



Original Article

Effects of Tooth Brushing and Mouth Washing on Leaching Bisphenol A Levels From an Orthodontic Adhesive: An In Vitro Study

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Cite this article as: Arslan B, Yıldırım E, Can Bodur O, Baloş Tuncer B, Çağrı Ulusoy M, Tuncer C. Effects of tooth brushing and mouthwashing on leaching bisphenol a levels from an orthodontic adhesive: An in vitro study. *Turk J Orthod.* 2022;35(1):27-32.

Main Points

- Bisphenol A (BPA) release from orthodontic adhesive samples after tooth brushing and mouth washing was investigated.
- Detected levels of BPA were found to be lower than referenced tolerable daily intake levels.
- Tooth brushing did not significantly affect the leaching BPA amount.
- Increased levels of leached BPA with alcohol-containing mouth washing solutions should be considered during fixed orthodontic treatment.

ABSTRACT

Objective: To assess the levels of bisphenol A (BPA) released from an orthodontic adhesive with respect to the effects of tooth brushing and mouth washing.

Methods: Three groups, each containing fifteen adhesive samples were prepared. In Group 1, samples were polymerized according to manufacturer instructions. In Group 2, after the same polymerization protocol, each sample was brushed with a fluoride-containing toothpaste. For Group 3, samples were immersed in a mouth washing solution after polymerization. Later, all samples were placed into glass tubes containing 5 mL distilled water. High-performance liquid chromatography (HPLC) measurements were performed to assess the leaching amount of BPA. Intergroup comparison was performed by one way ANOVA test.

Results: Mean amounts of BPA were found to be 0.2674 µg/L, 0.2692 µg/L, and 0.2705 µg/L, respectively. Only a significant difference was found between Group 1 and 3 ($P < .01$), revealing higher BPA levels with the mouth washing solution.

Conclusion: Measurable amounts of BPA release were observed in all groups of orthodontic adhesive samples, but the detected amounts were below the toxic levels. From a clinical point of view, alcohol-containing mouth washing solutions might increase the amount of leaching monomer, since alcohol is solvent of BPA.

Keywords: Bisphenol A, orthodontic adhesives, leaching, tooth brushing, mouth washing

INTRODUCTION

In recent years, peoples' concerns for the release of some chemicals from cosmetic and food products, kitchen tools, and/or various intraoral appliances have steadily been increasing. This is an important issue in terms of general public health. Endocrine-disrupting substances are chemicals that alter the development and functioning of the endocrine system.¹ They can be found in nature, synthetic and industrial products,² and affect production, release, binding, transport, activity, destruction, and excretion of hormones. One or more of these effects may be combined and clinical findings are based on the sum of all effects.³ Some substances are known to cause impairments in the endocrine system those are polychlorinated biphenyls, plastic-associated and household products, pesticides, and heavy metals.⁴

Among endocrine disruptors, bisphenol A (BPA) is a colorless and solid chemical that is well soluble in organic solvents but slightly soluble in water.⁵ It is used in the manufacturing of polycarbonates for hard plastic products and epoxy resin linings for metal food and beverage cans.⁶ Due to its wide application range, the demand and production for BPA are increasing year by year. It has been discovered that BPA is an artificial estrogen and can interact with other endocrine receptors such as the thyroid hormone receptor.⁷ In the human body, it has been determined in blood, urine, breast milk, and some other tissues.⁸

Bisphenol A is not a component of dental products by purely itself but its derivatives are used in dental materials. Therefore, BPA could be a clinical concern in dentistry, since exposure into the oral cavity can occur especially due to changes in intraoral temperature, salivary enzymatic structure, pH, or mechanical wear.^{9,10} Commonly used BPA-derivatives in dentistry are bisphenol A diglycidylmethacrylate (Bis-GMA), ethoxylatedbisphenol A glycidylmethacrylate (Bis-EMA), and bisphenol A dimethacrylate (Bis-DMA).¹¹ It is stated that toxic results can occur as a result of the dissolution of non-polymerized BPA monomers from resin-based dental materials; some studies were conducted in the field of orthodontics as well.¹²⁻¹⁵

This should be particularly taken into consideration for orthodontics, because early treatment is usually performed in most cases, declaring that most of the orthodontic patients are children and adolescents.¹⁶ Epidemiological and animal studies suggested that endocrine disruptors have adverse effects on birth weight, reproductive tract development and promote childhood obesity formation.¹⁷

Malkiewicz et al.¹² and Kloukos et al.¹⁸ suggested that mouth rinsing can be useful to prevent exposure to the potential hazard of leaching monomers after orthodontic bracket placement. In a relevant systematic review, several recommendations have been made to reduce the amount of exposed BPA.¹⁹ However, there is still no sufficient knowledge regarding a clinical way to prevent or decrease the possible release of BPA after orthodontic bonding. Therefore the aim of this in-vitro study was to assess

the levels of BPA released from an orthodontic adhesive by evaluating the effects of tooth brushing and mouth washing.

