

BIS-GMA—BASED RESINS IN DENTISTRY: ARE THEY SAFE?

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ABSTRACT

Background. The authors critically surveyed research dealing with the release of resin components from dental composites and the potential of these agents to mimic or disrupt estrogenic cell responses.

Types of Studies Reviewed. The studies reviewed included those on synthetic methods used to make bisphenol A glycidyl methacrylate, or BIS-GMA, and the biological effects of this resin in cell culture and animals. The estrogenic effect of bisphenol A was targeted because bisphenol A is present as an impurity in some resins (BIS-GMA) and as a degradation product from other resins (bisphenol A dimethacrylate, or BIS-DMA).

Results. The outcomes of this review revealed

that short-term administration of BIS-GMA and/or bisphenol A in animals or cell cultures can induce changes in estrogen-sensitive organs or cells.

However, considering the dosages and routes of administration and the modest response of estrogen-sensitive target organs, the authors conclude that the short-term risk of estrogenic effects from treatments using bisphenol A-based resins is insignificant. Long-term effects need to be investigated further.

Clinical Implications. Commonly used dental resins should not be of concern to the general public; however, pharmacological evaluation of dental materials is needed to ensure biologically safe and therapeutically effective substances.

“If we had a reliable way to label our toys good and bad, it would be easy to regulate technology wisely. But we can rarely see far enough ahead to know which road leads to damnation. Whoever concerns himself with big technology, either to push it forward or to stop it, is gambling in human lives.”

—Freeman Dyson, “Disturbing the Universe,” 1979

During World War II, German researchers developed a chemical process that could be used to cure dental methacrylates at room temperature.^{1,2} Despite initial successes, the clinical results reported during the mid-1950s showed that chemically cured methacrylate restorations were associated with increases in discoloration, recurrent tooth decay and pulp reactions.³⁻⁵ These initial side effects were attributed to polymerization shrinkage and monomer leaching.

To reduce polymerization shrinkage, researchers added inert filler particles to the self-curing methacrylate resin.^{6,7} To improve strength and adhesiveness of the resinous restorations, Bowen⁸ explored the possibility of using epoxy resins (diglycidyl ether of bisphenol A) mixed with silica particles. The *in vitro* results were promising, but the presence of moisture inhibited the

polymerization process of the epoxy resin. To overcome this problem, Bowen attached methyl methacrylate groups to the end groups of the epoxy resin, thereby converting the epoxy resin to a dimethacrylate.^{9,10} The experimental outcome was successful and resulted in a new resin called bisphenol A glycidyl methacrylate, or BIS-GMA, or just Bowen’s resin.

Bowen’s resin had some important advantages, including reduced shrinkage during polymerization and the ability to form cross-links (which are stronger than linear polymers) during polymerization. However, the higher viscosity of BIS-GMA made it more difficult to add filler to the monomer and to mix the chemically cured composite. To solve this problem, different monomers with lower viscosities (for example, triethyleneglycol dimethacrylate, or TEGDMA) were added

