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# PHARMACOKINETICS OF BISPHENOL A RELEASED FROM A DENTAL SEALANT

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## ABSTRACT

**Background.** Limited information is available regarding potentially estrogenic bisphenol A, or BPA, released from dental sealants. This study determined the rate- and time-course of BPA released from a dental sealant (Delton Opaque Light-cure Pit and Fissure Sealant, Preventive Care/Dentsply) when applied at a dosage of 8 milligrams (one tooth) or 32 mg (8 mg on each of four teeth) to 40 healthy adults. **Methods.** The authors recruited 40 healthy subjects (18 men and 22 women, 20-55 years of age) who did not have histories of pit and fissure sealant placement or composite resin restorations. The authors collected saliva (30 milliliters) and blood (7 mL) specimens from all subjects immediately before sealant placement (baseline) and at one hour, three hours, one day, three days and five days after sealant placement. They used high-pressure liquid chromatography to determine BPA (detection sensitivity 5

parts per billion, or ppb) in all specimens.

**Results.** The authors detected BPA in some saliva specimens (5.8-105.6 ppb) collected at one hour and three hours. The BPA, however, was not detectable beyond three hours or in any of the serum specimens. For the one- and three-hour saliva samples, the BPA concentration in the high-dose (32 mg) group was significantly greater than in the low-dose (8 mg) group ( $P < .05$ , Wilcoxon signed rank test). In the high-dose group, there was a significant decrease in saliva BPA concentrations from one hour to three hours ( $P < .01$ , Wilcoxon signed rank test).

**Conclusion.** This study showed that BPA released orally from a dental sealant may not be absorbed or may be present in nondetectable amounts in systemic circulation. The concern about potential estrogenicity of sealant may be unfounded.

**B**isphenol A-, or BPA-based epoxy resins are widely used in the manufacture of commercial products, including dental resins, polycarbonate plastics and the inner coating of food cans. The dental resin bisphenol A glycidyl dimethacrylate, or Bis-GMA (2,2-bis[4'-(2'-hydroxy-3'-methacryloxy) phenyl] propane), is the reaction product of diglycidyl ether of BPA and methacrylate.<sup>1</sup> Although BPA-based epoxy resins are relatively stable, in the laboratory the carbonate linkages can be hydrolyzed at high temperatures, resulting in the release of BPA.<sup>2,3</sup> BPA is a precursor to the resin monomer Bis-GMA and to bisphenol A dimethacrylate, or Bis-DMA. During the manufacturing process of Bis-GMA dental sealants,

BPA might be present as an impurity if the synthetic reactions do not stoichiometrically reach completion; BPA also might be present as a degradation product of Bis-DMA through esterases present in saliva, which can hydrolyze the susceptible ester bond contained in Bis-DMA monomers.<sup>4</sup>

Researchers found an estrogenic effect with BPA, Bis-DMA and Bis-GMA but not with triethylene glycol dimethacrylate, or TEGDMA, in an estrogen-sensitive cell line—MCF7.<sup>5</sup> Because BPA lacks structural specificity as a natural ligand to the estrogen receptor, the estrogenic potential of BPA has been reported to be much lower than that of the natural estrogen estradiol.<sup>6-8</sup>

A dental resin sealant serves as a protective coating or barrier that effectively isolates pits and fissures to help prevent caries in children and adults.<sup>9-11</sup> Once sealants are applied to tooth structures, they are polymerized in situ.<sup>12</sup> As there may be incomplete conversion to polymer, chemicals such as Bis-DMA and Bis-GMA might leach into the salivary fluid of the oral cavity.<sup>6,13</sup> Leaching of these monomers from resins can occur during the initial setting period and in conjunction with fluid sorption and desorption over time.<sup>14-16</sup> Thus, these chemical leachates from dental sealants may be bioactive.<sup>17-20</sup>

Little information exists regarding the potential health implications of BPA exposure from the environment or from dental sealants. Olea and colleagues<sup>6</sup> reported that 90 to 931 micrograms of BPA were detected in the saliva of patients in whom 50 milligrams of a sealant (Delton Opaque Light-cure Pit and Fissure Sealant, Preventive Care/Dentsply) had been placed one hour earlier. They also reported that BPA- and Bis-DMA-stimulated breast cancer cell MCF7 proliferation increased the number of progesterone receptors and showed competitive binding to estrogen receptors. These results generated considerable concern regarding the safety of dental resin materials.

