Bisphenol A release from an orthodontic adhesive and its correlation with the degree of conversion on varying light-curing tip distances

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Introduction: The aims of this research were to use high-performance liquid chromatography to assess the bisphenol A (BPA) released from an orthodontic adhesive (Transbond XT; 3M Unitek, Monrovia, Calif) with various light-curing tip distances and to correlate the release to the degree of conversion. **Methods:** One hundred thirty-eight premolar brackets were divided into 3 groups of 40 each for the high-performance liquid chromatography analysis and 3 groups of 6 each for assessing the degree of conversion. Fourier transform infrared spectroscopy was used for this purpose. Each group was studied at light-curing tip distances of 0, 5, and 10 mm. Statistical analyses were performed by using 2-way analysis of variance (ANOVA), post-hoc multiple comparisons Tukey HSD tests, and paired *t* tests. Pearson correlation was used to assess the correlation between the degree of conversion and BPA release. **Results:** BPA release was greater in specimens cured with a greater light-curing tip distance. The degree of conversion decreased with increased light-curing tip distances. A negative correlation was found between BPA release and degree of conversion. **Conclusions:** Clinicians should ensure that the adhesive is completely cured by keeping the light-curing tip as close to the adhesive as clinically possible. (Am J Orthod Dentofacial Orthop 2011;140:239-44)

Orthodontic bonding resins are exposed to oral fluids and are in contact with tissues throughout treatment. Leaching from resin can occur at 2 times: during the setting period of the resin and later when the resin is degraded. Leaching during the first process is related to the degree of conversion (DC).[1] The DC of adhesives is of particular importance, since it modulates the physical and mechanical properties of the material, especially solubility and degradation.[2]

Leaching results in the release of monomers such as bisphenol A (BPA). The implications of BPA released from dental biomaterials was first reported in a study that assessed dental sealants.[3] BPA causes skin allergies,[4] adverse effects on the reproductive systems of animals,[5] cell death via necrosis,[6] and high hemolytic activity.[7] Terhune et al[8] have suggested clinicians should be cautious in preventing extended contact of any of these materials with a patient's skin, mucosa, and gingiva.

The depth of cure of resin-based composite decreases with increasing irradiation distance.[9] The light-curing unit tip should ideally be in direct contact with the resin composite; however, this is not always clinically possible.[10] Inability to place thelight tip near the bonding material might reduce intensity and provide a lower degree of polymerization.[10,11]

Hence, the purposes of this study were to quantitatively assess the BPA released from the Transbond XT (3M Unitek, Monrovia, Calif) when varying the light-curing tip distances by 0, 5, and 10 mm with high performance liquid chromatography (HPLC) after accelerated chemical aging on days 1, 7, 21, and 35 and to evaluate the DC by using Fourier transform infrared (FTIR) spectroscopy on day 1. BPA release was also correlated to the DC.

MATERIAL AND METHODS

A visible light-cured adhesive, Transbond XT, was used. From 138 premolar stainless steel brackets (3M Unitek), 120 were used to assess BPA release with HPLC, and 18 were used for estimating the DC with the FTIR spectrometer (Perkin-Elmer, Norwalk, Conn).

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BPA released and the DC values were assessed at 3 light-curing tip distances from the bracket: 0, 5, and 10 mm.

The varying distances of the curing tip were standardized by using a specially fabricated wooden box on which a yellow tile was placed. Sample preparation and the DC assessment with the FTIR spectrometer were similar to those described by Gioka et al.[12] A 0.9-mm round metallic piece of wire with the predetermined distance from the bracket wing was used to confirm the curing distance. 0.9-mm diameter stainless steel wires 5 mm long (for the 5-mm group) and 10 mm long (for the 10-mm group) from the bracket wing were used to confirm the curing distance.

The brackets were pressed until they were seated on the tile. Excess resin was removed before polymerization. The yellow tile was covered by a cellulose film to facilitate detachment of the bracket-adhesive complex with recovery of the set material. Irradiation was performed from the labial surface of the bracket for 40 seconds with a halogen curing unit (3M ESPE, Seefeld, Germany).