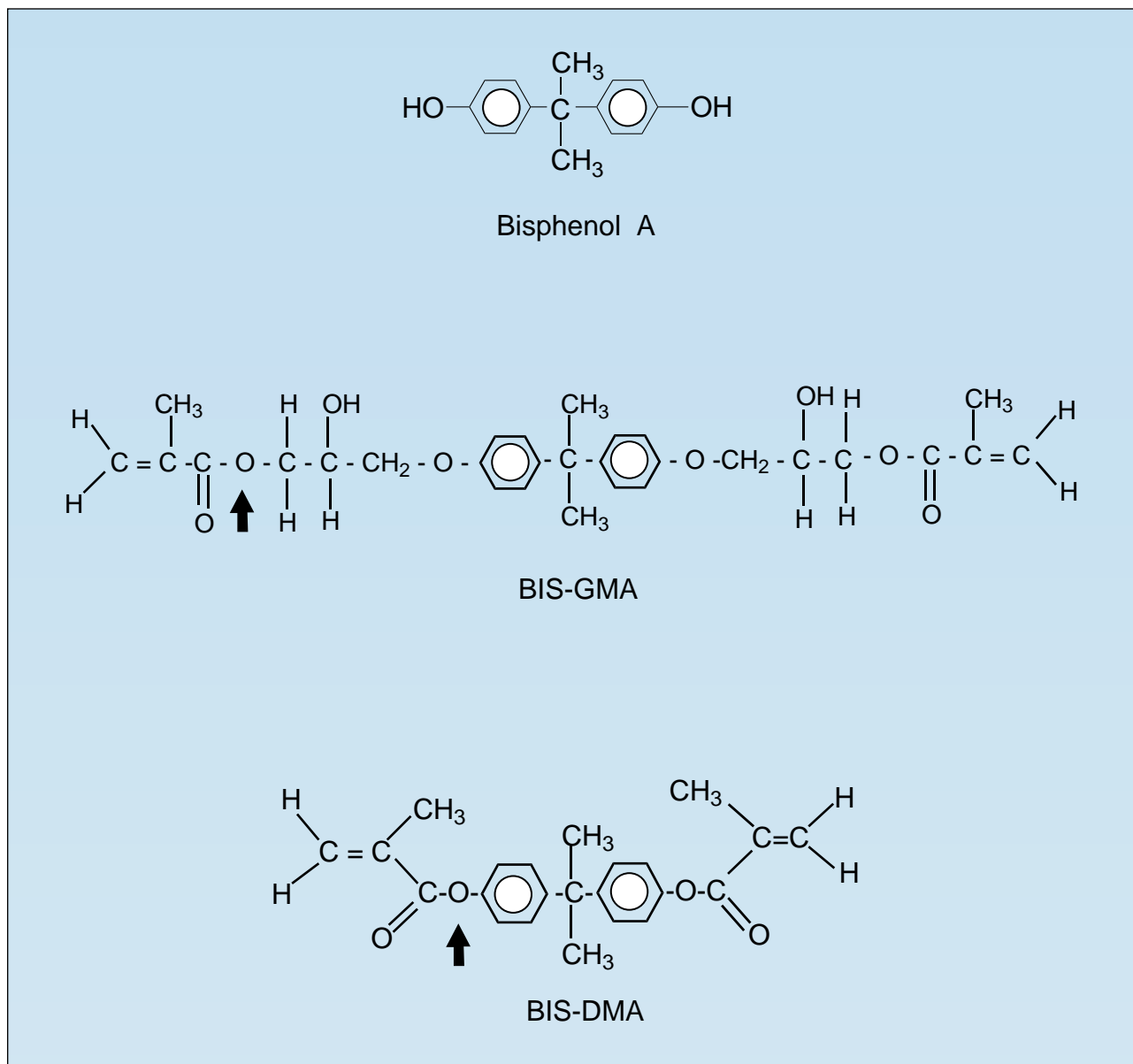


Figure 1. The bisphenol A molecule (top) is estrogenic. Under clinical conditions, esterase present in saliva can break ester linkages (arrows). If the bisphenol A glycidyl methacrylate, or BIS-GMA, molecule (center) is attacked by esterase, no bisphenol A will form. However, if the ester linkages of the bisphenol A dimethacrylate, or BIS-DMA, molecule (bottom) are attacked, bisphenol A can form.

to dilute the highly viscous BIS-GMA monomer and make it possible to incorporate more filler.

Bowen's composite material and resin have become more significant to dentistry than most other dental innovations. The combination of these materials with the acid-etching technique made new treatment op-

tions possible. Carious lesions could be prevented with sealants,¹¹ Class IV restorations and composite veneers could be placed successfully,¹² orthodontic brackets could be bonded,¹³ and metallic and ceramic constructions could be bonded by using the acid-etching technique with resin.¹⁴ By using composites rather than silicate

cements, clinicians could increase the longevity of Class III and V restorations¹⁵ and, during the last few years, more clinicians have found that posterior composites, when placed properly, can result in acceptable clinical restorations.¹⁶