METHODS

Ethical consent was not required since this was an experimental study. Three groups, each consisting fifteen samples of bonding adhesives were prepared at room temperature. All of the samples were prepared according to the experimental design by the same researcher at the same laboratory and under the same conditions. Samples were prepared in molds, made of Teflon matrices that were 5 mm in diameter and 2 mm in thickness, based on previous research.¹² Transbond XT Primer and Transbond XT Light Curing Adhesive (3M Unitek, Monrovia CA, USA) were chosen as primer and adhesive (Figure 1a). Primer was gently applied as a single layer with the help of an application brush (Figure 1b). Attention was paid to avoid exaggerated application. LED light source with 420-480 nm wavelength (COXO® DB-686 Cappu LED Curing Light; Foshan Coxo Medical Instrument CO. LTD, Guangdong Province, China) was used for photopolymerization. Light-curing was performed directly from the upper surface of the samples for 10 s.

Preparation of the Samples

The experiment procedures related to the preparation of the samples are shown in Figure 2.

In Group 1, adhesive samples were prepared, primer was applied gently with an application brush on each sample, and were polymerized. Samples were placed into glass tubes containing 5 mL distilled water (Figure 2a).

In Group 2, the polymerization process was performed in the same way as in Group 1. Then, each sample was manually brushed with a fluoride-containing toothpaste (Colgate® Triple Action Toothpaste; Colgate-Palmolive Company) and a toothbrush (Curaprox; Curaden International AG, Kriens, Switzerland). Standardization of the brushing force was targeted through manual brushing performed by the same researcher (BA), who was a right-handed 29-year-old woman, with gentle scrubbing

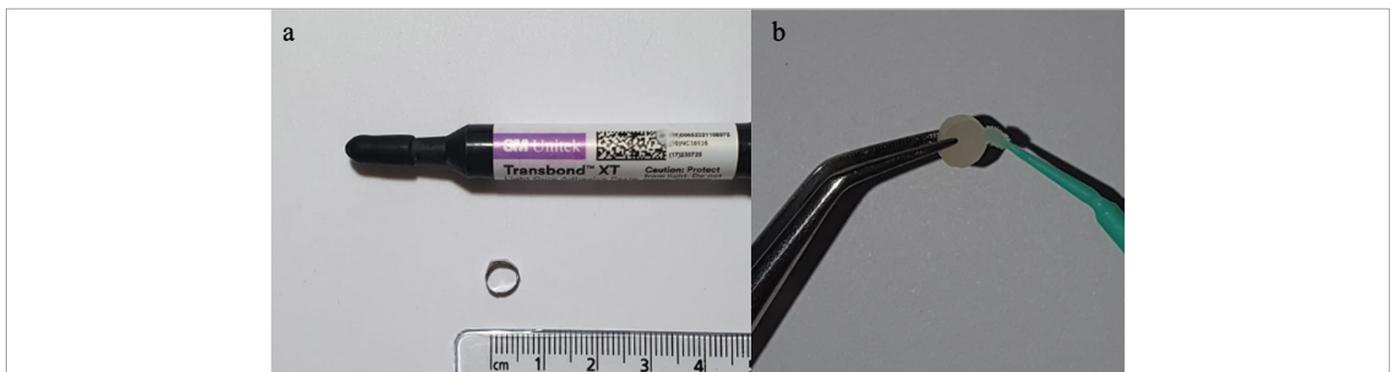


Figure 1. a, b. (a) Preparation of the adhesive samples (5 mm in diameter and 2 mm in thickness); (b) Application of primer

