24-hour and 48-hour extracts of polymerized composite released BPA in similar amounts, which supports the clinical findings that BPA exposure would be acute and limited to a short period after composite placement.²⁴

An explanation for short-duration BPA exposure might be found in the oxygen-inhibited layer of freshly cured composite.²⁷ Wear of the soft, underpolymerized surface layer on unpolished composite restorations could account for the clinical observation that the majority of BPA released into saliva occurs within 24 hours post-placement.²⁴

Tables 3 and 4 clearly show the maximum BPA content in unpolymerized composite dissolved in acetonitrile compared to the more clinically relevant polymerized composite extracts in artificial saliva. The ratio of extractable BPA from polymerized composite increases with increasing amounts of BPA in
unpolymerized material. Thus, the majority of a material’s BPA content is most likely “locked” inside the polymerized configuration, making BPA considerably less bioavailable. Therefore, curing the restorative material as completely as possible maximizes the material’s monomer-to-polymer conversion and should minimize its potential to release BPA. Furthermore, polishing the restoration may remove the oxygen-inhibited layer on the surface, and likely contributes to substantially reduced duration of BPA release into saliva immediately after restoration placement.27

Perhaps the most important source of BPA is from residual BPA left over from the bis-GMA synthesis process. Table 5 shows measurable amounts of BPA in raw bis-GMA that varies from supplier to supplier. However, these levels are not a significant source of BPA exposure (Tables 3 and 4).

Table 5 also shows that the highest level of residual BPA was found in a preparation of bis-DMA (112,797 ng/g). BPA in acetonitrile was released from bis-DMA without hydrolysis by esterases. Since esterases could not have contributed to the release of BPA from these raw materials, and since BPA is used to synthesize these compounds, the detectable BPA likely was from residual, unreacted BPA remaining after completion of the synthesis process.

Some manufacturers advertise “bis-GMA-free” resin restoratives; however, not all bis-GMA-free materials are actually free of BPA. One of these products, polymerized Venus Bulk Fill, showed a comparatively high potential for BPA release (80 ng/g) after extraction in artificial saliva (Table 4). When dissolved in acetonitrile (Table 3), unpolymerized Venus Bulk Fill had the highest BPA level at 12,225 ng/g. The explanation for this observation is that Venus Bulk Fill is a bis-EDMA-based material, which is synthesized from BPA. Although raw bis-EDMA was not investigated in this study for residual BPA, it is likely that the detectable BPA remained as an unreacted material used in the bis-EDMA synthesis. Therefore, clinicians should be skeptical of the claim that bis-GMA-free restoratives have no potential to release BPA.

What do these findings mean?

The EPA's maximum acceptable daily exposure limits are $3.5 \times 10^5$ ng of BPA per 24 hours for an adult weighing 70 kilograms and $0.5 \times 10^6$ ng of BPA per 24 hours for a child weighing 10 kilograms.28 Recently, a more conservative BPA exposure level was proposed by the European Food Safety Authority (EFSA) at 5,000 ng per kilogram per day and is 10 percent of the EPA’s limit and corresponds to 17,500 and 2,500 times lower than the maximum permissible daily limit for adults and children, respectively.27 According to the EPA's and EFSA's limits, respectively, one polymerized 250 mg Venus Bulk Fill composite extracted with artificial saliva after 24 hours was 175,000 and 17,500 times lower than the maximum permissible daily limit for adults and 25,000 and 2,500 times lower than the maximum permissible daily limit for children. Venus Bulk Fill had the highest potential to leach BPA than any other product tested, yet this product presented no concerns on the basis of the current established exposure limits.
Similarly, several polymerized bis-GMA-based products with the lowest extractable BPA levels (at or below 2.0 ng/g) after 24 hours in artificial saliva would produce daily exposure levels that are 7 million and 1 million times lower than the EPA maximum permissible daily limit, and 700,000 and 100,000 times lower than the EFSA’s maximum permissible daily limit for adults and children, respectively, from one composite.

**Conclusion**

This study shows that bis-GMA-based dental restorative materials have the potential to release BPA at a detectable level. Furthermore, bis-DMA and bis-EDMA also demonstrated a high potential to release BPA. All sources of raw bis-GMA had detectable levels of BPA. However, all of the tested dental restorative composites released BPA at levels that are far below the daily exposure limits set by the U.S. Environmental Protection Agency and the European Food Safety Authority.

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**References**
