International Journal Dental and Medical Sciences Research (IJDMSR)
ISSN: 2393-073X Volume 3, Issue 12 (December- 2019), PP 35-40
www.ijdmsr.com

Bisphenol A Release from Composite Resins Measured In Vivo with Gas Chromatography

Raoul Bationo[1], Wendpoulomdé Aimé Désiré Kaboré[2], Soumaïla Konaté[3], Monique Lydie Beugré-Kouassi[4], Jean Bertin Beugré[4], Fabienne Jordana[5]

[1]*CHU de Bogodogo, 14 BP 371 Ouagadougou 14, Burkina Faso*
Université Félix Houphouët-Boigny, 01 BP V34 Abidjan 01, Abidjan, Côte d'Ivoire
[2]*UFR Sciences de la Santé, Université Joseph Ki-Zerbo, 03 BP 7021 Ouagadougou 03, Ouagadougou, Burkina Faso*
[3]*Direction de la Toxicologie, du Contrôle de l'Environnement et de l'Hygiène Publique, Laboratoire National de Santé Publique, 09 BP 24 Ouagadougou 09, Ouagadougou, Burkina Faso*
[4]*UFR d'Odonto-Stomatologie, Université Félix Houphouët-Boigny, 01 BP V34 Abidjan 01, Abidjan, Côte d'Ivoire*
[5]*UFR d'Odontologie de Nantes, 1 Place Alexis Ricordeau, BP 84215, 44042 Nantes cedex 1, France*
Service d'Odontologie, CHU Nantes, 1 Place Alexis Ricordeau, 44093 Nantes cedex 1, France
***Corresponding author: Raoul Bationo, CHU de Bogodogo, Ouagadougou, Burkina Faso*

ABSTRACT:- *Some of resinous materials are based on bisphenol A, which is used as a precursor of bisphenol A glycidyl dimethacrylate or bisphenol A dimethacrylate during the production of many composite resins. Bisphenol A is one of the most common environmental endocrine disruptors, having an estrogenic action from competitive binding of estrogen like polymer molecules to natural hormone receptors.*
The aim of this study was to assess in vivo bisphenol A releasing from orthodontic and dental restorative resins (Grengloo, Blugloo, Transbond XT, Transbond LR, Clearfil ES-2, Clearfil ES Flow, Filtek Supreme XTE). One hour after resins placement in mouth, non-stimulated saliva was collected from patients to be analyzed by gas phase chromatography and mass spectrometry. The limit of quantification was 0.01 µg/mL. No bisphenol A has been detected in the saliva samples. Triethylene glycol dimethacrylate was present in two saliva samples, namely Grengloo and Transbond LR. Bisphenol A might be present at concentrations below of the detection and quantification limits. It would therefore have been desirable to also assess bisphenol A amounts in patients' urines.

Keywords:- *Bisphenol A, gas phase chromatography and mass spectrometry, Light-cured composite resin, saliva*

I. INTRODUCTION

BPA has been extensively studied as one of the most common environmental endocrine disruptors, having an estrogenic action from competitive binding of estrogen like polymer molecules to natural hormone receptors [1].

The environment-water, air, and soil-can be a route of exposure to BPA, but the primary source of exposure to BPA for most people is through the diet. BPA in food and beverages accounts for the majority of daily human exposure. BPA can migrate into food from food and beverage containers with internal epoxy resin coatings and from consumer products made of polycarbonate plastic such as baby bottles, tableware, food containers, and water bottles [2]. The daily human consumption of BPA is less than 1 $mg.kg^{-1}$, and greater doses may lead to destructive adverse effects on the endocrine system, especially during fetal development [1, 3, 4].
Dental composites are complex mixed materials which generally consist of an organic polymerizable matrix, reinforcing fillers, which are mainly inorganic and a silane-coupling agent [5].

The conversion of monomer to polymer during polymerization which is termed as the degree of conversion is always not complete. The unreacted monomers elute from resin-based composites as a result of chemical biodegradation in the presence of liquids such as water, saliva, ethanol, methanol, acetonitrile and bacterial enzymes [6-8].