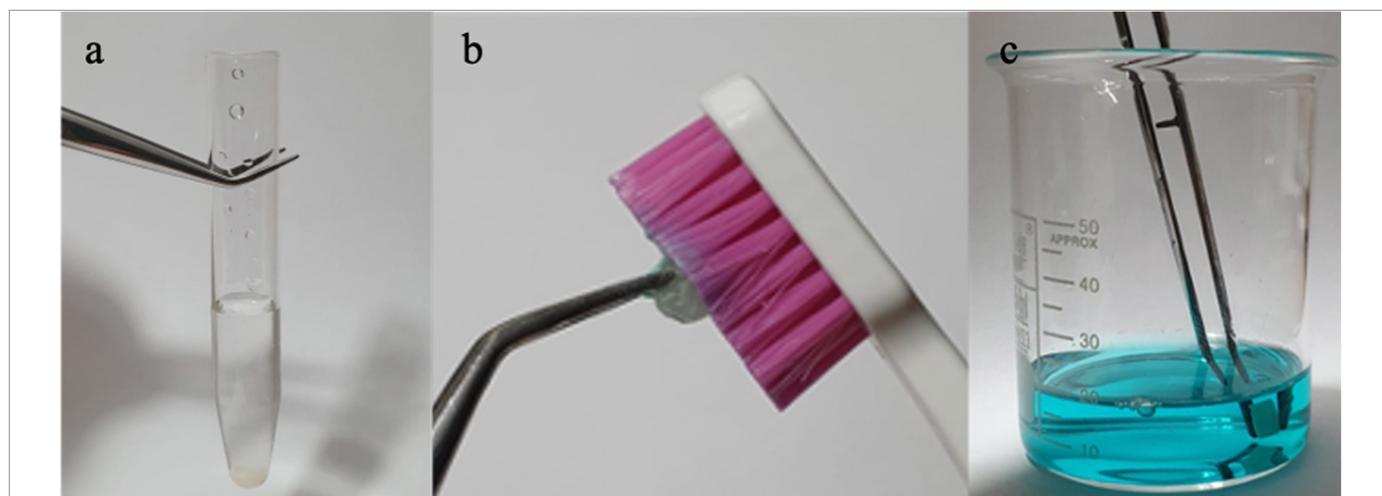


Figure 2. a-c. (a) Adhesive sample placed into glass tubes containing 5 mL distilled water in Group 1; (b) Adhesive for review the only sample manually brushed with a fluoride-containing toothpaste in Group 2; (c) Adhesive sample immersed and moved physically in 20 mL of mouth washing solution in Group 3

on the adhesive surfaces for 10 s. Later, samples were rinsed with water and placed in a glass tube containing 5 mL of distilled water (Figure 2b).

In Group 3, polymerization was performed with the same protocol. Then, each sample was immersed and moved physically in 20 mL of mouth washing solution (Cool Mint Listerine®; Johnson & Johnson Consumer Inc) in circular motions for 30 s and placed into a glass tube containing 5 mL of distilled water (Figure 2c).

In the second and third groups, a new glass of water and mouth-wash was used with each sample. During all experimental procedures, samples were held with a dental tweezer from a single point, and all surfaces were included in application procedures. Care was taken not to use any plastic material to prevent any monomer interference. High-performance liquid chromatography (HPLC) measurements were performed 1 h after the placement of composite samples to glass tubes with distilled water. Ingredients of the orthodontic primer and adhesive, toothpaste, and mouth washing solution are given in Table 1.

Chemical Analysis

High-performance liquid chromatography was used for the determination of BPA in samples. The HPLC system consisted of Shimadzu LC-2010 (Shimadzu Corp., Kyoto, Japan) and Shimadzu

HPLC solution software. Chromatographic separation was carried out on a C18 column (Phenomenex, 150 × 4.6 mm, 5 µm particle size), using a gradient solvent system comprising 80% acetonitrile acid (A) (for HPLC, gradient grade, ≥99.9%, Sigma-Aldrich) and 20% MeOH (B) (for HPLC, gradient grade, ≥99.9%, Sigma-Aldrich). Gradient profile: 0-20 min. The flow rate was adjusted to 1.0 mL/min, the detection wavelength was 228 nm and 2 µL of each sample was injected. All separations were performed at 25°C. Standard solutions were processed in a device according to this method and the time of peak for BPA was determined. The linear fittings of the calibration curves were used to calculate the concentration of BPA in the polymer solution based on the area of chromatographic peaks at the corresponding retention time. High-performance liquid chromatography 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 mg/L). Bisphenol concentration of the samples was determined by taking chromatograms on the HPLC instrument by autosampler.