We divided 120 brackets into 3 groups of 40 brackets each. Each group represented a light-curing tip distance of 0, 5, and 10 mm. The 40 brackets from each group were divided into 2 samples of 20 brackets each, representing the average number of brackets in a patient. These 20 brackets were immersed in a sterile glass tube containing 15 mL of absolute alcohol. Thus, each group consisted of 2 sterile glass tubes which in turn comprised 20 brackets per tube. The procedure used for assessing BPA release was similar to that described by Eliades et al.[13] Briefly, the bonded brackets were stored in an incubator for 5 minutes at 37°C and 50% relative humidity, and immersed in sterile glass tubes containing 15 mL of absolute alcohol (99% v/v) to induce accelerated aging. During the immersion period, the solution was agitated for 10 seconds, twice a day. The processed 50 μL amounts of alcohol solution from each group were removed at days 1, 7, 21, and 35 and filtered through 0.2-μm nylon filter paper injected in the instrument and analyzed with the HPLC device (model LC2010A-HT, Shimadzu, Kyoto, Japan) The column was calibrated with known concentrations (standard, 0.1–3 ppm) of BPA (Merck, Darmstadt, Germany) in ethanol. The linear fittings of the calibration curves were used to calculate the concentration of monomers in the alcohol solution based on the area of chromatographic peaks at the corresponding retention time. The HPLC assays were performed in triplicate for each time period, and the results were averaged. Under the conditions of the experiment, the detection threshold of BPA was estimated at 0.1 ppm (0.1 μg/L).

The DC assessment was done by using FTIR. Eighteen brackets were divided into the 3 groups of 6 brackets each to represent the light-curing tip distances of 0, 5, and 10 mm. Resin polymerization was performed at room temperature. Immediately after polymerization, the cellulose strip was discarded, and the light-cured specimens were stored in light-proof boxes to prevent further exposure to light irradiation.

The light-cured specimens, which in clinical conditions corresponded to the material in contact with the enamel, were measured and prepared as 5-mg resin disks that were then placed between 0.01-mm potassium bromide disks. The disks were then transferred to the FTIR spectrometer. Processing the samples and recording their spectra occurred within 24 hours. The spectrum was also obtained for uncured adhesive by smearing the paste onto the potassium bromide disks. Spectra obtained for the adhesive from FTIR were recorded under the following conditions: 4000 to 400 cm^{-1} wave number range, 4 cm^{-1} resolution, and 16 scans co addition. The DC was estimated on a relative percentage basis with the 2-frequency method and tangent baseline technique. Aliphatic (C=C) bond-stretching vibrations, with a characteristic infrared absorption peak about 1638 cm^{-1}, were chosen as the analytical frequency, and the aromatic (C..C) bond-stretching vibrations, which do not interfere with the polymerization reactions about 1609 cm^{-1}, were selected as the reference frequency. By using the change in the ratio of the aliphatic C=C to the reference frequencies before and after curing, the DC was calculated with the formula:

$$\%DC = 100\,(1 - RDB)$$

RDB is the residual double bond that is calculated as

$$RDB = \frac{Ap\,(C{=}C) \times Am\,(C..C)}{Am\,(C{=}C) \times Ap\,(C..C)}$$

where *Ap (C=C)* is the net peak absorbance area (NPAA) of set material at 1638 cm^{-1}; *Am (C..C)* is the NPAA of unset material at 1609 cm^{-1}; *Am (C=C)* is the NPAA of unset material at 1638 cm^{-1}; and *Ap (C..C)* is the NPAA of set material at 1609 cm^{-1}.

The mean and standard deviation values of the BPA release and the DC from the 3 groups were obtained. Statistical analysis for the BPA release was performed by using 2-way analysis of variance (ANOVA), post-hoc multiple comparisons Tukey HSD tests, and paired *t* tests. The samples obtained from the DC were analyzed separately with 1-way ANOVA. The Pearson correlation was used to assess the correlation between the DC and BPA released on day 1.

