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Research Article

Bisphenol A – A Possible Health Issue Arising from Dental Restoratives. A Review

Dmitrii Todirica[1], Ario Santini[2], Augustin Curticapean[3], Cristina Molnar Varlam[1], Székely Melinda[1] and Mohammed S. Aldossary[4]*

[1]Morphology of Teeth and Dental Arches, Technology of Dental Prostheses and Dental Materials Dept. Faculty of Dentistry, University of Medicine and Pharmacy, Tîrgu Mures, Romania

[2]The University of Medicine & Pharmacy, Targu Mures, Romania. Hon Fellow. The University of Edinburgh, Edinburgh, Scotland, UK

[3]General and Inorganic Chemistry Dept. Faculty of Pharmacy, University of Medicine and Pharmacy, TgMures, address: Gh.Marinescu No. 38, RO-540139, Tîrgu Mures, Romania

[4]Specialist in Pediatric Dentistry, Department of Dentistry, Ministry of Health, Riyadh, Saudi Arabia. P.O.Box 13743 Riyadh 11414, Saudi Arabia

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ABSTRACT

Dental resin-based materials are increasingly used in the contemporary dentistry. The elution of Bisphenol A from such materials is still gaining attention because of the potential biological effects on human. This review will discuss the possible health issue and the adverse effects on living organisms. Comprehensively, this review will discuss Bisphenol A elusion and release from different dental resin-based materials including composites, fissure sealants and orthodontic adhesives. Furthermore, the factors affecting the elution of Bisphenol A from dental materials will be explored, with an overview of its release into saliva and urine, and the methods of detection.

Introduction

The results of studies on tissues, organs, and systems have established the potential detrimental effects of Bisphenol A (BPA) and its derivatives. National and international organizations have unequivocally recognized this, acknowledging in official statements that BPA at levels as low as parts per billion have been detected in human blood and tissues. These are unconjugated, not metabolized and biologically active.

Evidence suggests that BPA can leach from plastic/polycarbonate products and recent studies indicate that it is considered a possible health risk substance [1]. BPA is best known for its xenoestrogenic effects and its ability to act as an endocrine disruptor [2].

* *Correspondence to: Mohammed S. Aldossary, BDS, MClinDent Specialist in Pediatric Dentistry, Department of Dentistry, Ministry of Health, Riyadh, Saudi Arabia. P.O.Box 13743 Riyadh 11414, Saudi Arabia; E-mail: msfd99@hotmail.com*

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One of the current controversies surrounding BPA is the possible violation of the principle of Paracelsus that states: "The dose makes the poison." Vandenberg stated that because BPA falls into the category of endocrine disrupting chemicals (EDC) it may elicit a possible non-monotonic dose response (NMDR) [3]. The fundamental principle that "the dose makes the poison" states that the higher the dose the greater the effect, resulting in a monotonic response which characterized by a linear response curve that moves along a diagonal in an upward trend. Non-monotonic dose response states (NMDR) that a response may be greater at a lower dose level than at a higher dose, which results in an unusual curve such as a "U" shaped one.

Because of the possible NMDR of BPA, the accepted tolerable daily intake (TDI) no longer applies and potential toxic effects may appear below these accepted exposure levels.

The Environmental Protection Agency established a lowest-observed-adverse-effect level for BPA A at 50 ug/kg bw/day [4].

BPA is a synthetic organic compound first synthesised by Pavlovich Dianin in 1891 [5]. It's a white solid compound having two hydroxyphenyl groups, with a high solubility in organic solvents and low soluble in water [6]. It belongs to the bisphenols and diphenylmethane derivatives group. It has the chemical formula $(CH_3)_2C(C_6H_4OH)_2$. It is synthesised by the condensation of two phenols with acetone in the presence of an acid, like hydrochloric acid (HCL) [7].

BPA is high production volume (HPV) chemical widely used in the production of polycarbonate plastics, polyacrylate resins and epoxy resins that are used in almost every industry. Polycarbonate plastic and epoxy resins are very often used in the food industry, as a component of different containers for food and beverage storages such as tin cans, water bottles etc. [1].