Considering the enormous success the dental profession has experienced with BIS-

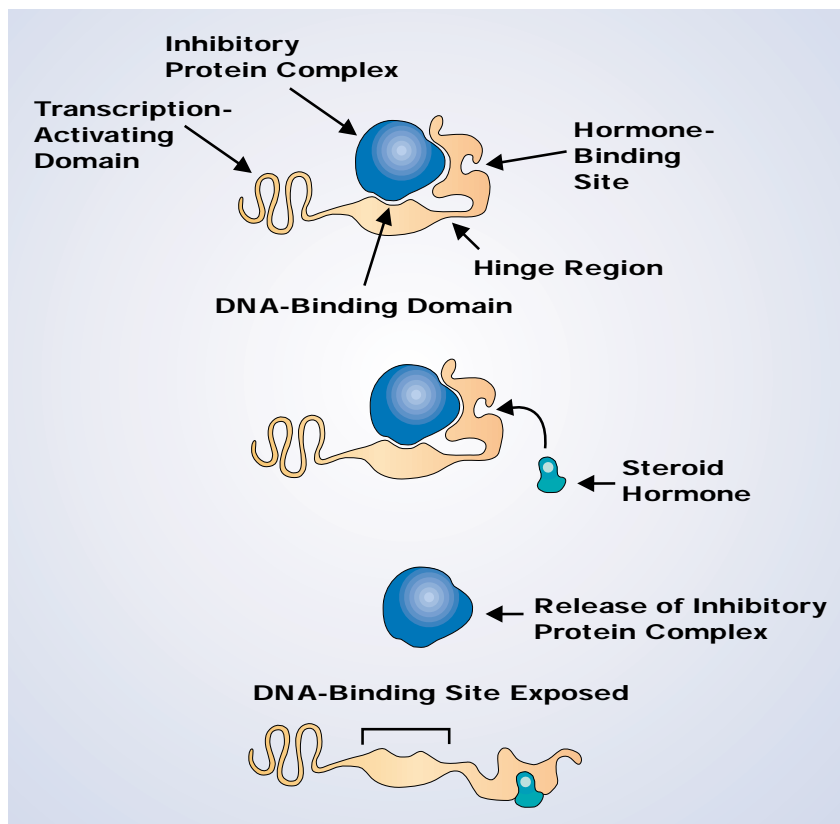


Figure 2. The unbound or free hormone enters the cell by diffusion and binds to macromolecules called receptors. When binding occurs, the receptor releases an inhibitory protein complex, and the DNA-binding site of the receptor becomes exposed and ready to bond to the DNA.

GMA-based materials, clinicians found it somewhat alarming that some of these materials, such as sealants, were implicated as being estrogenic.¹⁷ One study has shown that contaminants from a BIS-GMA-based sealant altered the proliferative nature of cultured human breast cancer cells.¹⁷ Olea and colleagues suggested that the changes in cellular behavior were due to the estrogenicity of bisphenol A and bisphenol A dimethacrylate, or BIS-DMA, components found in some dental sealants (Figure 1); these components were identified by mass spectrometry.

Because of the possibility that some components of modern dental resins are estrogenic, and the increasing use of

resin-based materials in dental practice, the need exists to review and discuss existing facts regarding these resins and their safety. Therefore, the goal of this article is to provide current information about the relationship of dental resin products used in the mouth with potential toxic effects, with the primary focus on estrogenic effects. To accomplish this, we will explore three principal areas: BIS-GMA chemistry and BIS-GMA uses in the mouth; steroid hormone action in the body and in the mouth; and various theories of how bisphenol A-based resins may affect estrogen-sensitive tissues in the body. We will also evaluate the data upon which these theories are based.

CREATING BIS-GMA

As early as 1936, Dodds and Lawson¹⁸ reported the estrogenicity of some diphenyl compounds containing two hydroxy groups in para positions. One such derivative, bearing two methyl groups and known as bisphenol A, has been used by some manufacturers during synthesis of BIS-GMA.¹⁰ Because of the estrogenic effect of bisphenol A, it seemed reasonable to suspect that at least in some BIS-GMA-containing materials, bisphenol A molecules could be present that could cause an estrogenic response.

The BIS-GMA molecule may not be an estrogen agonist if the bisphenol A groups present in the BIS-GMA molecule are sterically hindered and if the monomer is pure and does not contain any impurities from the synthesis. However, this conclusion may not be correct.¹⁹ To understand the potential risks associated with BIS-GMA, we must understand both the chemistry, including synthesis of BIS-GMA, and the biological interaction between the bisphenol A molecule (used during the synthesis of BIS-GMA) and estrogen receptors.

Chemistry. In 1965, Bowen received a patent¹⁰ in which the synthesis of BIS-GMA was described. According to that patent, there were three main ways to synthesize the monomer. The first way was based on attaching methacrylate groups to hydroxy glyceryl groups, which, in turn, were linked to phenoxy groups. When glacial methacrylic acid was reacted with the diglycidyl ether of a bisphenol and a tertiary amine was added to catalyze the above reaction, a BIS-GMA

molecule was formed.