Two recent in vitro studies examined components released from seven commercially available light-activated pit and fissure sealants and detected mainly TEGDMA and Bis-GMA.<sup>21,22</sup> As TEGDMA is a chemical that closely elutes with BPA in a gas or liquid chromatogram, its presence

may be identified mistakenly as BPA. An animal study showed that low doses of BPA administered to pregnant mice resulted in a significant increase in adult prostrate weight in male offspring compared with controls, although a dose-dependent relationship was not observed.<sup>23</sup> Other animal studies showed that BPA was effective in stimulating prolactin secretion from the pituitary glands<sup>24</sup> and increased proliferative activity in epithelial cells of the mammary glands.<sup>8</sup>

It remains uncertain if biological effects of BPA similar to

**Little information exists regarding the potential health implications of bisphenol A exposure from the environment or from dental sealants.**

those reported in cell-culture studies and in animals via systemic administration of BPA will occur in humans. Humans are exposed to BPA environmentally through food cans and dental restorative materials. A recent study suggested that the maximum potential dietary exposure to BPA from food and beverage cans that are coated with BPA-based epoxies to be about 2.2 parts per billion, or ppb.<sup>25</sup> Limited information is available, however, regarding the pharmacokinetic profile of BPA leaching from dental sealants in vivo.

We conducted this study to examine the rate- and time-course of BPA that might be

released from a light-cured pit and fissure sealant (Delton Opaque Light-cure Pit and Fissure Sealant, Preventive Care/Dentsply) when applied at concentrations of 8 or 32 mg on occlusal tooth surfaces of adults. We also sought to determine whether significant systemic absorption of BPA occurred by analyzing the concentrations of BPA in both saliva and serum specimens over a five-day period after the sealants were placed.

**MATERIALS AND METHODS**

**Subjects.** The study protocol was approved by the University of Nebraska Medical Center's institutional review board. Subjects included 40 adults, 20 to 55 years of age; 18 men (32.3 ± 2.3 years of age) (mean age ± standard error of the mean, or SEM) and 22 women (34.1 ± 1.9 years of age) recruited from the Lincoln, Neb., area.

We screened a total of 60 people to find the qualified subjects. We excluded people who had acute soft- or hard-tissue dental disease, existing composite resin restorations or a history of exposure to industrial or dental resins, as well as pregnant women. For subjects to be eligible, they had to have occlusal surface available to accommodate the selected sealant volume, as well as either one posterior tooth surface or four posterior teeth surfaces with unrestored fissures appropriate for placement of sealants. We fully explained the research protocol to the subjects and obtained written informed consent and brief medical histories from them before we began the study.

TABLE 1

BISPHENOL A RECOVERY RATES FROM BLANK SERUM SAMPLES.*			
SERUM TRIAL (ppb†)	AMOUNT RECOVERED (ppb)	PERCENTAGE	MEAN PERCENTAGE ± STANDARD ERROR OF MEAN
1 (5)	3.52	70	—
2 (5)	3.52	70	—
3 (5)	4.70	94	78.0 ± 7.8
1 (10)	10.97	110	—
2 (10)	8.19	82	—
3 (10)	11.31	113	101.7 ± 9.9
1 (20)	17.46	87	—
2 (20)	17.75	89	—
3 (20)	18.34	92	89.3 ± 1.5
			Average 89.7 ± 5.0

\* Known concentrations of bisphenol A were added to blood bank serum for analysis.  
† ppb: Parts per billion.

**Materials.** We selected a widely used commercially available dental sealant (Delton Opaque Light-cure Pit and Fissure Sealant) for this study. We analyzed sealant samples for commonly known components of composites and sealants using high-pressure liquid chromatography, or HPLC.