In the intraoral environment, these materials are exposed to extreme thermal changes, pH variances, mechanical erosion, and degradation occurrence from bacterial and salivary enzymes, which can cause BPA release [9, 10].
The presence of BPA in human saliva, urine, and blood after use of resinous restorative materials and pit-and-fissure sealants has been demonstrated [11-16].

For traditional and flowable resin composites used as lingual retainers, BPA release was confirmed in vivo as well [17], with the highest values in saliva measured immediately after polymerization.
The monomers also cause cytotoxicity [18-20], genotoxicity [21], mutagenicity [22] and toxic reactions to the reproductive system [23, 24]. It causes major cytotoxic reactions to the dental pulp and gingival fibroblasts [25-27].

BPA is never introduced intentionally in the composition of a composite resin. This compound can be a byproduct of degradation of bisphenol A-glycidyl methacrylate (Bis-GMA) or other components such as ethoxylated bisphenol A dimethacrylate (Bis-EMA), bis-dimethylaminopropyl (Bis-DMA), 2,2-bis-(4-(3-methacryloxypropoxy)phenyl)propane (Bis-PMA), and bisphenol a diglycidyl ether (BADGE) [11, 28].
There are some studies in the literature that have demonstrated the presence of BPA in human saliva, urine and blood after application of resin dental materials. The possibility of this chemical substance being absorbed systemically through the blood should be a concern to oral health care professionals [9, 10, 29].
Kang et al. [17] evaluated the release of BPA from a composite resin used to bond orthodontic lingual retainers, but no study has quantified the amount of BPA released either in vitro after different experimental periods or in vivo in the saliva and urine of patients after bonding of orthodontic brackets.
Based on these findings, the objectives of this study were to assess in vivo the levels of BPA in the saliva of patients after bracket bonding and after dental restoration placement from 7 contemporary composite materials.

II. METHODS

1. Clinical procedures

For standardization purposes, the same practitioner (RB) performed all procedures. Patients received either fixed orthodontic appliances or restorative dental treatment. All patients signed an informed consent and do not have any composite restoration and have not had any prior orthodontic treatment. Each treatment was performed in two different patients.

The composite materials used for the procedures are listed in Table 1. After placement resin was cured for 20 seconds by using BA Optima 10 LED Curing Light.

One hour after resins placement in mouth, 1 mL of non-stimulated saliva was collected from each patient to be analyzed.

Table 1: Specifications of resins used for study.

Product (Lot)	Resin matrix	Manufacturer
Clearfil Majesty ES-2 (4D0069)	Bis-GMA, Hydrophobic aromatic dimethacrylate Hydrophobic aliphatic dimethacrylate	Kuraray
Clearfil Majesty ES Flow (A60239)	TEGDMA, Hydrophobic aromatic dimethacrylate	
Grengloo (6623923)	TEGDMA, UDMA, HEMA, Bis-EMA6, GMA, EO-TMPTA, 3-trimethoxysilylpropyl methacrylate	Ormco
Blugloo (6556174)	UDMA, Bis-EMA6, GMA, EO-TMPTA, 3-trimethoxysilylpropyl methacrylate	
Transbond XT (N921496)	Bis-GMA, Bis-MEPP	3M
Transbond LR (N919866)	Bis-GMA, TEGDMA	
Filtek Supreme XTE (N879475)	Bis-GMA, UDMA, TEGDMA, Bis-EMA6, PEGDMA	

2. Analytical method

The monomers were extracted using solid phase extraction (NH_2 cartridge) and then analyzed by gas chromatograph mass spectrometer (Agilent 6890 Series – Agilent 7673). A capillary column 30 m in length, internal diameter of 320 µm, and film thickness of 0.25 µm was used with helium carrier gas at a flow rate of 1.2 mL per minute. The column temperature program was set as follows: initially, 80°C for 1 minute, increasing to 150°C at a rate of 20°C per minute, and then increasing to 280°C for 2 minutes at a rate of 10°C per minute. The temperature of the injector was 280°C, and the transfer line was 280°C. Mass spectra were obtained using electron impact ionization (69.9 eV, 34.6 µA, 230°C).