An advantage of this synthetic method was that no bisphenol A was involved in the synthesis of the BIS-GMA molecule. Any bisphenol A present in the final product of synthesized BIS-GMA was the carryover of unreacted impurities from the synthesis of diglycidyl ether of bisphenol A. Good quality control measures in manufacturing resolved this issue without complications, and today manufacturers of dental resins prefer this synthetic method because it excludes the use of bisphenol A. Resins manufactured with this method seldom contain more than a few parts per million bisphenol A.

The second synthetic method proposed by Bowen¹⁰ was to condense the sodium salt of bisphenol A with an equivalent amount of the reaction product of glycidyl methacrylate and anhydrous hydrochloric acid. During this reaction, sodium chloride was formed as a byproduct that could be washed away. A drawback of this synthesis is that residuals of the bisphenol A salt are left in the final product if excess glycidyl methacrylate is used during the reaction and if the reaction is not allowed to be completed.

The third synthetic method, which Bowen preferred when he submitted his patent application, was to combine two moles of glycidyl methacrylate with one mole of bisphenol A. A tertiary amine was added to catalyze the addition of the phenolic hydroxyl groups to the epoxide groups. A test to determine the completion of the reaction consisted of mixing a few drops of the monomer with a silica powder containing approximately 2 percent benzoyl

peroxide. This paste was then placed between two films of polyethylene and heated to 90 C. If the reaction was not completed, the paste failed to harden within 10 minutes.

Problems. From a biological point of view, several problems exist with the above syntheses of BIS-GMA monomer if strict product control measures are not performed. The first method can produce residuals of diglycidyl ether of a bisphenol in dental composites and cause allergic reactions.^{20,21} Method two results in residuals that can induce estrogenic effects¹⁷ (for example, the salt of bisphenol A) or produce allergic reactions (for example, glycidyl methacrylate). Finally, the third method could leave both glycidyl methacrylate and bisphenol A as impurities, causing allergic and estrogenic effects, respectively, from poorly purified BIS-GMA resins.

Dental monomers available on the market fulfill Environmental Protection Agency standards regarding maximal bisphenol A content; nonetheless, these standards are based on toxic effects of bisphenol A rather than on their estrogenic effects. Hence, the current controversy concerning bisphenol A revolves around two important observations: that bisphenol A can stimulate proliferation in estrogen-sensitive cell cultures²² and that bisphenol A contaminants can be present in dental sealants.¹⁷

THE ESTROGEN FAMILY AND HOW IT WORKS

Estrone, estradiol and estriol are the naturally occurring estrogen molecules found in humans. Estradiol is the most potent estrogen and can be

converted metabolically to estrone or estriol. Although estrogens are produced by both men (for example, in the testes and peripheral tissues) and women (for example, in the ovaries, placenta and peripheral tissues), estradiol is the most abundant estrogen found in premenopausal women, while estrone is the most abundant estrogen in postmenopausal women and in men.^{23,24}

Estrogens are secreted into the bloodstream in very low concentrations and are capable of regulating differentiation and growth of selected tissues distant from the site of secretion. The mechanism of action of estradiol²⁵ is thought to begin with the secretion of the hormone into the bloodstream, where the unbound or free hormone enters the cell by diffusion and binds to macromolecules called receptors (Figure 2).

Recent evidence suggests that there are two different estrogen receptors. The estrogen receptor alpha, or ER α , and the estrogen receptor beta, or ER β , are found in different tissues in the body and can act differently depending on the ligand. When estradiol is bound to the receptor, it transforms the receptor to an active configuration, and the activated receptor-estradiol complex binds with high affinity to specific nuclear sites (for example, discrete DNA sequences, the nuclear matrix, nonhistone proteins and the nuclear membrane), where gene activation and transcription of messenger RNA occurs. After the nuclear interaction, the receptor-hormone complex disassociates, leaving an unoccupied receptor and estradiol. Although the regulation of gene transcription by

hormone-receptor complexes in the nucleus appears to be the major biological action of estrogens, these molecules exhibit other behaviors that are independent of the genome.²⁶⁻²⁹