**Methods.** *Sealant placement.* We divided the subjects into two groups. Subjects in the low-dose group (seven men, 11 women) received a single 8-mg dental sealant dose on one surface, while subjects in the high-dose group (11 men, 11 women) received a total dosage of 32 mg of sealant—8 mg on each of four surfaces.

We isolated each tooth with absorbent cotton rolls and oral evacuation and conditioned the enamel using 37 percent phosphoric acid gel placed for 15 seconds against the enamel surface to be sealed. After conditioning

was completed, we rinsed the surfaces receiving the sealant for 10 seconds with air/water spray and thoroughly dried them. Using a primed pipette (Microman, Rainin Instrument), we delivered 7.3 microliters of the sealant to the conditioned tooth surfaces of subjects in the low-dose group and 29.2  $\mu$ L of the sealant to the conditioned teeth surfaces of the subjects in the high-dose group. (Pipette priming consisted of loading and discharging the positive displacement cylinder four times before loading for delivery to the tooth.) After sealant delivery, we removed excess sealant from the pipette tip by wiping the tip against the tooth surface receiving sealant. Each sealed surface was photoactivated by a hand-held visible light source for 60 seconds immediately after sealant application, per the manufacturer's instructions.

After we removed the isolation devices, we identified occlusal interferences using articulation paper. We removed occlusal prematurities using rotary burs with water spray and high-volume evacuation. Because we had screened subjects for the availability of occlusal surfaces to accommodate the selected sealant volume, the need for occlusal adjustment of sealants was minimal. At five days, we examined subjects for sealant retention. We found that the sealants were intact except in two subjects from the high-dose group, each of whom had one sealant missing from a maxillary second molar.

*Saliva and blood specimen collection.* We collected 20 to 30 milliliters of saliva and 5.5 to 7.0 mL of blood from each subject one hour before dental resin sealant placement. Each subject was asked to expectorate into a

TABLE 2

## BISPHENOL A RECOVERY RATE FROM SPIKED SALIVA.\*

SALIVA TRIAL (ppb†)	AMOUNT RECOVERED (ppb)	PERCENTAGE	MEAN PERCENTAGE ± STANDARD ERROR OF MEAN
1 (5)	4.278	85.6	—
2 (5)	4.718	94.4	—
3 (5)	4.847	96.9	—
4 (5)	4.600	92.0	—
5 (5)	3.742	74.8	88.7 ± 4.0
1 (10)	8.527	85.3	—
2 (10)	8.515	85.2	—
3 (10)	8.369	83.7	—
4 (10)	8.153	81.5	—
5 (10)	9.368	93.7	85.9 ± 2.1
1 (20)	17.729	88.6	—
2 (20)	17.281	86.4	—
3 (20)	16.832	84.2	—
4 (20)	16.735	83.7	—
5 (20)	19.145	95.7	87.7 ± 2.2
			Average 87.4 ± 1.6

\* Saliva was collected daily from among laboratory staff members who did not have restorative resin in their teeth. Known concentrations of bisphenol A were added to blank saliva for analysis.  
† ppb: Parts per billion.

50-mL plastic container for 30 minutes. A registered nurse drew about 7 mL of blood from each subject by venipuncture using serum-separator tubes containing ethylenediamine tetraacetic acid. We repeated the same procedure for collecting saliva and blood at one hour, three hours, one day, three days and five days after the sealants were placed. We centrifuged all of the saliva and serum specimens and froze them at -70 C until we assayed them for the concentrations of BPA.

*Sample extraction procedure.* We used a solid-phase extrac-

tion cartridge packed with 500 mg of reverse phase C18 (Extract-Clean C18, Alltech) to extract organic components from saliva and serum specimens. The eluted acetonitrile solution was directly injected into a column for HPLC analysis.

The cartridge was conditioned with 5 mL of methanol followed by 5 mL of deionized water. After loading 1 mL of saliva or serum, the cartridge was washed with 2.5 mL of deionized water four times, and the organic components were eluted with 0.5 mL of acetoni-

trile twice and collected for analysis.

