Bisphenol A in Hawley and Vacuum-Formed Retainers: An In-Vitro Study

Arnis Jenny A[1], Purwanegara Miesje K[2]*, Anggani Haru S[3]

1. Arnis Jenny A, Orthodontic resident (author), Department of Orthodontics, University of Indonesia, Indonesia
2. Purwanegara Miesje K, co-author, Department of Orthodontics, University of Indonesia, Indonesia.
3. Anggani Haru S, co-author, Department of Orthodontics, University of Indonesia, Indonesia.

Abstract

Chemical materials based on bisphenol A (BPA) are commonly used in orthodontic practices. The environmental conditions of the oral cavity, especially saliva, can influence retainer material properties, causing degradation and release of BPA monomers. The usage of retainers for a long period of time can cause the release of BPA in a small dose and is believed to become potentially hazardous for health.

This study is aimed to analyze of BPA concentration within Hawley self-cure, heat-cure, and vacuum-formed retainers (VFR) in artificial saliva.

There were sixty-three specimen of acrylic resin fabricated by self-cure (n=21), acrylic resin fabricated by heat-cure (n=21), and also VFR (n=21). Each of the specimen about 1 gram was submerged into 10 mL of artificial saliva for the period of 1 hour, 7 days, and 30 days at 37°C incubators. Afterwards, the liquid samples of 5 mL were taken to determine the BPA concentration using liquid chromatography mass spectrometry (LC-MS/MS). Statistical analyses were performed using "Kruskal Wallis" test. Results: There were statistically significant differences of BPA concentration between all groups within 7-day and 30-day submersion, where the highest concentration was noted in the VFR group while the lowest concentration was in the Hawley retainer fabricated by self cure.

The amounts of BPA released from three material retainers were no more than 11.98 ng/mL. The numbers were further below the reference dose for daily intake.

Experimental article (J Int Dent Med Res 2022; 15(1): 61-65)

Keywords: Bisphenol A, Hawley retainer, vacuum formed retainer.

Received date: 13 August 2021 **Accept date:** 14 November 2021

Introduction

A retainer is needed during the retention phase to prevent relapse until reformation of gingival and periodontal tissues until the process of growth and development are completed. There are two types of retainers, removable and fixed retainers. The removable retainers commonly used by patients are Hawley and vacuum formed retainers. Hawley retainers have advantages in terms of durability, while VFR is becoming more popular because it is made of transparent plastic so that it has advantages in terms of aesthetics and comfort.[1,2]

Corresponding author:
Prof. Dr. Miesje K Purwanegara
Departmen of Orthodontics, Faculty of Dentistry
University of Indonesia, Jl. Salemba Raya no.4, Jakarta Pusat 10430, Indonesia
E-mail: miesjekar@gmail.com

There is concern regarding the release of chemical substances based from multiple plastic products used in daily basis. Based on the data from World Health Organization (WHO) in 2011, BPA is the chemical that is most commonly used on plastic products such as polycarbonate and resin epoxy that have negative impact for health.[3] Adverse effects of BPA for health can influence the endocrine system by binding and activating estrogen receptor in human and also contribute to abnormality of pathogenesis endocrine such as infertility in men and women, early pubertal stages and other metabolism abnormality, and BPA is reported to have carcinogenic impact.[3,4] BPA is easily found on plastic based packaging of food products, drinking bottles, lunch boxes, and coatings for food. According to some research, BPA is also found in medical and dental tools, such as orthodontic materials. [4,5]

The United States Food and Drugs Administration (USFDA) prohibited the usage of epoxy resin based on BPA material as coating for

baby canned food on July 2012.[6,7] The safety dose for BPA usage in the United States is 50 µg per kg body weight per day. Then the decision was changed to 4 µg per kg body weight per day on January 2015. In few European countries, the usage of BPA is prohibited for food products.[6]

BPA-based chemical substances are often used in orthodontic materials, for example in Acrylic base plate material from Hawley retainers. Based on American Dental Association (ADA) there are two types of acrylic which are self curing and heat curing, both of which contains powder and liquid. Hydroquinone material within the liquid component of acrylic is believed to contain BPA. Besides that, BPA are often added into thermoplastic material with the purpose to increase material strength and durability, like the one found in VFR.[3] The environmental conditions of the oral cavity especially saliva can influence the characteristics of retainer material, causing material degradation and release of BPA monomer.[8]

The usage of a retainer for a longer period causing the release of BPA in a lower dose has became an attention because this is believed to have a potential risk for health. There have been many research conducted using in vivo or in vitro regarding the release of BPA from orthodontic materials, such as polycarbonate braces, composite resin, fixed lingual retainer, and clear aligner. However not many research have been conducted on BPA release on Hawley retainers and VFR.[5,6,9,10] ADA gives supports on all research regarding safety dosage of BPA, even though it is believed that BPA exposure from orthodontics material do not have negative effects for health.[11] This is the reason for researchers to conduct further research using the in vitro approach to identify differences of BPA containment base of immersion of Hawley retainers and VFR in artificial saliva.