BPA is also a component of bisphenol A diglycidylether methacrylate (Bis-GMA) which is a monomer in the production of dental resin based materials (RBMs). BPA is not present in a pure form in these materials but may be present as an impurity, because of incomplete polymerisation or because of product degradation [8]. BPA can also be released from RBMs because of the enzymatic salivary hydrolysis of BPA derivatives, such as bis-GMA or bis-DMA [9].

In 2012, the worldwide production of BPA exceeded 4.6 Mt/year (million tons per year) with Asia being the top BPA manufacturer [8]. It is estimated that the global production of BPA will increase to 8 Mt/year in 2018.

Pure BPA is not used in the manufacturing of dental materials. However, its derivatives, ethoxylated bisphenol-A glycol dimethacrylate (BisEMA), bisphenol- A dimethacrylate (BisDMA) and the most popular BPA derivative, bisphenol A diglycidyl methacrylate (BisGMA) are used. These derivatives can be categorised as BPA derivatives with ether linkage (-O-), such as BisGMA and BisEMA or BPA derivatives with ester bond (-O-CO-), such as BisDMA and polycarbonate. The derivatives with the ether linkage do not hydrolyse into BPA while the derivatives with ester bond hydrolyse into BPA, because of this, some of the derivatives can contain a small amount of BPA (at a ppb or ppm level) [6, 10].

A possible health issues?

Until recently, the effects of BPA were unknown or uncertain. Recent *in vivo* and *in vitro* studies have shown possible effects of BPA on the living organism. These can be developmental and reproductive effects, metabolic diseases, endometrial disorders, miscarriage, alterations in birth weight, cardiovascular diseases, ADHD, diabetes, DNA double-strand breaks, mammary and prostate cancer, dental fluorosis, interference in the development of the neocortex in prenatal exposure, or increased infertility for women [11-17].

Studies have shown that perinatal and early life exposure to BPA can have harmful multisystemic effects [18-20]. Because of its multiple sources of exposure, tracking BPA intake in humans is difficult. The most vulnerable categories are infants and children to whom the lowest-observed-adverse-effect level dose for BPA is much smaller than 50 ug/kg bw/day, making them more predisposed to BPA health risks [21, 22].

In 2006, EFSA, based on the adverse systemic effects of BPA in rats, stipulated a tolerable daily intake (TDI) of 50 ug/kg bw/day. In 2012, an appraisal by a Swedish Chemical Agency (KEMI) suggested that there is a need for a much lower TDI dose. In 2014, the EFSA stated that the TDI dose of BPA should be reduced temporarily to 5 ug/kg bw/day (t-)TDI [23].

Research has demonstrated that BPA can migrate from consumer goods into food items, and that the environment and humans are exposed daily to a variety of BPA sources, each varying in the quantity of released BPA [24, 25].

Possible Exposure Sources

BPA represents 75% of polycarbonate plastic and 17% in epoxy resins [25].
BPA exposure sources include:

1. Food products such as dairy products, canned drinks, and canned food
2. Non-food products such as dermal contact-cosmetics, toys, and thermal papers
3. Medical devices including dental materials
4. Occupational exposure through dermal, oral, and inhalation routes.

Goetz et al. concluded the most common pathway of BPA exposure is consumer food representing 5% to 10% of total exposure [25]. The most susceptible consumer groups are the infants and children, and the most important sources for BPA exposure for them are polycarbonate baby bottles. The most critical BPA source for teenagers and adults are canned food products [25].

Monomer release from dental materials

Modern resin-based dental materials (RBMs) have successfully replaced amalgam in many dental procedures. With a survival rate of more than seventeen years and a success rate of 75 %, RBMs have earned a rightful place in the dental treatments [26]. However, unanswered questions regarding their toxicity and biocompatibility still exist and require

explanations. These RBMs include resin composites, dental sealants and adhesives, liners and orthodontic cement. They consist of organic resin monomers and co-monomers such as Bis-GMA, Bis-EMA, Bis-DMA, TEGDMA, solvents and reinforcing fillers. These materials are hardened by a polymerisation process, chemically activated by mixing a base and a catalyst or light cured [27, 28]. RBMs have disadvantages such as polymerization shrinkage, the incomplete degree of cure and release of monomers from the cured material [27]. Polymerization of RBMs is invariably incomplete, and as a consequence, unbound monomers can elute from polymerised dental materials.