RESULTS

Table 1 shows the mean concentrations of BPA released on days 1, 7, 21, and 35 at the 3 light-curing

Table I. Means and standard deviations (in ppm) of the BPA readings in each group evaluated on days 1, 7, 21, and 35

Light-curing tip to bracket distance (mm)	*Day 1 Mean ± SD*	*Day 7 Mean ± SD*	*Day 21 Mean ± SD*	*Day 35 Mean ± SD*
0	14.38 ± 0.010	21.55 ± 0.015	31.79 ± 0.007	30.31 ± 0.007
5	38.31 ± 0.004	49.46 ± 0.012	54.21 ± 0.010	50.39 ± 0.005
10	63.69 ± 0.008	65.67 ± 0.011	64.76 ± 0.009	60.81 ± 0.008

Table II. Two-way ANOVA: dependent variable, BPA release

Day	*Light-curing tip to bracket distance (mm)*	*Mean ± SD*	*n*
1	0	14.38 ± 0.01	6
	5	38.31 ± 0.00	6
	10	63.69 ± 0.01	6
	Total	38.79 ± 20.72	18
7	0	21.55 ± 0.02	6
	5	49.46 ± 0.01	6
	10	65.67 ± 0.01	6
	Total	45.56 ± 18.75	18
21	0	31.79 ± 0.01	6
	5	54.21 ± 0.01	6
	10	64.76 ± 0.01	6
	Total	50.26 ± 14.147	18
35	0	30.31 ± 0.01	6
	5	50.39 ± 0.01	6
	10	60.81 ± 0.09	6
	Total	47.17 ± 13.03	18
Total	0	24.51 ± 7.19	24
	5	48.09 ± 6.05	24
	10	63.73 ± 1.87	24
	Total	45.44 ± 17.13	72

tip distances of 0, 5, and 10 mm. Tables II through VII show the BPA release from days 1 to day 35 in the 3 groups and between the 3 groups. Greater amounts of BPA were observed at the 10-mm light-curing tip distance. There was a statistically significant increase in the mean release of BPA as the light-curing tip distance was increased ($P < 0.05$). A significant reduction ($P < 0.05$) in the DC was observed as the light-curing tip distance increased (Table VIII).

DISCUSSION

Leaching of BPA was observed on day 1 even at the 0-mm light-curing tip distance; this substantiates the observation of Thompson et al[14] that cured orthodontic adhesives are far from inert. Tip distances of 0 and 5 mm showed increases of BPA released up to day 21, after which the concentration showed a marginal decrease. At 10 mm, the day 1 release was not only greater, but this increased release remained more or less constant until day 35. This could be because of the depletions in the concentration of the BPA in the adhesive. Furthermore, the chemical polymerization reactions initiated by the initially polymerized compounds on the unpolymerized part of the orthodontic adhesive can be quite slow and therefore might lead to the complete polymerization of the resin only by day 35. The maximum monomer leaching was experienced during the initial period of bonding but before day 35. Thus, it can be interpreted that the exact period of the beginning of the reduction is between days 21 and 35 at the 0-mm and 5-mm distances. Further studies should be directed to accurately assess the exact period of this reduction.

The increased BPA release with increased light-curing tip distances could also be due to decreased DC of the resin. Therefore, the DC was evaluated with FTIR, where decreases in the mean percentages of the DC as the light-curing tip distances were increased were seen. Post-hoc multiple comparisons Tukey HSD analysis showed a statistically significant difference when the light-curing tip distances were increased from 0 to 5 mm and from 0 to 10 mm, but not when the distance was increased from 5 to 10 mm (Table IX).

The DC decrease was severe when the light-curing tip distance was increased from 0 to 5 mm, but it was minimal when the light-curing tip distance was increased from 5 to 10 mm. Thus, a 5-mm increase in distance might significantly influence the DC and thereby the BPA release.

On correlating the DC with the BPA release, a moderate negative correlation between the BPA and its DC at 0 mm ($r = -0.683$), a weak negative correlation between the BPA and its DC ($r = -0.441$) at 5 mm, and a negligible negative correlation between the BPA and its DC ($r = -0.171$) at 10 mm were obtained (Table X). Although this means that with a lower DC there was a greater BPA release, these correlations were not statistically significant. This could be because factors other than polymerization, such as the presence of an activator or a primer, and the solubility of the components, might also have roles in the cytotoxicity of the materials.[15]

A yellow tile was used to ensure that the influence of the background surface was limited to a standardized extent of backing reflectance of the activating light as proposed by Eliades et al.[2] Although HPLC is a valid technique, since it can detect a potential toxic release in an orthodontic adhesive, this release must be further assessed for cytotoxicity. HPLC was used because this

Table III. Test between the subjects: dependent variable, BPA release

Source	Type III sum of squares	df	Mean square	F	Significance	Partial eta squared
Corrected model	20825.987*	11	1893.272	2E + 007	0.00	1.00
Intercept	148715.836	1	148715.836	2E + 009	0.00	1.00
Day	1266.688	3	422.229	4330133	0.00	1.00
LCTBD	18716.200	2	9358.100	1E + 008	0.00	1.00
Day* LCTBD	843.099	6	140.517	1441054	0.00	1.00
Error	0.006	60	9.75E-005			
Total	169541.829	72				
Corrected total	20825.993	71				

LCTBD, Light-curing tip to bracket distance.
*R squared = 1.000 (adjusted R squared = 1.000).

