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ORIGINAL ARTICLE

Release of bisphenol A and its derivatives from orthodontic adhesive systems available on the European market as a potential health risk factor

Konrad Małkiewicz[1], Jadwiga Turło[2], Anna Marciniuk-Kluska[3], Kinga Grzech-Leśniak[4], Magdalena Gąsior[4], Mariusz Kluska[5]

[1] *Department of Orthodontics, Medical University of Warsaw, Poland*
[2] *Department of Drug Technology and Pharmaceutical Biotechnology, Medical University of Warsaw, Poland*
[3] *Faculty of Management, Siedlce University of Natural Sciences and Humanities, Poland*
[4] *Lubuski College of Public Health, Zielona Góra, Poland*
[5] *Institute of Chemistry, Siedlce University of Natural Sciences and Humanities, Poland*

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Abstract

Introduction. Treatment with fixed orthodontic appliances requires the application of adhesive systems to enable secure fastening of brackets and retainers to the surface of tooth enamel. The orthodontic bonding systems are similar in terms of chemical composition to dental filling materials, the chemical stability of which is not satisfactory. Particularly alarming is the release of bisphenol A and its derivatives to the external environment, which has been well-documented for materials used in conservative dentistry.

Objectives. The aim of the study was an *in vitro* assessment of the release of biologically harmful bisphenol A and its derivatives from orthodontic adhesives available on the European market, as a potential health risk factor for orthodontic patients.

Material and methods. The study assessed levels of BPA, BPA polymers and Bis-GMA resin in eluates of six commonly used orthodontic adhesives: Light Bond, Transbond XT, Resilence, Aspire, GrěnGloo and ConTec LC, obtained after one hour, 24 hours, 7 days and 31 days of material sample storage in water. The presence and concentration of the studied chemicals in the obtained solutions were identified using the HPLC method.

Results. The highest ($p \leq 0.05$) concentration of BPA at 32.10µg/ml was observed in the Resilence material eluates. The highest concentration of poly-bisphenol A was found in solutions obtained after incubation of ConTec LC adhesive at 371.90µg/ml, whereas the highest amount of Bis-GMA resin (425.07µg/ml) was present in Aspire material eluates.

Conclusions. 1) In conditions of the current experiment it was demonstrated that most of the assessed orthodontic adhesive resins available on the European market and released into the outside environment – biologically harmful bisphenol A or its derivatives, posing a potential threat to the patients' health. 2) Release of BPA and its derivatives into aqueous solutions is the highest in the early stages of sample incubation.

Key words

bisphenol A, orthodontic adhesives, HPLC, health risk factors

INTRODUCTION

Orthodontic treatment with fixed appliances requires the application of materials securing effective fastening of brackets to the tooth enamel. With the development of adhesive technologies, orthodontic adhesives polymerized with visible light in the clinical conditions are used more and more commonly, replacing systems where bonding proceeded through a chemical reaction initiated by mixing base paste with a catalyst. Orthodontic adhesives are also used to attach fixed retainers to the surfaces of teeth. While the positioning of resin between the bracket base and enamel makes it less accessible in the oral environment, the whole mass of adhesive used to attach a retainer is affected by the oral environment [1].

Light-polymerized orthodontic adhesive systems structurally resemble the composite materials used in conservative dentistry to restore lost tooth tissue. The properties of composite materials result from two major components used in their production, namely, inorganic fillers, such as silica, barium-aluminum glass, or powdered ceramics and an organic matrix made of oligomers and monomers, of which the most frequently used include: glycidyl methacrylate (BIS-GMA), DEGMA 2-(2-hydroxyethoxy) ethyl methacrylate, triethyleneglycol dimethacrylate (TEGDMA), or urethane dimethacrylate (UDMA). In addition, composite materials also contain dyes as well as polymerization inhibitors and catalysts [2].