Studies have shown that the most common monomers leaching from RBMs are Bis-GMA, TEGDMA, UDMA, HEMA and BPA and can have a cytotoxic effect on human cells. Allergy to bis-GMA monomer can occur, and allergic contact stomatitis has been reported two days after placement of an RBM [29].

The ingestion of leached dental monomer is one source of BPA exposure contributing to the increase of the TDI. Leaching of monomers depends on their molecular weight. Monomers with low molecular weight like TEGDMA may leach out much faster than high molecular weight monomers such as BisGMA and UDMA, which are trapped in the polymer network matrix and need the degradation of the resin matrix to leach out [30].

Bis-GMA and TEGDA are two of the most common monomers used in RBMs. These monomers contain ester linkages bonding BPA and triethylene glycol fragments to polymerisable resin segments. The mentioned ester groups are susceptible to hydrolysis by the salivary enzymes, consisting of the protein albumin, resulting in toxic products such as triethylenglycol (TEG) and methacrylic acid (MA) [31].

BPA can become part of the dental materials in three ways: 1) as a direct constituent, 2) as a byproduct of degradation of other constituents and 3) as trace material from the manufacturing process [32]. In a study conducted by Michelsen et al., a variable amount of bisphenol-A diglycidyl methacrylate (Bis-GMA), 2-hydroxyethyl methacrylate (HEMA), and urethane dimethacrylate (UDMA) was detected immediately in the patient's saliva after dental restorative treatment. The detected amount ranged from 0.028 to 9.65 μg ml (-1) for Bis-GMA, from 0.015 to 0.19 μg ml (-1) for HEMA, and from 0.004 to 1.2 μg ml (-1) for UDMA [33]. The mentioned monomers could not be detected in saliva samples before the dental treatment [33].

A meta-analytical study concluded that monomers were released from dental resin restoration materials and the quantity of the released monomers was higher when samples were stored in an organic storage medium rather than an inorganic medium. The first monomer to elute was HEMA. The study also concluded that, depending on the extraction solvent, up to 11% weight could be extracted from the resin-based sample. BPA may be released in a quantity of 132.36 μmol in the first 24 h from a full molar crown resin restoration [34].

Fleisch et al. concluded that BPA is detectable in saliva for up to three hours after dental resin restoration placement. RBMs, containing bisphenol A dimethacrylate (bis-DMA) are more likely to be hydrolysed into BPA and have a stronger estrogenic effect compared to dental products that contain the bisphenol A derivative glycidyl dimethacrylate (bis-GMA) [2]. Because of the inaccurate data related to the possible quantity of eluted monomers and the many factors affecting their release, it was concluded that precautionary measures are required to reduce BPA exposure. These include removing the oxygen inhibition layer with a pumice stone, having the patient gargle for a few seconds after placement of a restoration and limiting the use of RBMs during pregnancy.

There is an increased number of reports on BPA exposure and adverse perinatal development. Molar Incisor Hypomineralisation is a recently reported condition which affects the first molars and the permanent incisors. Random white opacities are present on the enamel, and a variable prevalence of 2.4% to 40% is reported in children aged 6 to 8 years. Although there are some possible causes for this condition, an association with postnatal BPA exposure has been made and is possibly related to the fact that ameloblasts are susceptible to BPA exposure [35].

Release from resin-based materials

RBM consist of a polymerisable organic matrix, of monomers such as Bis-GMA, TEGDMA, UDMA, HEMA, reinforcing inorganic fillers, a coupling agent, usually silane, and photoinitiators that initiate and modulate the polymerisation reaction [36].