The principal biological activities of estrogens in women include development, growth and maintenance of secondary sex characteristics; stimulation of uterine growth; control of the pulsatile release of luteinizing hormone from the central nervous system; thickening of the vaginal mucosa; and ductal development in the breast. In men, the physiological significance of estrogens is largely unknown, but they may be involved in the regulation of androgen and estrogen levels as well as sexual behavior.³⁰

Evidence suggests that stomatic tissues in the mouth are modulated by estrogens.²⁵ For example, during pregnancy, the prevalence and severity of gingivitis has been reported to be elevated,³¹⁻³⁴ leading to greater gingival probing depths,^{31,33,35} increased bleeding on probing or toothbrushing,^{34,35} localized gingival overgrowths^{31,36} and elevated gingival crevicular fluid production.³³ The reasons for these biological changes in the mouth are multifactorial and probably result from the actions and interactions of cells and microbio-
ta with estrogens.²⁵

BIS-GMA EPISTEMOLOGY

In many ways, the interest in BIS-GMA toxicity is rooted in research that examined the effects of ecological agents on the reproductive systems of animals.³⁷ Since the early 1980s, the observation that pesticides such as dichlorodiphenyl-trichloroethane, or DDT, could behave in a similar manner as

estrogen has perked interest in environmental compounds that stimulate the estrogen receptor.

Estrogen receptors, or estrophiles, located in specific target tissues are known to react with only a limited number of structurally related compounds. Structure-activity relationships (that is, the physical structure of the compound and how effective it is in activating the receptor) for estrogens have shown selective, high-affinity binding of steroidal and nonsteroidal compounds that contain the phenolic A ring of the cyclopentanoperhydrophenanthrene structure. Hence, agents containing a phenolic ring, such as diethylstilbestrol or bisphenol A, have the ability to activate estrophiles.

However, not all chemicals with a phenolic ring possess the ability to induce estrogenic responses in biological systems. Research has shown that biological responses to chemicals depend on a number of pharmacological considerations, including delivery of the unbound or free drug in proper concentration to the site of action, binding and activation of the receptor, as well as the amount of time the free drug spends at the site of action.³⁸⁻⁴⁰

If specific chemicals found in resin-based materials possess estrogenic activity, what does this portend for their application in the mouth? The simple response would be to discontinue their use; however, the appropriate answer is far more complex, since the risk-to-benefit ratio of any agent introduced into the mouth must be carefully examined. For example, some drugs used to control dental plaque promote calculus formation and extrinsic staining dur-

ing use.⁴¹ Hence, the benefit of controlling plaque growth must be weighed against the possibility of untoward events in the patient. Two common methods used to assess drug toxicity involve evaluating the effects of agents on cells in culture or in cells found at the site of action in the body.

Cell culture experiments.

As noted above, BIS-GMA-based resins contain many chemicals, including BIS-GMA and minor amounts of impurities such as bisphenol A and/or diglycidyl ether of bisphenol A. Other monomers, such as TEGDMA, BIS-DMA and bismethacryloyloxyethoxyphenylpropane, are also added to the BIS-GMA monomer to change the rheology of the resin phase. To date, only one cell-culture experiment has been conducted to assess the estrogenic effects of dental resins, and it evaluated only one cell line (obtained from a pleural effusion derived from a human breast adenocarcinoma) and a small number of parameters.¹⁷

Taking these limitations into account, Olea and colleagues¹⁷ found that BIS-GMA, by itself, was unable to stimulate proliferation of breast cancer cells in culture. In contrast, bisphenol A was shown to be an estrogenic compound capable of stimulating the number of cells and the progesterone receptor content of breast cancer cells, but at 2,500 times the concentration necessary for estradiol to produce similar effects.²²

It is not surprising that recent data have shown bisphenol A to be a weak estrogen agonist for either ER α or ER β .⁴² Using observed log relative binding affinity values, researchers found that bisphenol A bound

with greater affinity to ER α than to ER β ; however, the affinity of bisphenol A for either ER α or ER β was low, as evidenced by the fact that it bound estrogen receptors with an affinity similar to that of many androgenic hormones.⁴²

Although the estrogenicity of BIS-GMA-based dental resins is not well-defined, *in vitro* experiments have identified components that are released from such resins. If cured commercial BIS-GMA-based resins are stored in organic solvents, small amounts of TEGDMA, BIS-GMA and BIS-DMA residuals have been identified.^{17,43} Moreover, one of these studies¹⁷ found bisphenol A in the solvent, while the other study could not.⁴³

In recent studies,^{44,45} similar resins were stored in distilled water or artificial saliva, and TEGDMA was the main substance detected in the storage media.^{44,45} These findings show that the composition of the medium (for example, saliva) surrounding a restoration will have a significant impact on the amount of leachable components of the resin.