*High-pressure liquid chromatography method.* In this study, we used HPLC with an online photodiode array ultraviolet visible, or UV/VIS, detector (SPDA-M10Avp, Shimadzu) with a 190- to 800-nanometer wavelength range and a fluorescence detector (RF-10A, Shimadzu) for most known dental sealant components. We analyzed the samples with a reversed-phase HPLC column (Supelcosil LC-18, Supelco) (300 × 4 millimeters, with a particle size of 5 mm) and a guard

column backed with the same material. An automatic injector with a 50-mL sample loop was used. Elution was performed with 50 percent acetonitrile and water at a flow rate of 1.0 mL/minute. The fluorescence detector was set at an excitation wavelength of 278 nm and an emission wavelength of 315 nm with detection sensitivity up to 5 ppb, or nanograms per milliliter, for BPA with a 30- $\mu$ L injection. We quantified components in the samples based on the standard curve. All determinations were conducted at room temperature.

*Standard calibration curves and recovery rate.* We performed external calibration using the chromatographic responses of six standard concentrations in their corresponding solvent. We processed standard solution signals using a linear regression program. Chromatographic standards were prepared with known resin components—TEGDMA, Bis-DMA, BPA and Bis-GMA. We prepared initial standard stock

TABLE 3

HIGH-PRESSURE LIQUID CHROMATOGRAPHY OF STANDARDS.*			
STANDARD	DETECTOR	RETENTION TIME (MINUTES)	DETECTION LIMIT† (ppb‡)
<b>Bisphenol A</b>	Fluorescence	5.8	5
<b>Triethylene glycol dimethacrylate</b>	Ultraviolet visible photodiode array	8.4	200

\* We analyzed standards that included bisphenol A and triethylene glycol dimethacrylate for retention time and the lowest level of detection.  
 † Thirty-microliter injection with a signal-to-noise ratio of 3:1.  
 ‡ ppb: Parts per billion.

solutions of each component in methanol at 0.5 mg/mL, as well as serial dilutions from these stock solutions in acetonitrile and water (1:1) for the HPLC study.

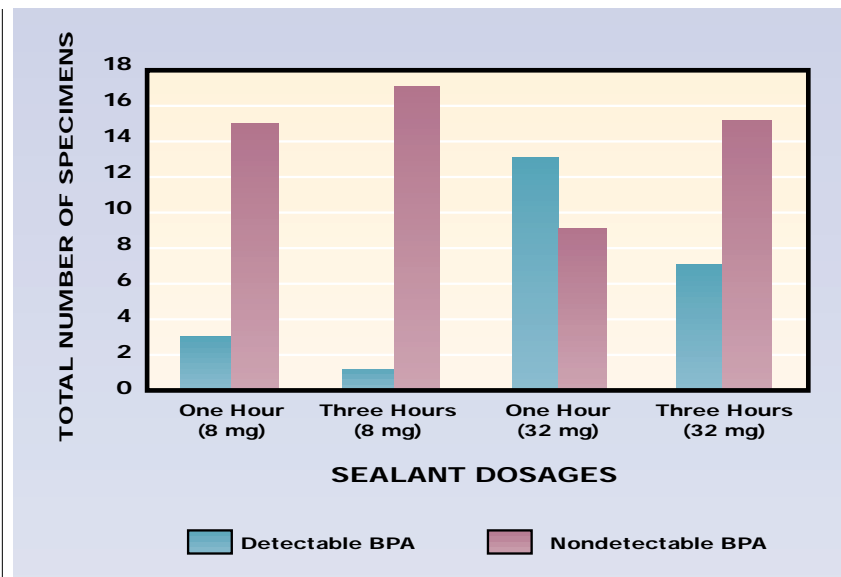
*Sealant product analysis.* We analyzed three samples from the same batch of sealant that we used for placement in this study. We prepared sealant sample disks using a 2-mm thick  $\times$  10-mm diameter stainless steel setting mold and then used a visible light-curing unit (Visilux-2, 3M Dental Products)

to polymerize the sealant samples for 50 seconds per surface. Each sample was placed in a vial containing 5 mL of saline solution. After the sample underwent 10 seconds of sonication in an ultrasonic bath, we withdrew a 200- $\mu$ L sample of extraction solvent as the control at baseline. We then incubated the vials containing the samples at 37 C in a water bath and took another 200- $\mu$ L sample at 24 hours. All collected samples were refrigerated until we conducted the HPLC analysis.