Elution of constituents occurs by diffusion through the matrix or after degradation of the matrix. Recent studies have proven that elution of monomers from RBM is possible and this has raised concerns about health risks. Ferracane et al. stated that factors affecting elution include the extent of the polymerisation reaction, the chemistry of the solvent and the size and chemical nature of the released constituents. Other influencing factors are the light wavelength, light source, light intensity, exposure time, and the light curing tip to material distance [37-39]. Simultaneously obtaining ideal conditions for all of these factors is difficult and consequently, it is estimated that the number of unreacted monomers in polymerised RBMs is below 10% and the degree of conversion is between 50% and 70% [40].

Cokic et al. investigated the possibility of monomer elution from composite dust. High concentrations of unpolymerized methacrylate monomers, including BPA, may be released in water [41]. Higher amounts released when samples are immersed in ethanol. The composite dust particles were between 6nm and 5μm, facilitating the transport of monomers into the respiratory system, and indicate a possible source of health concern for the increasing incidence of respiratory disease in dental personnel.

Restoration of teeth with RBMs is associated with higher BPA levels in saliva and urine immediately after placement, which decreases over time [42-44]. Lee et al. obtained saliva samples from 30 volunteers. Before restoration placement, they detected a BPA saliva level of 0.15 μg/L, 5 min, whereas after restoration placement the salivary BPA levels rose to 3.64 μg/L [45]. BPA elution increased in proportion to the number of filled surfaces. Seven days after placement, the BPA level decreased to 0.59 μg/L indicating that the highest quantity of BPA is released in the first 7-14 days after placement in the oral cavity.

Polymerisation conditions or degree of conversion (DC) are known to affect the elution of monomers. Shorter light curing unit tip to material distances and longer curing time determined higher DC with a concomitant decreased elution of TEGDMA and UDMA. There was,

however, no effect on Bis-GMA. Contrary to TEGDMA and UDMA, the release of BPA increased with a decrease in light curing unit tip to material distances and longer curing time. Individual polymerisation conditions are needed for each monomer to decrease its elution [40].

Maserejian et al. stated that exposure to BPA during pregnancy is associated with weaker neuropsychological development in children [46]. In a randomised clinical trial, 534 children were placed into two groups; the first group has been treated with amalgam and the second with RBMs. The results had no statistically significant associations, and only slightly poorer intelligence test results were seen in the group treated with RBC.

Another study, looking for an association between RBM dental treatment for children and psychosocial problems, concluded that children treated with bis-GMA containing RBM, especially on posterior teeth, had poorer scores in intelligence and emotional tests, as well as lower psychosocial function. In comparison, children treated with amalgam or urethane dimethacrylate-based compomer had no adverse psychological outcome [47].

The idea that one type of light curing unit (LCU) affects elution of constituents was questioned by the study of Polydorou et al., when the elution of constituents was found to be not only dependent on the type of LCU but also was material dependent [48]. The conclusion was that each material having its own composition might have specific monomer elution kinetics. This is supported by Santini et al., who reported that different OAs exhibited individual monomer elution kinetics during a seven-day immersion in 75% ethanol/water [49].

Regarding the different elution kinetics from different materials Polydorou et al., related that nanohybrid resin composite materials when cured for more extended periods. Bis-GMA was seen to elute even after one year of storage in 75% ethanol [50].

Release from resin fissure sealants

Dental resin-based sealants (RBS) have been proven to be effective in preventing dental caries and are an approved and commonly used treatment to combat dental decay in children [51, 52]. RBSs are widely used because they can be light cured, are user-friendly and have had a high retention score [53].

Although BPA is not used as a raw material in the production of RBSs, its derivatives, Bis-GMA and Bis-DMA are. As with other monomer-containing RBSs, elution of BPA can occur due to degradation, incomplete polymerisation or the presence of an impurity from the manufacturing process [2, 34].

Han et al., investigated the possibility of a relationship between salivary BPA and RBSs. Children with dental sealants had a salivary BPA level from 0.002 to 8.305 µg/L, compared to the control group who had a lower salivary BPA of 0.40 µg/L [54].

BPA and BPA analogues were detected in 65 RBSs sold on the U.S market. From the analysed sealants, 46% leached BPA in quantities up to 1070 µg/g [55]. The highest release of monomers occurred when a plasma arc LCU was used [56].