The compounds constituting organic matrix of composite materials are not biologically inert substances. Numerous studies conducted in the laboratory involving tests on

Address for correspondence: Konrad Małkiewicz, Department of Orthodontics, Medical University of Warsaw, Nowogrodzka 59, 02-006 Warsaw, Poland
E-mail: konrad.malkiewicz@interia.pl

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animals have shown that the compounds have cytotoxic and mutagenic potential, and that they are potent allergens. Some of them also indicate para-hormonal action by activating receptors for the estrogen group of hormones. Unfortunately, it has also been confirmed that polymers used for reconstruction of hard tissues of teeth are not completely stable chemically after the cross-linking reaction, and emit potentially harmful components to the external environment [3]. Two more phenomena contribute to this: incomplete polymerization [4] of composite materials, as well as their secondary decomposition under the influence of physical and chemical agents present in the oral environment [5].

Among the potentially harmful bio-chemicals released from polymers used in the oral environment, bisphenol A deserves special attention – it is a decomposition product from Bis-GMA which is widely-used in the manufacture of composite resin fillings. Studies evaluating the effect of BPA on living organisms have shown its adverse effects on the reproductive system of laboratory animals, and confirmed the hypothesis that exposure of pregnant females to this compound may cause foetal birth defects, as well as damage to the nervous system and behavioural changes in animals born during the experiments [6–8].

The results of studies documenting potential biological harmfulness of BPA caused legislative changes, both in individual countries and international organizations. Canada was the first country to qualify BPA as a toxic substance. France and Denmark have introduced legislation banning the use of BPA in the production of bottles for feeding children. Legal regulations in force in the above-mentioned countries also prevent the import and sale of such products. In addition, Danish legislation disallows the use of bisphenol A and its derivatives in production processes of any articles that have contact with food for children up to three years of age. Similar legislation has also been introduced in Australia and some States of the USA.

The presence of BPA in composition of materials that are in contact with food is allowed in the European Union under European Commission Directive 2002/72/EC of 6 August 2006. The exception are baby feeding bottles, in which the presence of bisphenol A has been legally prohibited since March 2011 [9]. It has been proved that BPA released from plastic bottles easily penetrates into the baby's body [10].

Despite many studies documenting harmful biological effects of bisphenol A, it is widely-used for the production of epoxy and polycarbonate resins used in manufacturing of food storage containers, eyeglass lenses, water bottles and medical materials. Bisphenol A is also an essential component of Bis-GMA resin, constituting up to 70% weight of materials used as enamel and dental fillings [11]. Many published studies have confirmed the cytotoxicity of Bis-GMA resin in tissue cultures. It has also been demonstrated that the compound has mutagenic properties and causes damage to the structure of cell nuclei [12, 13].

Objectives. The aim of the study was an assessment – conducted in *in vitro* conditions – of the release of biologically harmful bisphenol A and its derivatives from orthodontic adhesive systems available on the European market, as a potential health risk factor for orthodontic patients.

MATERIALS AND METHOD

The study evaluated the release of bisphenol A, poly-bisphenol A and Bis-GMA resin from six orthodontic adhesives based on light-cured polymers: Light Bond (Reliance, USA), Transbond XT (3M, USA) Resilence (Ortho Technology, USA), Asphire (Ortho Classic, USA), GrěnGloo (Ormco, USA), ConTec LC (Dentaurum, Germany).

Samples of the evaluated materials were prepared in teflon matrices 5mm in diameter and 2mm deep, washed in HPLC grade methanol and water (Sigma Aldrich, USA). Orthodontic adhesive resins were polymerized with the use of an LED 55 lamp (TPC Advanced Technology, USA) for 20 seconds at 1200mW/cm2. Seven samples of each evaluated material were prepared. The samples were then removed from the matrices and placed in individual, sterile glass containers with 10 ml of HPLC grade water (Sigma Aldrich, USA) and 0.1 ml of Antibiotic Antimycotic preparation (Gibco, USA), used to inhibit the growth of microorganisms. The containers were placed in an incubator shaker oscillating at 20 cycles per minute at a constant temperature of 36 °C. After one-hour incubation, the eluates were retrieved from the above the samples, the containers were rinsed with HPLC grade water, dried in a stream of compressed air, and the samples again poured over with 10 ml of HPLC grade water with 0.1ml of AA poly-antibiotic mixture. The described procedure was repeated after 24 hours, 7 days and 30 days of observation, in each case collecting eluates from dental materials. The solutions were frozen at -18 °C immediately after collection from the containers to prevent secondary chemical reactions of the compounds released from dental materials.