TABLE 4

ANALYSIS OF SEALANT.*							
SEALANT SAMPLE	LOT	EXPIRATION DATE	SAMPLE WEIGHT (GRAMS)	BPA† (ppb‡) BASELINE	BPA (ppb) 24 HOURS	TEGDMA§ (ppm**)	TEGDMA (ppm) 24 HOURS
<b>Light Cure 1</b>	961024	10/2000	0.196	ND††	ND	26.60	107.44
<b>Light Cure 2</b>	961024	10/2000	0.195	ND	ND	84.98	243.55
<b>Light Cure 3</b>	961024	10/2000	0.188	ND	ND	57.94	167.42

\* We analyzed samples of sealant (Delton, Preventive Care/Dentsply) for the presence of bisphenol A and triethylene glycol dimethacrylate.  
 † BPA: Bisphenol A.  
 ‡ ppb: Parts per billion.  
 § TEGDMA: Triethylene glycol dimethacrylate.  
 \*\* ppm: Parts per million.  
 †† ND: Not detectable.



**Figure.** Detection of bisphenol A, or BPA, in saliva specimens collected from subjects at one and three hours after placement of a low dose (8 milligrams) and a high dose (32 mg) of a dental sealant (Delton Opaque Light-cure Pit and Fissure Sealant, Preventive Care/Dentsply).

**Statistical methods.** We used a nonparametric test to determine if there was a significant difference between the high- and low-dose groups in this study. We used Statistical Analysis System (Release 6.12-TS020, SAS Institute) to run the Wilcoxon signed rank test and significance was determined at the  $P = .05$  level. For detectable and nondetectable readings, we used the  $\chi^2$  test ( $P = .05$ ) to determine if the two dose groups differed at the one- and three-hour readings.

**Recovery.** We prepared standard solutions of known concentrations of BPA and mixed different amounts in blank serum obtained from a blood bank or saliva for 30 minutes (spiked samples). We then extracted spiked samples using the previously described sample extraction procedure.

## RESULTS

**HPLC analysis of recovery rate.** Tables 1 and 2 represent the recovery rates of spiked

BPA in blank serum and spiked saliva, respectively. The overall average recovery rate was  $89.7 \pm 5.0$  percent (mean  $\pm$  SEM) for the blank serum sample and  $87.4 \pm 1.6$  percent for the spiked saliva sample extracted using the previously described solid-phase sample extraction method.

**Analysis of standards and sealant.** In Table 3, the fluorescence detector data showed that BPA was detectable at excitation wavelength of 278 nm and an emission wavelength of 215 nm. Because fluorescence detection is up to 1,000-fold more sensitive than traditional UV/VIS detection, it allowed us to detect BPA at the 5-ppb level. The other common components in dental composites—TEGDMA and BisDMA—are not detectable by fluorescence, but we were able to detect them using a photodiode array UV/VIS detector at 195 nm with a detection limit at 200 ppb.

In Table 4, we concluded that BPA was not present in dental sealants above the 5-ppb

detection limit.

**Analysis of saliva and serum specimens.** We found BPA (5.8-105.6 ppb) in some saliva specimens collected at one and three hours but did not detect it at 24 hours or in any of the serum specimens. Since BPA was detectable in saliva specimens in only the one- and three-hour samples, we analyzed only specimens from these periods.

To see if there was a difference in BPA concentrations between the low-dose patient group and the high-dose patient group, we used a nonparametric test based on ranks. The Wilcoxon signed rank test indicated significant differences in BPA levels between the low- and high-dose groups ( $P = .0293$  and  $P = .0350$ , respectively) at one and three hours. When we used only the presence or absence of BPA as a response variable (detectable or nondetectable), the  $\chi^2$  statistic also showed differences between the low- and high-dose groups ( $P = .006$  and  $P = .020$ , respectively) for the one- and three-hour readings.