Data were acquired by selected ion monitoring mode and processed with the software MSD ChemStation.

The calibration curve and response factor were computed with reference BPA in different concentrations from 0.01 to 50 µg/mL with caffeine as internal standard. Linear correlation with efficiency of 0.996 was obtained between the amount of BPA and the corresponding peak area.

III. RESULTS AND DISCUSSION

Most resins used in dentistry contain BPA derivatives; for this reason, they have attracted the attention of dental researchers as an additional source of exposure to humans [13]. The release of BPA from composites may occur at 2 moments: during or just after resin placement, caused by incomplete monomer polymerization, and later, as a result of material degradation [14]. In the intraoral environment, these materials are exposed to extreme thermal changes, mechanical erosion, pH alterations, and enzymatic degradation from bacterial and salivary enzymes, which can cause BPA release. Incomplete polymerization of adhesive systems can also cause BPA release [15, 30, 31].

Some studies noted that 20-45% of monomer remains unpolymerized after curing and has the direct potential to leach into saliva [32-34].

In a study by Joskow et al. [13], which measured BPA amounts in saliva and urine using gas chromatography and high-resolution mass spectrometry, BPA was found in saliva immediately and 1 hour after placement of sealants.

The in-vivo assessment of BPA released from orthodontic adhesives was assessed in a study by Kang et al. [17] They evaluated the changes in BPA levels in saliva and urine before and after placing a lingual bonded retainer on the mandibular dentitions. Samples were obtained immediately before placement of the retainer and 30 minutes, 1 day, 1 week, and 1 month after placement. The only significant level of BPA was detected in the saliva collected immediately after lingual retainer placement.

In our study, no BPA has been detected in the saliva samples.

Triethylene glycol dimethacrylate (TEGDMA) was present in two saliva samples, namely Grengloo and Transbond LR. It is one of the most commonly released co-monomers by the composite resins. The eluted TEGDMA comes from un-polymerized molecules. It has been demonstrated [35, 36] that the amount of eluted TEGDMA decreases when a composite is subjected to a longer light irradiation.

Table 2 shows in vivo studies measuring BPA amounts released by resin composites and sealants in saliva.

Table 2: In vivo studies evaluating BPA amounts in saliva.

Author	**Human sample that was analyzed**	**Product Name**	**Time of analyses**	**BPA measurement method**	**Highest amount of BPA traced in saliva**
Olea et al., 1996 [16]	Saliva	Delton LC.	1 h before placement. 1 h after.	High performance liquid chromatography	30 mg/mL 1 h after placement.
Arenholt-Bindslev et al., 1999 [37]	Saliva	Delton LC. Visio-Seal.	Before placement. Immediately after. 1 h after. 24 h after.	High performance liquid chromatography	2.8 ppm for samples taken immediately after placement of Delton LC.
Fung et al., 2000 [11]	Saliva and blood	Delton LC.	Immediately before placement. 1 h after. 3 h after. 24 h after. 3 days after. 5 days after.	High- pressure liquid chromatography	105.6 ppb at 1 and 3 h after placement.
Joskow et al., 2006 [13]	Saliva and urine	Delton LC. Helioseal.	Before placement. Immediately after placement. 1 h after. 24 h after.	Gas chromatography and high-resolution mass spectrometry	42.8 ng/mL in Delton LC group immediately after placement.
Zimmerman-Downs et al.,	Saliva and blood	Delton LC.	1 h before placement.	BPA Elisa Kit	At all post-treatment time:

2010 [38]			1 h after. 3 h after 24 h after		3.98 ng/mL in low-dose group; 9.08 ng/mL in high-dose group.
Kang et al., 2011 [17]	Saliva and urine	Filtek Flow. Z250.	Before placement. Immediately after placement. 1 day after. 1 week after. 1 month after.	Liquid chromatography/mass spectrometry	5.042 ng/mL immediately after placement.
Han et al., 2012 [10]	Saliva	Not given.	Survey study. Not interventional sealant placement	BPA Elisa Kit	8.305 mg/L

Concerning the BPA safety issues, the European Food Safety Authority announced an initial risk assessment, based on a tolerable daily intake (TDI) of 50 µg/kg body weight/day [39]. Several scientists arguably disputed the use of TDI for risk assessments on endocrine disruptor chemicals, suggesting that their effects are observed at very low doses, non-monotonic dose-response curves, as well as on effects occurring from very specific windows of exposure [40].