Statistical Analysis

Data analysis was performed using the Statistical Package for Social Sciences software (SPSS, Version 21.0, USA). The Shapiro-Wilk test was used to determine the homogeneity of the data. Descriptive statistics were presented as mean and standard

Table 1. Ingredients of orthodontic primer and adhesive, toothpaste and antiseptic mouthwashing solution

	Ingredients
Transbond XT Primer	Bisphenol A Diglycidyl Ether Dimethacrylate (BISGMA), Triethylene Glycol Dimethacrylate (TEGDMA), 4-(Dimethylamino)-Benzeneethanol
Transbond XT Light Curing Adhesive	Silane Treated Quartz, Bisphenol A Diglycidyl Ether Dimethacrylate (BISGMA), Bisphenol A Dimethacrylate, Silane Treated Silica, Diphenyliodonium Hexafluorophosphate, Triphenylantimony
Colgate® Triple Action Toothpaste	Sodium Monofluorophosphate, Glycerin, Hydrated Silica, Water, Sodium Bicarbonate, PEG-12, Sodium Lauryl Sulfate, Flavor, Sodium Hydroxide, Cellulose Gum, Carrageenan, Sodium Saccharin, FD&C Blue No 1., D&C Yellow No.10
Cool Mint Listerine®	Eucalyptol, Menthol, Methyl Salicylate, Thymol, Water, Alcohol, Sorbitol, Poloxamer, Benzoid Acid, Sodium Saccharin, Sodium Benzoate, Flavor, Green 3

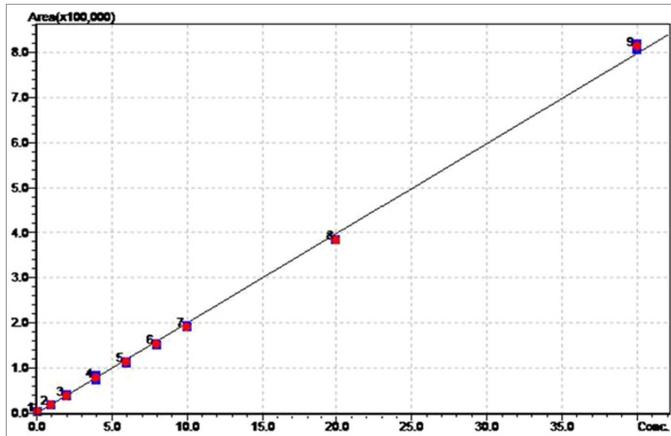


Figure 3. Standard calibration curve for high-performance liquid chromatography for detection of Bisphenol A in the polymer solution

deviation. Intergroup comparison was performed by one way ANOVA test. A *P* value of < .05 was considered to be statistically significant.

30 RESULTS

Bisphenol A amount in distilled water was analyzed with HPLC. The standard calibration curve for HPLC of detection of BPA was given in Figure 3. The calibration method was used for the determination of BPA in the prepared solution sample. The peak intensities that vary linearly with BPA concentration were given in the calibration curve, and it was proved appropriate that peak areas change linearly with concentration. In addition, the fact that the regression number was close to 1 ($R^2 > 0.99$) made it possible to analyze BPA with this method.

Descriptive statistics and comparison between groups are given in Table 2. The results declared that measurable amounts of BPA release were observed in all groups. Mean amounts of BPA was found to be 0.2674 , 0.2692, and 0.2705 µg/L, respectively in Group 1 to 3. The highest amount of BPA release was found in Group 3, whereas the lowest was detected in Group 1.

According to one way ANOVA test, no statistically significant differences were found between Group 1 and Group 2, as well as between Group 2 and Group 3. However, there was a significant difference between Group 1 and Group 3 ($P < .01$), declaring significantly higher BPA levels in mouth washing group.

DISCUSSION

Studies on the leaching of BPA monomer from dental materials were first performed on dental sealants in the 1990s.²⁰ In this in-vitro study we aimed to evaluate the amount of BPA released from a standard orthodontic adhesive under the influence of tooth brushing and mouth washing. Although tooth brushing did not reveal significant differences relative to the leaching BPA amounts, increased levels were noted with the mouth washing solution. Previous studies have shown that BPA was released into the oral environment after adhesive polymerization and it was noted that the highest BPA release occurred immediately after polymerization.^{12,21,22} Inline, measurements were made immediately after polymerization in our study.