After 30-day observation the eluates were thawed and analyzed for presence of BPA, BPA polymers and Bis-GMA by means of high performance liquid chromatography (HPLC).

Chromatographic measurements were performed with a SPD-6A liquid chromatograph (Shimadzu, Kyoto, Japan) equipped with a LC-6A gradient pump, UV detector, a Rheodyne sampling valve (Berkeley, CA, USA), model 7125, with a 20µl sample loop, and a Shimadzu C-R6A data recorder. The obtained chromatogram describing the presence of identified compounds was processed with the use of OriginPro 7.5 program (OriginLab Corporation, Northampton, USA).

The results, expressed in µg/ml, describing concentration of bisphenol A, poly-bisphenol A and Bis-GMA in solutions obtained after incubation of the assessed orthodontic adhesives and filling materials, were input to Microsoft Excel 2000 spreadsheet (Microsoft, USA), and after extracting data sets, they were analyzed using Statistica 8.0 programme (StatSoft, Poland). In the process of statistical hypothesis testing, the significance level of $\alpha = 0.05$ was assumed. The tool used for analysis of means was ANOVA single-factor analysis of variance model, and for multiple-choice testing, the Newman-Keuls test and the Tukey's test were used.

RESULTS

Of the six evaluaed orthodontic materials, the presence of bisphenol A was confirmed in eluates of one of them. In all observation periods, BPA was detected in solutions originating from Resilence adhesive samples which were stored in water. The compound was not observed in eluates originating from incubation of the other dental polymers.

Significantly ($p \leq 0.05$) the highest concentration of bisphenol A at 32.1μg/ml was observed in Resilence material eluates obtained after 1 hour of sample storage in water. Significantly ($p \leq 0.05$) lower concentrations were detected in solutions after 24-hour, 7-day and 31-day observation, and were respectively, 8.4μg/ml, 6.4μg/ml and 1.7μg/ml. Data analysis showed that the amount of BPA released into the external environment during the first hour was significantly higher than the amounts emitted in the subsequent observation periods.

Figures describing the mean and limiting concentrations of bisphenol A identified in the eluates of the assessed dental materials at different periods of observation are shown in Table 1.

Bisphenol A polymers were identified in eluates from three evaluated orthodontic adhesives: Resilence, Light Bond and ConTec LC. Significantly the highest ($p \leq 0.05$) concentration of poly-BPA at the level of 371.9μg/ml was observed in solutions obtained after 1 hour storage in water of ConTec LC samples. In eluates obtained after one hour of observation of Resilence and Light Bond adhesives, BPA polymers' concentrations were respectively 9.24μg/ml and 28.63μg/ml.

Concentrations of BPA polymers released from these materials were significantly the highest after 1 hour compared with identifiable quantities after other periods of observation.

Values of average and limiting concentrations of poly-BPA detected in eluates of the assessed orthodontic adhesives after 1 hour, 24 hours, 7 days and 31 days of sample storage in water are shown in Table 2.

Analysis of the solutions obtained after storage of orthodontic adhesive samples in the aqueous environment demonstrated the presence of Bis-GMA only in eluates of the Aspire material. Significantly, the highest concentration of the identified compound, compared to other periods of observation, was detected in samples obtained after 1 hour incubation at 425.0μg/ml. After 24 hours, 7 days and 31 days of observation, Bis-GMA concentration in Aspire eluates amounted to 198.84μg/ml, 68.91μg/ml and 14.94μg/ml, respectively.