The amount of BPA released from resin composites has been assessed in many studies [41]. Although much lower than the TDI, the 24-h release of BPA from dental materials was pertinent in patients with multiple or large restorations, representing a significant source of BPA in such patients [41].

Even though the patient may come in contact with considerable amounts of unpolymerized monomers during the placement of composites, the release of uncured monomers after polymerization has been postulated to cause most of the unwanted effects [42].

The aqueous environment of the oral cavity, encouraging chemical degradation and softening, and therefore corrosion-wear, is a critical factor that alters the mechanical properties of resin composites [43].
The salivary composition and the degradation of the material may be affected by physiologic variables such as time of collection of saliva, diet, and salivary flow rate [44].

IV. CONCLUSION

Although BPA is not used by itself as a raw material in composite resins, it is likely to be present as an impurity from the synthesis process [29, 41].

No BPA amount was detected in saliva but this compound might be present at concentrations below of the detection and quantification limits.

The usual biological matrix used to characterize exposure to BPA is urine [45]. It would therefore have been desirable to also assess BPA amounts in patients' urines.

ACKNOWLEDGEMENTS

The GC-MS analyses were performed to the Laboratoire National de Santé Publique (LNSP) of Ouagadougou.

CONFLICT OF INTEREST

The authors declare no potential conflicts of interest.

REFERENCES

[1]. Gao H, Yang B, Li N, Feng L, Shi X, Zhao W, et al. Bisphenol A and hormone-associated cancers: current progress and perspectives. Medicine 2015;94: e132.

[2]. Howe SR, Borodinsky L, Lyon SR. Potential exposure to Bisphenol A from food contact use of epoxy coated cans. J Coatings Technol 1998;70: 69-74.

[3]. Soto AM & Sonnenschein C. Environmental causes of cancer: endocrine disruptors as carcinogens. Nat Rev Endocrinol 2010;7: 363-370.

[4]. Frye C, Bo E, Calamandrei G, Calzà L, Dessì-Fulgheri F, Fernández M, et al. Endocrine disrupters: a review of some sources, effects and mechanisms of actions on behaviour and neuroendocrine systems. J Neuroendocrinol 2012;24: 144-159.