Table IV. Post-hoc multiple comparisons Tukey HSD test: comparison of BPA released in the 4 time periods with those of the other time periods at the light-curing tip to bracket distance of 0 mm

				95% CI	
Day (a)	Day (b)	Mean difference (a−b)	P value	Lower bound	Upper bound
1					
	7	−7.17	0.00*	−7.19	−7.16
	21	−17.41	0.00*	−17.43	−17.39
	35	−15.93	0.00*	−15.95	−15.91
7					
	1	7.17	0.00*	7.16	7.19
	21	−10.24	0.00*	−10.25	−10.23
	35	−8.76	0.00*	−8.78	−8.74
21					
	1	17.41	0.00*	17.39	17.43
	7	10.24	0.00*	10.22	10.25
	35	1.48	0.00*	1.46	1.50
35					
	1	15.93	0.00*	15.91	15.95
	7	8.76	0.00*	8.74	8.78
	21	−1.48	0.00*	−1.50	−1.46

*Mean difference is significant at the 0.05 level.

Table V. Post-hoc multiple comparisons Tukey HSD test: comparison of BPA released in the 4 time periods with those of the other time periods at the light-curing tip to bracket distance of 5 mm

				95% CI	
Day (a)	Day (b)	Mean difference (a−b)	P value	Lower bound	Upper bound
1					
	7	−11.15	0.00*	−11.16	−11.14
	21	−15.90	0.00*	−15.91	−15.88
	35	−12.08	0.00*	−12.09	−12.06
7					
	1	11.15	0.00*	11.14	11.16
	21	−4.75	0.00*	−4.76	−4.73
	35	−0.93	0.00*	−0.94	−0.91
21					
	1	15.90	0.00*	15.88	15.91
	7	4.75	0.00*	4.73	4.76
	35	3.82	0.00*	3.81	3.84
35					
	1	12.08	0.00*	12.06	12.09
	7	0.93	0.00*	0.91	0.94
	21	−3.82	0.00*	−3.84	−3.81

*Mean difference is significant at the 0.05 level.

is more ideal than gas chromatography or mass spectrometry for quantitative assessment of BPA release. Pulgar et al[16] evaluated BPA release using HPLC and subsequently confirmed the release with gas chromatography or mass spectrometry, thus showing that HPLC is sufficient for eliciting BPA release. The minimum detection limit of BPA is .2 μg per milliliter.

Alcohol immersion facilitates a potent aging medium because it induces potent plasticizing of polymers and allows the effects to be studied.[13] Orthodontic-adhesive exposure to the oral environment involves only the peripheral margins of the bracket with an average thickness of 150 to 250 μm, and thus the effect of aging might not be potent. To facilitate a larger safety window, brackets bonded to free adhesive surfaces were used; therefore, the entire surface of the adhesive, which corresponds to the surface bonded to the enamel, was exposed to the aging medium.

Considerable quantities of resins leach from cured products.[2,5,12-14,16,17] The significance of leaching relates to the formation of formaldehyde, a potentially hazardous by-product, which is a result of the oxidation of pendant C=C. Caldas et al[10] and Radzi et al[18] showed decreases in the hardness and intensity on increasing the light-curing distances. Sfondrini et al[19] checked the bond strength with various light-curing tip distances. To date, no study has tested the DC for varying light-curing tip distances. The DC was assessed, since bond

Table VI. Post-hoc multiple comparisons Tukey HSD test: comparison of BPA released in the 4 time periods with those of the other time periods at thye light-curing tip to bracket distance of 10 mm

				95% CI	
Day (a)	*Day (b)*	*Mean difference (a−b)*	P *value*	*Lower bound*	*Upper bound*
1					
	7	−1.98	0.00*	−2.00	−1.97
	21	−1.07	0.00*	−1.09	−1.06
	35	2.88	0.00*	2.86	2.89
7					
	1	1.98	0.00*	1.97	2.00
	21	0.91	0.00*	0.90	0.93
	35	4.86	0.00*	4.85	4.88
21					
	1	1.07	0.00*	1.06	1.09
	7	−0.91	0.00*	−0.93	−0.909
	35	3.95	0.00*	3.94	3.97
35					
	1	−2.88	0.00*	−2.89	−2.86
	7	−4.86	0.00*	−4.88	−4.84
	21	−3.95	0.00*	−3.97	−3.94

*Mean difference is significant at the 0.05 level.