Figures describing the mean and limiting concentrations of Bis-GMA resin identified in eluates of the assessed dental materials at subsequent periods of observation are shown in Table 3.

DISCUSSION

In the available literature there are very few reports describing research on the chemical stability of orthodontic adhesive resins. In contrast to composite restorative materials, which undoubtedly release potentially harmful chemical compounds into the external environment, it is assumed that orthodontic adhesives are biologically safe. This belief is justified by the argument that in clinical conditions adhesive resins, compared to the mass of the filling, are applied in relatively small quantities, and additionally they are located between the base of the bracket and tooth enamel, which minimizes their contact with the external environment. However,

Table 1. Values of average and limiting concentrations of BPA detected in eluates of the assessed orthodontic adhesives after one hour, 24 hours, 7 days and 31 days of sample storage in water

Material	BPA concentrations according to observation period											
	1 hour			24 hours			7 days			31 days		
	mean concentration μg/ml	range μg/ml	SD	mean concentration μg/ml	range μg/ml	SD	mean concentration μg/ml	range μg/ml	SD	mean concentration μg/ml	range μg/ml	SD
Light Bond	0.00	#	0.00	0.00	#	0.00	0.00	#	0.00	0.00	#	0.00
Transbond XT	0.00	#	0.00	0.00	#	0.00	0.00	#	0.00	0.00	#	0.00
Resilence	32.10	28.90 – 37.30	3.10	8.40	6.90 – 12.40	1.94	6.40	2.20 – 10.40	2.47	1.71	0.90 – 2.40	0.55
Aspire	0.00	#	0.00	0.00	#	0.00	0.00	#	0.00	0.00	#	0.00
GrěnGloo	0.00	#	0.00	0.00	#	0.00	0.00	#	0.00	0.00	#	0.00
ConTec LC	0.00	#	0.00	0.00	#	0.00	0.00	#	0.00	0.00	#	0.00

Table 2. Values of average and limiting concentrations of polymers of BPA detected in eluates of the assessed orthodontic adhesives after one hour, 24 hours, 7 days and 31 days of sample storage in water

Material	poly-BPA concentrations according to observation period											
	1 hour			24 hours			7 days			31 days		
	mean concentration μg/ml	range μg/ml	SD	mean concentration μg/ml	range μg/ml	SD	mean concentration μg/ml	range μg/ml	SD	mean concentration μg/ml	range μg/ml	SD
Light Bond	28.63	23.10 – 37.30	4.71	8.84	6.10 – 13.20	2.95	1.96	1.10 – 3.90	0.97	1.36	0.20 – 3.20	1.07
Transbond XT	0.00	#	0.00	0.00	#	0.00	0.00	#	0.00	0.00	#	0.00
Resilence	9.24	8.40 – 10.60	0.92	4.09	2.50 – 7.60	1.76	2.07	0.50 – 2.60	0.73	1.10	0.20 – 1.70	0.51
Aspire	0.00	#	0.00	0.00	#	0.00	0.00	#	0.00	0.00	#	0.00
GrěnGloo	0.00	#	0.00	0.00	#	0.00	0.00	#	0.00	0.00	#	0.00
ConTec LC	371.90	355.60 – 417.20	23.27	152.09	146.70 – 163.20	6.31	59.13	55.70 – 66.20	3.65	9.26	6.70 – 13.20	2.49

Table 3. Values of average and limiting concentrations of Bis-GMA detected in eluates of the assessed orthodontic adhesives after one hour, 24 hours, 7 days and 31 days of sample storage in water