The tolerable daily intake level of BPA detected by the European Food Safety Authority is limited to 50 mcg/kg bw/day.²³ The current results declared that the detected BPA levels were considerably lower than the mentioned tolerable daily intake levels. Furthermore, all the adhesive surfaces were in contact with distilled water in this study, but in reality, only the adhesive parts at the bracket margins are in contact with the oral cavity. Previous studies mentioned the low dose effects of BPA.^{24,25} Despite the small amounts of this chemical, long treatment durations of fixed orthodontic appliances might reveal clinical importance in this sense. Therefore, even small amounts of monomer may be considered to be important. It was mentioned that rinsing the mouth with water after orthodontic bonding could be useful to take the excess monomer out.^{12,18} It is obvious that this process can be beneficial since the results of our study also showed measurable amounts of BPA. In addition, it can be explained that pure water was preferred in our study to avoid the interference effects of different substances in tap water.

Current results showed that brushing teeth with a fluorized toothpaste did not seem to affect the amount of leaching BPA monomer when compared to Group 1, in which samples had no additional process after polymerization. However, it was noted that antiseptic mouthwash can increase the amount significantly. This finding might be attributed to the fact that most of the mouth washing solutions—including the one used in this study—contain alcohol, which is an organic solvent for BPA.²⁵ Eliades et al.¹³ investigated the effect of alcohol-containing rinsing solution and water on the release of BPA after orthodontic bonding in an in vivo study and demonstrated higher levels of BPA in patients who only rinsed their mouth with water, which was against the result they expected. They

Table 2. Descriptive values of data and comparison between groups with one way ANOVA test

	Group 1	Group 2	Group 3	<i>P</i> between Groups		
	Mean ± SD	Mean ± SD	Mean ± SD	Group 1-Group 2	Group 1-Group 3	Group 2-Group 3
BPA levels (mg/L)	0.2674 ± 0.000632	0.2692 ± 0.002549	0.2705 ± 0.003796	.146	.007*	.402

Group 1, samples were placed into pure water after polymerization.

Group 2, samples were brushed with a fluoride-containing tooth-paste, rinsed, and placed in pure water after polymerization.

Group 3, samples were immersed in mouth washing solution and placed in pure water after polymerization.

**P* < .05.

SD, standard deviation; BPA, bisphenol A.

related their results to the reduced contact of the adhesive with the solution, which was limited with bracket–enamel margin, and also to the insufficient rinsing of patients due to the taste of ethanolic solutions.

Since alcoholic compounds contain a hydrocarbon group instead of hydrogen atom in water, alcohol is chemically less polar solvent than water. While the hydrocarbon part of alcohol gets van der Waals interaction with the hydrocarbon part of BPA, hydroxyl group of alcohol makes a hydrogen bond with the hydroxyl group of BPA which enhances the solubility of BPA in alcoholic compounds. Hence, using an alcohol-containing mouthwash after orthodontic bonding may increase the amount of BPA in the oral cavity. Many studies have been performed with mouth washing solutions and the results declared that these products provide antimicrobial and antigingivitis activities.²⁶⁻²⁸ On the other hand, it should be considered that systematic toxicological studies have to be conducted to sustain the total safety of these products. Since in vivo reactions could provide different results, future studies with alternative strategies should be investigated to provide better knowledge on this topic.

The study had some limitations. First, the measurements were performed with HPLC at only 1 time interval, but the recurring brushing and rinsing procedures were not simulated, therefore further researches could be planned in this regard. Second, the clinical practice of bonding was not fully imitated in this experiment. Again, BPA release with different adhesives and mouth washing solutions could be addressed in the future.

CONCLUSION

The present in vitro study provided a basis for the measurable amounts of BPA release with orthodontic adhesive samples. However, the detected levels of BPA were below the referenced tolerable daily intake levels. Results declared no significant difference relative to the leaching BPA amounts. However, orthodontists should consider the increased levels of leached BPA with alcohol-containing mouth washing solutions during fixed orthodontic treatment.

Ethics Committee Approval: Ethical consent was not required since this was an experimental study.

Informed Consent: N/A.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - B.B.T., C.T., M.Ç.U.; Design - B.B.T., C.T., M.Ç.U.; Supervision - B.B.T., C.T., M.Ç.U.; Fundings - B.A.; Materials - B.A., E.Y., O.C.B., M.Ç.U.; Data Collection and/or Processing - B.A., E.Y., O.C.B., M.Ç.U.; Analysis and/or Interpretation - B.B.T., C.T., E.Y., O.C.B., M.Ç.U.; Literature Review - B.A., B.B.T., C.T., M.Ç.U.; Writing - B.A., B.B.T., C.T., M.Ç.U.; Critical Review - B.B.T., C.T., M.Ç.U.

Conflict of Interest: The authors have no conflicts of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

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