In the low-dose group, only three of 18 subjects' samples changed from detectable to nondetectable limits; therefore, there were not enough observations to run a valid statistical test to see if the one- and three-hour readings differed for this group.

In the high-dose group, we conducted the Wilcoxon signed rank test. This test indicated a significant difference ( $P < .01$ ) between the one- and three-hour readings for the high-dose group. The data are summarized in the figure, which illustrates the number of subjects with detectable and nonde-

tectable BPA in saliva specimens for each of the one- and three-hour readings.

## DISCUSSION

In this study, we were able to determine BPA at a detection limit of 5 ppb with a recovery rate in both saliva and blood specimens close to 90 percent. In a similar study, our research group detected the release of TEGDMA from seven commercially available dental pit and fissure sealants *in vitro*.<sup>26</sup> TEGDMA elutes chromatographically close to BPA; therefore, we specifically analyzed TEGDMA to avoid misidentifying it as BPA. Thus, our results concur with other studies showing the release of TEGDMA from pit and fissure sealant.<sup>21,22</sup> We also were able to detect trace amounts of BPA in the saliva of some of the subjects. In contrast with Hamid and Hume's<sup>21</sup> finding that BPA was not found in water eluates from sealant sampled from extracted third molars, we detected low concentrations of BPA in saliva specimens collected at both one and three hours immediately after sealant placement. *In vitro* studies such as that conducted by Hamid and Hume<sup>21</sup> do not take into consideration *in vivo* factors such as mastication and incidental tooth contact during swallowing, as well as saliva, which may enhance the release of unpolymerized components from sealant. These factors may explain the difference between Hamid and Hume's study and our finding.

The concentration of BPA in saliva reported in this study (5.8-105.6 ppb) is more than 250 times less than the values reported by Olea and colleagues

(3.3-30.0 ppm).<sup>6</sup> Arenholt-Bindslev and colleagues<sup>27</sup> also reported lower levels of BPA ( $\leq 0.3$ -2.8 ppm) in saliva immediately after placement of sealant (Delton Opaque Light-cure Pit and Fissure Sealant) in four subjects.

The same brand of sealant was used in the two previously mentioned studies and our study. Thus, the excessively large amount of sealant (50 mg) placed per subject in Olea and colleagues'<sup>6</sup> study compared with the amounts placed in our study (8 or 32 mg) might account for greater leachability and incomplete polymerization of the sealant. The source of BPA in our study remains uncertain. BPA can be present as an impurity from the chemical synthesis of Bis-GMA-based resin or can be derived from enzymatic degradation of Bis-DMA—an ester bond-specific monomer—by esterase enzymes present in saliva.<sup>4,24</sup> Evidence for the latter is found in an *in vitro* study,<sup>22</sup> which showed that Bis-DMA, but not BPA, could be leached from a sealant (Delton Opaque Light-cure Pit and Fissure Sealant) in ethanol solutions.<sup>22</sup>

Our clinical protocol indicated that a maximum of 8 mg of sealant could be placed on the occlusal surface of an average molar without significant occlusal adjustment. This study was designed to examine the dose-dependent, time-dependent release of BPA after placement of sealant in the oral cavity during a five-day period.

Our results suggested that BPA decreased to nondetectable levels in saliva after three hours in some subjects and was not detectable in any serum specimens. This finding implies that

when BPA is released orally from sealant, it may not be absorbed systemically, the quantity absorbed is minute and below our detection limit, or BPA absorbed into systemic circulation possibly is being metabolized.

Regardless of the possibilities, the amount of BPA derived from the sealant used in this study was low and comparable to the environmental exposure from food products.<sup>27</sup> Although BPA may have low estrogenic potency, there is no solid evidence that it is absorbed or causes adverse health effects.

## CONCLUSION

Our findings are significant, as we have demonstrated that BPA released orally from a common dental sealant may not be absorbed or may be present in nondetectable quantities in systemic circulation. Furthermore, we reported a dose-dependent, time-dependent release of BPA from one brand of pit and fissure dental sealant into saliva during initial placement and up to three hours postplacement. Thus, the recent concern regarding the potential estrogenicity of sealants may be unfounded. ■



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