Material	Bis-GMA concentrations according to observation period											
	1 hour			24 hours			7 days			31 days		
	mean concentration µg/ml	range µg/ml	SD	mean concentration µg/ml	range µg/ml	SD	mean concentration µg/ml	range µg/ml	SD	mean concentration µg/ml	range µg/ml	SD
Light Bond	0.00	#	0.00	0.00	#	0.00	0.00	#	0.00	0.00	#	0.00
Transbond XT	0.00	#	0.00	0.00	#	0.00	0.00	#	0.00	0.00	#	0.00
Resilence	0.00	#	0.00	0.00	#	0.00	0.00	#	0.00	0.00	#	0.00
Aspire	425.07	379.20 – 487.50	31.85	198.84	185.80 – 227.20	17.38	68.91	55.10 – 87.20	10.17	14.94	13.80 – 17.20	1.40
GrěnGloo	0.00	#	0.00	0.00	#	0.00	0.00	#	0.00	0.00	#	0.00
ConTec LC	0.00	#	0.00	0.00	#	0.00	0.00	#	0.00	0.00	#	0.00

this assumption seems to be too optimistic. Observations suggest that in practice, excess orthodontic adhesive that escapes from the bracket base during its positioning on the tooth surface, is not completely removed prior to resin polymerization. There are also doubts about the cross-linking process of orthodontic adhesives polymerized with visible light. During the polymerization process, they are located under the bracket base, so that access of light to the resin is provided only by transillumination through the enamel tissue. It should also be noted that orthodontic adhesive resins used for attaching fixed retainers on tooth surfaces are directly exposed to the complex oral environment.

The phenomenon of basic monomer release from polymerized orthodontic adhesive to the external environment was described by Eliades et al [14] in an article published in 1995.

Eliades et al [14] reported release of Bis-GMA resin from Transbond orthodontic adhesive samples (3M, USA). The authors polymerized the material with visible light and then extracted it in 75% aqueous ethanol solution for 15 days. The presence of Bis-GMA in eluates of the assessed orthodontic adhesive was confirmed by the high performance liquid chromatography method (HPLC). The above results demonstrate the chemical instability of orthodontic adhesives. In the presented study, Transbond material's chemical stability was also evaluated, but no Bis-GMA molecules were observed in eluates of the tested orthodontic adhesive. Perhaps the differences in the results obtained are due to the fact that Eliades et al. [14] used 75% ethanol for elution of the adhesive resin's components, which is a strong plasticizer intensifying disintegration of dental composite materials. Water, in which the evaluated orthodontic adhesives were stored in the present study, has much less potential for dental polymer degradation than alcohol, therefore Tranbond material's structure could remain intact, and Bis-GMA resin was not released into the external environment.

In the presented study, the presence of Bis-GMA resin in eluates of the Aspire material in four consecutive observation periods, i.e. after 1 hour, 24 hours, 7 days and 30 days of sample storage in water, was detected. This confirms that orthodontic adhesives can release Bis-GMA molecules into the external environment, and the applied analytical method is sufficiently sensitive to detect this phenomenon.

To-date, few studies have been published on the evaluation of the phenomenon of bisphenol A – a decomposition product of Bis-GMA resin – release from orthodontic adhesive resins.

The hypothesis of BPA release from chemically-cured Rely-a-Bond (Reliance, USA) orthodontic adhesive was re-examined in the study by Eliades et al [15] conducted in 2007. The authors evaluated the release of bisphenol A from Rely-a-Bond samples from samples of another Reliance adhesive (light cured), the name of which was not mentioned. The authors stored samples in 99% ethanol, analyzing materials' eluates after 1 day, 7 days, 21 days and 35 days of incubation. Eliades et al. [15] ruled out the presence of bisphenol A in the solutions and presented a hypothesis that orthodontic adhesives do not release potentially harmful BPA.

However, the quoted authors [15] changed their position in 2011, publishing an article [16] describing the identification of bisphenol A in eluates from Transbond XT orthodontic adhesive (3M, USA), polymerized with visible light. Eliades et al. [16] detected BPA in aqueous solutions obtained after 10, 20 and 30 days of sample storage, using gas chromatography and mass spectrometry GC/MCLS. The authors described BPA presence in the eluates at 0.0029µg/ml concentrations.