Materials and methods

This study used a self-curing acrylic resin (Ortho Resin, Dentsply, England), heat curing (ADM, Tricodent, England), and VFR thermoplastic resin (Essix ACE, Dentsply International, Florida, USA).

Acrylic material from the Hawley retainer was made by mixing polymer and acrylic resin monomer into an aluminum mold measuring 60 x 70 x 15 mm in a ratio according to the manufacturer's instructions, and the surface was then polished until smooth, flat, and not porous. Group A used a Hawley retainer acrylic material with chemical polymerization, while the material for Group B was a Hawley retainer acrylic material with polymerization using heat. Group C, on the other hand, used a VFR material made by heating a thermoplastic sheet, and it was then vacuum-pressured (vacuum-pressure machine, Denshine, China) according to the manufacturer's instructions. The retainer material was cut into squares and weighed 1 gram with 21 specimens in each group, and they were divided into 3 so that there were 7 specimens in each group of immersion time (1 hour, 7 days, 30 days).

A total of 63 specimens were stored in a 15 ml glass vial and incubated at 37°C for 24 hours. The specimens were removed from the incubator for 1 hour, immersed in artificial saliva, and incubated at 37°C. During the immersion, the glass vial was shaken for 10 seconds twice a day. Samples were stored in a refrigerator at -80°C (Chest freezer, Vestfrost, Denmark) after the specified immersion time until the analysis process started. The sample was processed by mixing and vertexing 5 mL of artificial saliva with 0.1 N Hydrogen Chloride and 5 ml of methyl t-butyl ether. Then it was centrifuged at 3000 rpm for 10 minutes, and after that, 5 mL of the supernatant was taken and evaporated. Then 10 µL of the dry residue was taken and transferred to an auto sampler vial to be injected into the liquid chromatography column. The BPA content in artificial saliva was analyzed according to the method presented in the journal article written by Kang et al[9] using liquid chromatography/mass spectrometry (Agilent USA type 6470 Triple Quad).

Statistical Analysis

Data processing and analysis were done using SPSS 23.0 software. The Bivariate analysis was done to measure the BPA level within the groups of self-cure, heat cure Hawley retainer and VFR material during 1 hour, 7 days, and 30 days of immersion. The analysis began with doing Shapiro-Wilk normality test and then followed by Kruskal Wallis test because data distribution was abnormal. P value less than 0.05 shows statistically significant differences, while p value less than 0.01 shows very significant differences.

Results

Analysis results of LC/MS-MS show that all artificial saliva samples contained BPA, as shown at the descriptive analysis in figure number 2. As seen, the highest concentration of BPA was at VFR group, while the lowest was at Hawley self-cure retainer group. From duration of immersion, there was an increase of BPA concentration with straight comparison to the amount of duration of immersion, except at Hawley self cure retainer group where decreasing BPA concentration occurred at 7 days of immersion, but during 30 days of immersion the BPA concentration increased again.

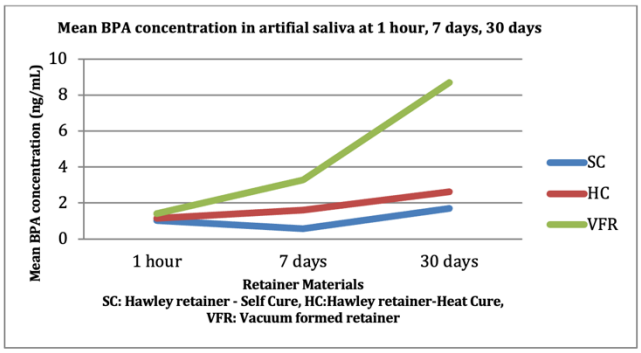

Figure 1. Mean BPA levels in artificial saliva at 1 hour, 7 days, and 30 days.

Hawley self-cure retainer, heat-cure retainer, and VFR materials comparison studies based on duration of immersion (1 hour, 7 days, 30 days) can be seen at table 1. For 1 hour of immersion, there is no significant difference of BPA concentration between all three retainer materials. However, during 7 and 30 days of immersion there were significant differences, where the most significant differences of BPA concentration occurred during the 30 days of immersion ($p<0.01$).