[5]. Craig RG. Restorative dental materials. Chap 9. 11th edition, United States, Mosby Inc; 2002.
[6]. Ferracane JL & Condon JR. Rate of elution of leachable components from composite. Dent Mater 1990;6: 282-287.
[7]. Hegde MN, Bhat GT, Nagesh SC. Release of monomers from dental composite materials - an in vitro study. Int J Pharm Pharmaceut Sci 2012;4: 500-504.
[8]. Manojlovic D, Radisic M, Vasilijevic T. Monomer elution from nanohybrid and ormocer- based composites cured with different light sources. Dent Mater 2011;27: 371-378.
[9]. Moreira MR, Matos LG, De Souza ID, Brigante TA, Queiroz ME, Romano FL et al. Bisphenol A release from orthodontic adhesives measured in vitro and in vivo with gas chromatography. Am J Orthod Dentofac Orthop 2017;151: 477-483.
[10]. Han DH, Kim MJ, Jun EJ, Kim JB. Salivary bisphenol-A levels due to dental sealant/resin: A case-control study in Korean children. J Korean Med Sci 2012;27: 1098-1104.
[11]. Fung EY, Ewoldsen NO, St Germain HA Jr, Marx DB, Miaw CL, Siew C, et al. Pharmacokinetics of bisphenol A released from a dental sealant. J Am Dent Assoc 2000;131: 51-58.
[12]. Kingman A, Hyman J, Masten SA, Jayaram B, Smith C, Eichmiller F, et al. Bisphenol A and other compounds in human saliva and urine associated with the placement of composite restorations. J Am Dent Assoc 2012;143: 1292-1302.
[13]. Joskow R, Barr DB, Barr JR, Calafat AM, Needham LL, Rubin C. Exposure to bisphenol A from bis-glycidyl dimethacrylate-based dental sealants. J Am Dent Assoc 2006;137: 353-362.
[14]. Pulgar R, Olea-Serrano MF, Novillo-Fertrell A, Rivas A, Pazos P, Pedraza V, et al. Determination of bisphenol A and related aromatic compounds released from bis-GMA-based composites and sealants by high performance liquid chromatography. Environ Health Perspect 2000;108: 21-27.
[15]. Goldberg M, Dimitrova-Nakov S, Schmalz G. BPA from dental resin material: where are we going with restorative and preventive dental biomaterials? Clin Oral Investig 2014;18: 347-349.
[16]. Olea N, Pulgar R, Pérez P, Olea-Serrano F, Rivas A, Novillo-Fertrell A, et al. Estrogenicity of resin-based composites and sealants used in dentistry. Environ Health Perspect 1996;104: 298-305.
[17]. Kang YG, Kim JY, Kim J, Won PJ, Name JH. Release of bisphenol A from resin composite used to bond orthodontic lingual retainers. American Journal of Orthodontics and Dentofacial Orthopedics 2011;140: 779-789.
[18]. Ahmed RH, Aref MI, Hassan RM, Mohammed NR. Cytotoxic effect of composite resin and amalgam filling materials on human labial and buccal epithelium. Nature and Science 2010;8: 48-53.
[19]. Kehe K, Reichl FX, Durner J, Walther U, Hickel R, Forth W. Cytotoxicity of dental composite components and mercury compounds in pulmonary cells. Biomaterials 2001;22: 317-322.
[20]. Ratanasathien S, Wataha JC, Hanks CT, Dennison JB. Cytotoxic interactive effects of dentin bonding components on mouse fibroblasts. J Dent Res 1995;74: 1602-1606.
[21]. Durner J, Debiak M, Burkle A. Induction of DNA strand breaks by dental composite components compared to Xray exposure in human gingival fibroblasts. Arch Toxicol 2011;85: 143-148.
[22]. Schweikl H, Hiller KA, Bolay C, Kreissl M, Kreismann W, Nusser A et al. Cytotoxic and mutagenic effects of dental composite materials. Biomaterials 2005;26: 1713-1719.
[23]. Michelsen VB, Moe G, Strom MB, Jensen E, Lygre H. Quantitative analysis of TEGDMA and HEMA eluted into saliva from two dental composites by use of GC/MS and tailormade internal standards. Dent Mater 2008;24: 724-731.
[24]. Miletic V, Santini A, Trkulja I. Quantification of monomer elution and carbon–carbon double bonds in dental adhesive systems using HPLC and micro-Raman spectroscopy. J Dent 2009;37: 177-184.
[25]. Issa Y, Watts DC, Brunton PA, Waters CM, Duxbury AJ. Resin composite monomers alter MTT and LDH activity of human gingival fibroblasts in vitro. Dent Mater 2004;20: 12-20.
[26]. Michelsen VB, Kopperud HB, Lygre GB, Bjorkman L, Jensen E, Kleven IS et al. Detection and quantification of monomers in unstimulated whole saliva after treatment with resin-based composite fillings in vivo. Eur J Oral Sci 2012;120: 89-95.
[27]. Moharamzadeh K, Van Noort R, Brook IM, Scutt AM. Cytotoxicity of resin monomers on human gingival fibroblasts and HaCaT keratinocytes. Dent Mater 2007;23: 40-44.
[28]. McKinney C, Rue T, Sathyanarayana S, Martin M, Seminario AL, DeRouen T. Dental sealants and restorations and urinary bisphenol A concentrations in children in the 2003-2004 National Health and Nutrition Examination Survey. J Am Dent Assoc 2014;145: 745-750.
[29]. Fleisch AF, Sheffield PE, Chinn C, Edelstein BL, Landrigan PJ. Bisphenol A and related compounds in dental materials. Pediatrics 2010;126: 760-768.
[30]. Sunitha C, Kailasam V, Padmanabhan S, Chitharanjan AB. Bisphenol A release from an orthodontic adhesive and its correlation with the degree of conversion on varying light-curing tip distances. Am J Orthod Dentofacial Orthop 2011;140: 239-244.