In a study by McKinney et al., although children who had dental sealants had a BPA concentration from 20% to 25% higher than children with no dental sealants, there was no statistically significant association between the number of RBSs and urinary BPA concentrations [57].

BPA can be detected in saliva before RBSs placement or RBC placement. This can be explained by the multiple sources of exposure to BPA other than RBCs. The detection level can range from 0.07 to 6.00 ng/ml at baseline. After RBS placement, BPA concentrations can peak at 9.08 ng/ml, after especially after three hours, returning to baseline level within 24 hours [58].

Contrary to the above, a systemic review concluded that patients are not at risk to BPA exposure from RBSs and dental practitioners should adopt a protocol of using an abrasive pumice to clean sealants' surfaces or have children and teenagers rinse with tepid water for 30 seconds [59].

A survey study from South Korea examined 495 children aged 8-9 years old. Depending on the number of RBSs and RBC restorations present in their oral cavity, the children were classified into four groups. The group of children with more than 11 sealants/restorations showed a BPA urine concentration level of 9.13 µg/g, which was much higher than the control group [60].

Release from orthodontic materials

BPA has been in the center of attention especially in the orthodontic field as it is known that BPA derivate based composites can affect the psychophysical health of children. One opinion is that that the quantity of the orthodontic adhesive (OA) placed between the bracket and the enamel is a small one and therefore poses no harm. Also, the location of the OA, between the bracket base and the enamel reduces the contact area of the material and the oral environment. Malkiewicz et al., stated that this assumption might be too optimistic since, in clinical practice, excess OA may escape from the bracket base during positioning and might not be entirely removed by the practitioner prior polymerisation [61]. Another contra-argument is the fact that the OA is located under the bracket base, and during the polymerisation process, UV light access is debatable, especially in the case of metallic brackets where UV light to the resin is provided only by the transillumination through the enamel [61]. Santini et al., evaluated the total light energy (TLE) transmission through ceramic brackets [62]. They concluded that, following the curing recommendations of the manufacturers, insufficient TLE was delivered to the OA and to obtain a better degree of cure increased exposure duration was recommended.

In a systemic study, Halimi et al., concluded that the rate of released BPA from OAs was 11,000 times lower than the DTI [63]. Kang et al., researched the saliva and urine level of BPA from 22 volunteers fitted with a fixed, lingual mandibular retention device [64]. The authors stated that the only significant high level of BPA (in a maximum dose of 20,889 ng/ml) was detected in the collected saliva immediately after the placement of the retainer.

Kloukos et al., assessed the amount of released BPA *in vivo* from a light-cured OA immediately after bracket bonding to 20 recruited patients. Group A (11 patients) had to rinse their mouth with 25 ml of water after

bonding, Group B (9 patients) had to rinse the mouth with a simulated mouth rinse formulation containing a mixture of 20 ml de-ionised water plus 5 ml absolute ethanol [65]. The rinsing solutions were collected in glass tubes before, immediately after bonding the brackets and after a second mouth rinse. In both groups, BPA was detected. Higher levels were discovered after the first post-bonding rinse and decreased after the second post-bonding mouth rinse. Higher BPA concentration levels could be established in the water rinsing solution than in the de-ionised water/ethanol solution. The authors concluded that BPA concentration followed a pattern of initial increase after which it decreased to the base-line values. The detected BPA concentration was far below the reference limit for the TDI.

Malkiewicz et al., assessed *in vitro*, the release of BPA and its derivatives from six OAs. Using HPLC, they assessed the eluted levels of BPA. Samples were stored in water, and BPA polymers and Bis-GMA concentrations evaluated at one hour, 24 hours, seven days and 31 days [61]. All three possible eluted substances were un-detected in all of the samples. In only one adhesive the highest concentration of BPA, at 32.1 µg/ml, was detected one hour after sample storage. Significantly lower concentrations were detected after 24 hours (8.4 µg/ml), 7 days (6.4 µg/ml) and 31 days (1.7µg/ml) storage period. The eluted quantity of BPA in the first hour was higher than the combined released quantity from the other storage periods. BPA polymers were detected in three out of the six orthodontic adhesives. Again, the highest concentration of BPA polymers was detected after one hour of storage, at 371.9 µg/ml. At 24 hours, seven days and 31 days, BPA polymer concentrations were much lower, ranging from 152.09 µg/ml after 24 hours, 59.13 µg/ml after seven days and 9.26 µg/ml after 31 days of storage. The eluted Bis-GMA material had the highest concentration 1 hour after storage and was highest in the water solution, 425.0 µg/ml. The conclusion was that BPA and its derivatives are released into the environment, and the highest released quantity in aqueous solution is immediately after placement [61].