Duration of immersion	Group	N	BPA concentration		95% CI		P values*
			Mean (ng/mL)	SD (ng/mL)	Lower	Upper	
1 hour	**SC**	7	1.03	0.43	0.64	1.43	0.27
	HC	7	1.14	0.21	0.95	1.33	
	VFR	7	1.36	0.36	0.98	1.74	
7 days	**SC**	7	0.54	0.22	0.34	0.74	0.03*
	HC	7	1.64	0.95	0.77	2.51	
	VFR	6	3.28	1.85	1.34	5.22	
30 days	**SC**	7	1.69	0.54	1.19	2.19	0.00*
	HC	7	2.63	0.49	2.18	3.09	
	VFR	7	9.05	2.80	6.11	11.98	

Table 1. Comparison results of BPA concentration of Hawley self cure retainer, heat cure retainer and VFR base on duration of immersion using Kruskal Wallis test.

Discussion

BPA is an industrial chemical compound that is widely used in plastic materials that can be found every day because of its light weight, not easy to break, and low production costs. For this reason, BPA is often added to orthodontic materials, including retainers.[4,5]

According to the US Environmental Protection Agency (US-EPA), BPA is an endocrine disrupting chemical (EDC).[12] In addition, the release of BPA in low doses over a long period of time is also believed to cause adverse health effects.[13] Therefore, the release of BPA from orthodontic retainer material is of concern to researchers, since retainers are used for a long time.

Evaluation of the concentration of BPA in this study was carried out by analyzing the immersion of the self-cure Hawley retainer, heat-cured, and VFR material in artificial saliva. This study was conducted in vitro where the retainer material was immersed in artificial saliva, and the treatment was adjusted to the oral environment. In accordance with the observations of Söderholm et al., more ion release occurred when the composite resin was immersed in artificial saliva than in water. This was also done to resemble the actual environment in the mouth so that the results obtained were not biased.[14]

All artificial saliva samples in this study showed a certain amount of BPA content (Table 1). Artificial saliva has almost the same composition as saliva in the oral cavity. Saliva consists of mostly water (99%) and a small part of protein and inorganic substances (1%).[15] Water is the main factor that causes biodegradation. When the polymer material is immersed in water, the water will diffuse into the polymer material, causing the release of the weakest monomers, including monomers that are not completely polymerized and additive components such as BPA. The composition of monomers forming a chemical structure is a factor that affects the resistance of the material to the degradation process. Meanwhile, the level of BPA released from the retainer material depends on the chemical solvent, the pH of the solution, and the immersion time.[8]

The Hawley retainer consists of an acrylic

base and a VFR of thermoplastic polyethylene terephthalate (PET) which is a polymer material. Based on the ADA, there are two types of acrylic resins, namely self-curing where the polymerization uses a chemical reaction and heat curing where the polymerization uses heat. Furthermore, there are two types of polymer structures, namely amorphous and crystalline structures. The polymer chains are related to one another to form an irregular pattern in the amorphous structure, while the crystalline structure has the polymer chains forming an orderly arrangement. [8,16]

The highest concentration of BPA in this study is in the VFR group, while the lowest is in the self-cure Hawley retainer group (Figure 1). This is because Polyethylene terephthalate (PET) in VFR has more amorphous structure and less crystalline structure so that it has softer properties.[17,18] Crystallinity is a factor that determines polymer properties, for example determining whether a polymer is more opaque or transparent, and softer or harder. One polymer material may have two structures, amorphous and crystalline with varying compositions.[16] Meanwhile, the acrylic resin on the Hawley retainer has a crystalline structure so that it has higher strength, stiffness, hardness, and melting temperature.[16] The higher the crystalline content of a polymer, the more difficult it is to break the polymer chain bonds, and this is advantageous because less amount of monomer is released.

Table 1 shows that there was no significant difference in the concentration of BPA between the three retainer materials after the one-hour immersion, while after 7 days and 30 days of immersion, there was a significant difference where the most significant difference in BPA concentration occurred at 30 days of immersion ($p<0.01$). This was due to the accumulation of BPA content that was released from the retainer material and the amount increased with the increase in immersion time. However, this is a drawback of this study, because it does not fully reflect the use of retainers in the oral cavity, where the patient can remove and reinstall the retainer device. Therefore, in an in vivo study, the opposite result was obtained where in that study the highest amount of BPA detected was within 1 hour after installing a retainer, namely in the VFR group, but the longer the release, the less BPA was found.[5,9]

The limitations of this study include the small number of samples so that the data distribution was not normal. In addition, the maximum immersion time in this study was 30 days so that the amount of BPA release longer than 30 days was unknown.