We can conclude that the potential release of bisphenol A from sealants should vary depending on the amount of time and the type of medium in which the sample was incubated. In the study by Nathanson and colleagues,⁴³ seven different sealants incubated in 95 percent ethanol for four minutes produced no detectable levels of bisphenol A in the eluate.⁴⁰ However, in a study by Olea and colleagues,¹⁷ saliva samples collected one hour after sealants were placed (approximately 50 milligrams of sealant per subject) contained variable

amounts of bisphenol A (ranging from 3.3 to 30 micrograms per milliliter).

It is interesting to note that one of the collected saliva samples was able to stimulate cell proliferation in human breast cancer cells.¹⁷ Notwithstanding the bisphenol A measured in the saliva sample, it is well-known that saliva contains many other growth factors and hormones⁴⁶⁻⁴⁸ that may have been responsible for the proliferation of cells. To determine the effects of salivary-derived bisphenol A on cells in culture, researchers must obtain additional data from many subjects to evaluate the responses of the hormone-responsive cells incubated with aliquots of saliva samples harvested before and after the application of BIS-GMA-based sealants.

In situ experiments.

Experiments initiated on the benchtop offer valuable information about the effect of individual agents on specific cells but often lack the ability to evaluate the pharmacokinetic and/or pharmacodynamic properties and interaction of drugs with humoral components in a healthy animal model. In a recent study using a murine animal model, Mariotti and colleagues⁴⁹ investigated the physiological and biochemical effects of commercially used BIS-GMA to determine if estrogen-sensitive reproductive tissues, such as the uterus, could be stimulated to grow. Their experiments showed these BIS-GMA solutions to be marginally estrogenic in the uterus.⁴⁹ More specifically, BIS-GMA injected subcutaneously at concentrations far higher than those monitored in saliva were unable to stimulate increases in the

cell number or cell size of reproductive organs in mice, but were able to stimulate modest increases in the weight and collagen content of the uterus.⁴⁹

Practitioners should place these data into proper perspective, considering that the route of administration of BIS-GMA in mice is different from that in humans, the concentrations injected were pharmacological in nature, the duration of the experiment was only three weeks and the positive results obtained from reproductive tissue weight and collagen content were small in comparison with the effects of a similar dose of estradiol in mice. The *in vivo* effects of BIS-GMA and associated chemicals may be of even less consequence when the amount of chemical components released from BIS-GMA resins in the mouth, the duration of this release and the absorption of these chemical components from the gastrointestinal, or GI, tract are determined. We should note that the current data from animal studies suggest that the potentially harmful effects of a short-term exposure to BIS-GMA are inconsequential.⁴⁹

Absorption, metabolism and excretion: pharmacokinetics. Questions remain concerning the potential long-term effects of BIS-GMA-based resins in the human body. Clinical experience tells us that sealants and composites wear over time and that the worn components, including bisphenol A-based resin molecules, are swallowed and must pass through the GI tract. What happens during the absorption of these chemicals is poorly understood.

Climie and colleagues⁵⁰

showed that 80 percent of a single oral dose of glycidyl ether of bisphenol A was eliminated in the feces and 11 percent in the urine zero to three days after administration in mice. These findings suggest that most of glycidyl ether of bisphenol A was not easily absorbed from the GI tract, but it is unclear if BIS-GMA and BIS-DMA (BIS-DMA being another bisphenol A-based resin present in some sealants and composites) would be equally difficult to absorb.

We do know that BIS-GMA does not dissolve in water easily,⁵¹ but it may dissolve quite extensively in other solvents,⁵² such as alcohol and lipids. Therefore, some BIS-GMA may be absorbed as a result of its lipophilic nature, but the amount of BIS-GMA and BIS-DMA that is transported from the intestinal tract to the site of action depends on a number of factors, including capillary permeability, blood flow, extent of plasma protein and specific organ binding, regional differences in pH, available transport mechanisms and permeability characteristics of specific tissue membranes,⁵³ all of which are unknown for BIS-GMA and BIS-DMA.