Table VII. Paired *t* test: correlation of BPA release

Pair	*Mean ± SD for day 1 (t value)*	*Mean ± SD for day 7 (t value)*	*Mean ± SD for day 21 (t value)*	*Mean ± SD for day 35 (t value)*
0 mm	14.38 ± 0.01	21.55 ± 0.02	31.79 ± 0.01	30.31 ± 0.01
5 mm	38.31 ± 0.00	49.46 ± 0.01	54.21 ± 0.01	50.39 ± 0.01
	−4000.86*	−2491.22*	−3897.20*	−3965.23*
0 mm	14.38 ± 0.01	21.55 ± 0.02	31.79 ± 0.01	30.31 ± 0.01
10 mm	63.69 ± 0.01	65.67 ± 0.01	64.76 ± 0.01	60.81 ± 0.01
	−7778.25*	−5636.07*	−7026.63*	−6061.60*
5 mm	38.31 ± 0.00	49.46 ± 0.01	54.21 ± 0.01	50.39 ± 0.01
10 mm	63.69 ± 0.01	65.67 ± 0.01	64.76 ± 0.01	60.81 ± 0.01
	−9470.82*	−2151.55*	−4765.69*	−2416.58*

*Significance was found at $P < 0.00$ for all values.

Table VIII. Means and standard deviations of the DC of the 3 groups (n = 6 for all readings)

Light-curing tip to bracket distance (mm)	*Mean ± SD (%)*
0	27.4733 ± 4.76
5	3.2233 ± 0.23
10	2.7933 ± 0.20

Table IX. Post-hoc multiple comparisons Tukey HSD test: comparison of percentages of DC of the 3 groups with those of the other groups

Group (i)	*Group (j)*	*Mean difference (i−j)*
0 mm	5 mm	24.2500*
	10 mm	24.6800*
5 mm	0 mm	−24.2500*
	10 mm	0.4300
10 mm	0 mm	−24.6800*
	5 mm	−0.4300

*Mean difference is significant at the 0.05 level.

strength and hardness measurements have many drawbacks.

In a clinical situation, the irradiation is usually done for 10 seconds each on the mesial, distal, occlusal, and gingival aspects. However, in our study, the irradiation was performed on the labial aspect only to ensure elimination of distance errors while moving the source to various aspects of the bracket. To reduce the potential for BPA release, several precautionary measures can be followed. Only as much material as necessary should

Table X. Pearson correlation between the BPA released and the DC at day 1 for the 3 groups

Light-curing tip to bracket distance	*Pearson correlation r value*	*Significance P value*
0 mm	−0.683	0.135
5 mm	−0.441	0.382
10 mm	−0.171	0.746

$P > 0.05$ is insignificant.

be used, and excess polymerized adhesive should be removed, particularly in areas where the adhesives could come into intimate contact with the subgingival and interproximal tissues. Once the adhesive has set, excess activator material must be removed thoroughly by washing the tooth with a water spray. Other factors such as light intensity play a role in the BPA release. Hence, the clinician should take every step to ensure maximum curing of the resin. Keeping the light-curing tip as close to the adhesive is 1 such step. If it is clinically difficult, as in rotated or malposed teeth, additional irradiation might be considered.

Although in clinical situations the volume of liquids passing through the oral cavity might dilute the leached components and thus reduce their concentration, the prolonged exposure of tissues and organs to such noxious materials must not be overlooked.

CONCLUSIONS

Release of BPA was detected from Transbond XT with light-curing tip distances of 0, 5, and 10 mm, thus indicating that the cured composite is not inert. Increasing the light-curing tip distance caused a decrease in the DC, and this led to greater BPA release. The clinician should keep the light-curing tip as close to the adhesive as clinically possible.

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