[31]. Gioka C, Bourauel C, Hiskia A, Kletsas D, Eliades T, Eliades G. Light-cured or chemically cured orthodontic adhesive resins? A selection based on the degree of cure, monomer leaching, and cytotoxicity. Am J Orthod Dentofacial Orthop 2005;127: 413-419.
[32]. Komurcuoglu E, Olmez S, Vural N. Evaluation of residual monomer elimination methods in three different fissure sealants in vitro. Journal of Oral Rehabilitation 2005;32: 116-121.
[33]. Sasaki N, Okuda K, Kato T, et al. Salivary bisphenol-A levels detected by ELISA after restoration with composite resin. Journal of Materials Science Materials in Medicine 2005;16: 297-300.
[34]. Rueggeberg FA, Dlugokinski M, Ergle JW. Minimizing patients' exposure to uncured components in a dental sealant. Journal of the American Dental Association 1999;130: 1751-1757.
[35]. Fadini L, Brambilla R, Cagetti MG, Gagliani M : G It Cons 2006 Suppl vol lV n.1, January-March
[36]. Polydorou O, König A, Hellwig E, Kümmerer K. Long-term release of monomers from modern dental-composite materials. Eur J Oral Sci 2009;117: 68-75.
[37]. Arenholt-Bindslev D, Breinholt V, Preiss A, Schmalz G. Time-related bisphenol-A content and estrogenic activity in saliva samples collected in relation to placement of fissure sealants. Clinical Oral Investigations 1999;3: 120-125.
[38]. Zimmerman-Downs JM, Shuman D, Stull SC, Ratzlaff RE. Bisphenol A blood and saliva levels prior to and after dental sealant placement in adults. Journal of Dental Hygiene 2010;84: 145-150.
[39]. EFSA – European Food Safety Authority. Opinion of the scientific panel on food additives, flavourings, processing aids and materials in contact with food on a request from the commission related to 2,2-bis(4-hydroxyphenyl) propane (Bisphenol A). Question number EFSA-Q-2005-100. The EFSA Journal 2006;428: 1-75.
[40]. ANSES – French Agency for Food, Environmental and Occupational Health & Safety. Opinion of 29 January 2010 of the French Agency for Food Safety on the critical analysis of the results of a toxicity study on the development of the nervous system as well as other recently published data on the toxic effects of bisphenol A. Paris: ANSES; 2010.
[41]. Van Landuyt KL, Nawrot T, Geebelen B, De Munck J, Snauwaert J, Yoshihara K, et al. How much do resin-based dental materials release? A meta-analytical approach. Dental Materials 2011;27: 723-747.
[42]. Mohsen NM, Craig RG, Hanks CT. Cytotoxicity of urethane dimethacrylate composites before and after aging and leaching. Journal of Biomedical Materials Research 1998;39: 252-260.
[43]. Cilli R, Pereira JC, Prakki A. Properties of dental resins submitted to pH catalysed hydrolysis. Journal of Dentistry 2012;40: 1144-1150.
[44]. Navazesh M. Methods for collecting saliva. Ann N Y Acad Sci 1993;694: 72-77.
[45]. CDC, 2009. Fourth National Report on Human Exposure to Environmental Chemicals. Department of Health and Human Services: Centers for Disease Control and Prevention, National Center for Environmental Health, Atlanta, Georgia (Available on http://www.cdc.gov/exposurereport)

Corresponding author: Raoul Bationo,
CHU de Bogodogo, Ouagadougou, Burkina Faso