Conclusions

Based on the findings of this study, it can be concluded that all retainers, whether self-curing Hawley retainers, heat curing, and VFR, when immersed in artificial saliva will release BPA. There is a very significant difference between the BPA content of the three retainer groups after 7 and 30 days, with the highest total BPA concentration at VFR. However, the amount of BPA released from the three retainer materials was not more than 11.98 ng/mL. This amount is still far below the safe limit tolerance issued by the EFSA, which is 4 micrograms per kilogram of body weight per day (µg/kgBB/day).

Declaration of Interest

The authors report no conflict of interest.

References

1. Johnston CD, Littlewood SJ. Retention in orthodontics. *Br Dent J*. 2015;218(3):1-4.
2. Xian L, Ashari A, Mohamed AM, et al. Relapse in modified vacuum-formed and Hawley retainers for transverse expansion: A multicenter randomized control trial. *J Int Dent Med Res*. 2020;13(2):614-621.
3. Food I, Authorities S. BISPHENOL A - Current state of knowledge and future actions by WHO and FAO. *Int Food Saf Authorities Netw (INFOSAN)*. 2009;23(11):1-6.
4. Geens T, Aerts D, Berthot C, et al. A review of dietary and non-dietary exposure to bisphenol-A. *Food Chem Toxicol*. 2012;50(10):3725-3740.
5. Raghavan AS, Sathyanarayana HP, Kailasam V, Padmanabhan S. Comparative evaluation of salivary bisphenol A levels in patients wearing vacuum-formed and Hawley retainers: An in-vivo study. *Am J Orthod Dentofac Orthop*. 2017;151(3):471-476.
6. Pelourde C, Bationo R, Boileau M-J, Colat-Paros J, Jordana F. Monomer release from orthodontic retentions: An in vitro study. *Am J Orthod Dendofacial Orthop*. 2018;153(1):248-254.
7. Chen L, Suh B. Bisphenol A in Dental Materials: A Review. *JSM Dent*. 2013;1:1004-1008.
8. Bettencourt AF, Neves CB, Almeida MS De, et al. Biodegradation of acrylic based resins : A review. *Dent Mater*. 2010;6:171-180.
9. Kang Y, Kim J, Kim J, Won P, Nam J. Release of bisphenol A from resin composite used to bond orthodontic lingual retainers. 2011;140(6):779-789.
10. Paramita NA, Purbiati M, Ismaniati NA. Antibacterial Effects of Silver and Titanium Dioxide Nanoparticle Solutions on Streptococcus mutans on Thermoplastic Retainers. *J Int Dent Med Res*. 2019;12(3):907-911.
11. A D A. ADA Statement on Bisphenol A and Dental Materials.

Counc Sci Aff. 2013;(4):1-2.
12. Aschberger K, Castello P, Hoekstra E, Karakitsios S, Munn S, Sarigiannis D. *Bisphenol A and Baby Bottles : Challenges and Perspectives*. Italy; 2010.
13. Soriano S, Alonso-Magdalena P, Garcia-Arevalo M, Novials A, Muhammed S, Salehi A. Rapid insulinotropic action of low doses of bisphenol-A on mouse and human islets of Langerhans: role of estrogen receptor beta. *PLoS One*. 2012;7:311.
14. Soderholm J, Mukherjee R, Longmate L. Filler Leachability of Composites Stored in Distilled Water or Artificial Saliva. *Am Assoc Dent Res*. 1996;9(1):152-158.
15. Yoshizawa J, Schafer C, Schafer J, Farrell J, Paster B, Wong D. Salivary Biomarkers: Toward Future Clinical and Diagnostic Utilities. *Journals ASM*. 2013;26(4):781-791.
16. Anusavice K, Shen C, Rawls H. Phillips Science of Dental Materials. In: 12th Ed. St. Louis: Elsevier; 2013:475.
17. Silva GG, Valente ML da C, Bachmann L, dos Reis AC. Use of polyethylene terephthalate as a prosthetic component in the prosthesis on an overdenture implant ☆. *Mater Sci Eng C*. 2019;99(1):1341-1349.
18. Bach C, Dauchy X, Chagnon M, et al. Chemical migration in drinking water stored in polyethylene terephthalate (PET) bottles : a source of controversy. *jWatres*. 2012;46(3):571-583.