Research by Eliades et al. in 2011 [16] confirms BPA release from orthodontic adhesive resins. In the presented study, bisphenol A was observed in the Resilence adhesive eluates at concentrations of 32.10µg/ml after 1 hour, 8.40µg/ml after 24 hours, 6.40µg/ml after 7 days, and 1.71µg/ml after 30 days of sample incubation in water. Although it is not possible to directly compare the results of both studies in the qualitative (different types of adhesives were assessed) and quantitative context (different research methods were used to identify the presence and concentration of BPA), the results indicate the need for further research on the bio-safety of orthodontic adhesive resins.

In the available literature there are no reports describing release of bisphenol A polymers from orthodontic adhesive resins. This phenomenon may result from the fact that research teams focus on bisphenol A monomers because of their widely documented adverse biological effects. BPA polymers also carry the potential danger of the spectrum corresponding to the effects of bisphenol A monomers. It was proved that polycarbonates made from bisphenol A degrade to single BPA molecules, and the process is favoured by increased temperature [17]. The oral environment, which is characterized by wide temperature and pH changes, by the presence of digestive enzymes and periodic supply of a variety of chemical compounds, can promote poly-BPA molecule degradation to bisphenol A.

It is also possible that poly-bisphenol A molecules identified in the presented study may be created by polymerization of BPA initially released into the external environment from the assessed orthodontic adhesives.

The phenomenon of bisphenol A polymer release from dental materials undoubtedly requires further study and further clarification, whether their presence in orthodontic adhesives' eluates results directly from degradation of materials, or from BPA secondary polymerization in aqueous solutions.

Orthodontic adhesive decomposition in the oral cavity conditions, and Bis-GMA resin release into the external environment, can also cause a potential biological threat. Tests carried out in laboratory conditions confirmed the cytotoxic effect of Bis-GMA in relation to fibroblasts, dental pulp cells, and oral epithelial cells [18]. Bis-GMA resin released from dental materials has a genotoxic effect in cultured cells, causes degradation of nucleic acids, and disrupts their repair mechanisms [19].

The release of bisphenol A and its derivatives from orthodontic adhesives assessed in the presented study can raise doubts as to their safety. Although BPA is released from dental composite materials in relatively small quantities, it remains a potentially biologically harmful chemical compound because its effect is not limited to direct induction of cytotoxicity at the cellular level.

It has been shown that even small amounts of BPA indicate paracrine activity [20] by affecting receptors from the estrogens group, disrupting spermatogenesis and oogenesis.

The study by Bouskine et al. [21] showed that bisphenol A can act not only through hormonal activity, but also disturb maturing and differentiation of tissues through pathogenic effect on the processes of intercellular communication.

Reports on the effects of bisphenol A on developing animal embryos are particularly alarming. Durando et al. [22] reported pro-oncogenic effects of BPA on embryos of rats whose mothers were exposed to this chemical compound during pregnancy. The publication by Poimienova et al. [23] described impairment of nervous system development, impairment of cognitive skills and severe behavioural changes in rats exposed to bisphenol A during prenatal development. An experiment by Salian et al. [24] demonstrated the impact of prenatal exposure to BPA on decrease in reproductive capacity of successive generations of animals through impaired spermatogenesis in newborn male rats, and an increased tendency in newborn females to miscarry.

Recognizing bisphenol A as a potentially biologically dangerous compound was reflected in a document published by the US Food and Drug Administration (FDA) in 2010, entitled 'Update of Bisphenol A for Use In Ford Contact Applications' [25]. The FDA declared their intention to take steps to withdraw BPA from food packaging production processes, and to tighten legal regulations on its use.

CONCLUSIONS

The results of this study allow formulation of the following conclusions:

1) In conditions of the current experiment it was demonstrated that most of the assessed orthodontic adhesive resins available on the European market release to the outside environment the biologically harmful bisphenol A or its derivatives, posing a potential threat to patients' health.
2) Release of BPA and its derivatives into aqueous solutions is the highest in the early stages of sample incubation.

In the context of the results of the presented study, and reports published in the available literature, it seems necessary to undertake further research on the safety of light-polymerized orthodontic adhesives, especially in the treatment of pregnant women and the treatment of children.

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