The estrogenic effect observed in mice injected with commercial BIS-GMA may be a result of impurities (bisphenol A and diglycidyl ether of bisphenol A) or the biodegradation of BIS-GMA to bisphenol A. Little is known about BIS-GMA metabolism in the human body. Subcutaneous implant studies do not suggest that cured BIS-GMA-containing materials are toxic, which may indicate that biodegradation is limited.⁵⁴ Nonetheless, the metabolic conversion of BIS-GMA to more ac-

tive compounds may occur through different enzymatic reactions. We know that enzymes, such as esterases, attack dental composite resins.⁵⁵ These proteins catalyze the hydrolyses of ester linkages. Researchers believe that they function by attaching to the molecule at a specific molecular site, so that the electrostatic forces of nearby atoms sharply reduce the energy needed to cleave and re-form the appropriate chemical bonds.

Munksgaard and Freund⁵⁵ incubated a variety of dental resins (diethyleneglycol dimethacrylate, urethane dimethacrylate, TEGDMA, decylmethacrylate, laurylmethacrylate, BIS-GMA and 2-hydroxypropylmethacrylate) with porcine liver esterase. They found that methacrylic acid, or MAA, was released as a byproduct, suggesting that the resins were hydrolyzed. Of all the resins, BIS-GMA released the least MAA, suggesting that the hydroxy group present in BIS-GMA forms a steric hindrance, thereby making it more difficult for the esterase to attack this molecule. From this study, one can conclude that a hydrolase (porcine liver esterase) was able to catalyze the hydrolysis of monomethacrylates and dimethacrylates in the polymerized and unpolymerized state, and that the hydrolytic process would increase the hydrophilicity of the resin surface.

Because of the possibility that esterase may degrade some dental resins, we suspected that some of the findings presented by Olea and colleagues¹⁷ related to the presence of BIS-DMA rather than BIS-GMA in some of the sealants investigated. An attack of the ester linkages in BIS-DMA could result in the formation of

bisphenol A, but a similar attack on BIS-GMA will not result in the formation of bisphenol A.

The excretion of BIS-GMA and BIS-DMA and associated metabolites is poorly understood. We do know that the rate of clearance of BIS-GMA and BIS-DMA from the mouth depends on the salivary flow. However, once the BIS-GMA and BIS-DMA are ingested and absorbed, renal and/or biliary excretion of these compounds is unclear, but undoubtedly depends on a variety of physicochemical characteristics (for example, lipid solubility, degree of ionization, drug pKa [that is, the negative logarithm of the ionization constant of an acid]) that govern the transfer of BIS-GMA, BIS-DMA and metabolites from the internal to external environment.

Despite the fact that bisphenol A may be released from dental resins under certain conditions, it is important to realize that release of monomer components from dental resins follows a logarithmic function. For example, the amounts of bisphenol A and BIS-DMA identified in the study by Olea and colleagues¹⁷ were collected one hour and 24 hours after resin sealants were placed. Their results show a clear decrease in component concentrations over time, a finding that has been known for years.⁵⁶ The conclusion we can draw from these findings is that it is likely that the estrogenic effect that might be induced from a newly placed restoration or sealant will decrease over time. However, such a conclusion cannot exclude some additive or synergistic effect with other xeroestrogens present in the mouth.

CONCLUSION

Based on existing research, we must accept that certain impurities may be present in some BIS-GMA-based resins, and these impurities, when released from restorations, are potentially estrogenic. Under extreme conditions, these impurities are capable of inducing weak estrogenic effects on target tissues. However, the amounts of bisphenol A that may be present as an impurity or produced as a degradation product from dental restorations, including sealants, are quite small and far below the doses needed to affect the reproductive tract.

However, although current findings suggest that the short-term risk of estrogenic effects from dental treatments using BIS-GMA-based resins is insignificant, research should be directed at evaluating the pharmacokinetics and pharmacodynamics of the long-term release of contaminants from BIS-GMA-based resins (that is, bisphenol A) used in the mouth. In addition, organizations such as the International Standardization Organization should consider including in future standards tests that determine the bisphenol A content of dental resins and their ability to release the bisphenol A that may form during degradation of the polymer structure. ■

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