The distance of the light curing lamp and the orthodontic adhesive has also been a shown to affect the elution of BPA. Sunitha et al. concluded that BPA eluted concentration levels were much higher when the distance of the curing lamp was greater from the sample and that there was a direct correlation with the increase of the released BPA and the increasing light curing tip distance [66]. Even when the light curing tip distance was 0 mm in controlled *in vitro* conditions, BPA leached on day one showing that orthodontic adhesives are not inert materials and full degree of cure is very hard to achieve [66].

In a study by Purushothaman et al., the quantified release of BPA from orthodontic adhesives cured with halogen light or electroluminescent diodes (LED) at three distances (0 mm, 5 mm, 10 mm) was assessed [67]. The authors concluded that the LED curing lamp caused less BPA release and improved the degree of cure than the halogen light. Also, a smaller distance from the light curing tip to the sample determined a higher degree of cure and far less BPA leaching. Purushothaman et al., implied that self-polymerising composites have a higher degree of cure and release less BPA than light-cured composites [67].

Klokos et al., assessed the short- and long-term elution of BPA from OAs and polycarbonate brackets [68]. He concluded that BPA was released from OAs in concentrations of between 0.85 and 20.88 ng/ml *in vivo* and up to 65.67 ppm *in vitro*. Polycarbonate brackets eluted up to 22.24 µg/g of BPA in ethanol solution and 697 µg/g in water solution after a 40 months storage [68].

Pelourde et al. evaluated the release *in vitro* of monomers from the OA bonded brackets using a reproducible model of bonded retentions and calibrated molds. BPA concentrations did not surpass the 0.02 ppm detection limit. Iodobenzene, iodobiphenyl, and triphenyl stibine were also detected [69].

Kotyk et al., determined the quantity of BPA eluted from OAs during simulated intraoral exposure [70]. Samples of OAs were immersed in artificial saliva for two weeks. The highest quantity of released BPA was detected from a thermoformed orthodontic retainer was 7.63 µg/g and from an OA 2.75 µg/g. The detected quantities were below the daily intake reference dose [70].

Release into saliva

Through the placement of RBMs in the oral cavity different monomers, including BPA may be eluted into saliva, especially since unspecific enzymes from saliva can have a hydrolysis effect on the dimethacrylate resin matrix of RBMs [6].

Moreira et al., assessed in vivo BPA salivary concentrations, after bracket bonding with OAs. Saliva samples were collected before and 30 minutes, 24 hours, one day, one week and one month after bracket bonding [71]. The authors concluded that BPA salivary levels were highest 30 minutes after bonding but did not surpass the reference dose for daily intake.

Another *in vivo* study evaluated and compared the BPA salivary levels of patients wearing vacuum-formed retainers or Hawley retainers [72]. After their completed fixed orthodontic treatment, 45 patients were randomly allocated to one of three groups; a vacuum-formed retainer, Hawley retainer fabricated by chemical cure and Hawley retainer made by heat curing. Before and after placement, saliva samples were collected, the collection period was 1 hour, one week and one month after placement. BPA was found in all three groups, with the highest salivary BPA level of 2.38420 ppm, observed in patients wearing vacuum formed retainer seven days after placement. The authors suggested that although vacuum-formed retainers composed of polypropylene/ polyethene have aesthetic, maintenance, fabrication and, durability advantages over Hawley retainers, consideration should be given to the use of a Hawley retainer fabricated by heat cure. To reduce BPA elution into saliva, Valittu et al., recommended the immersion of the retainer in water at 37°C for a whole day before placement [73].

Dental fillings may be associated with higher concentrations of unconjugated BPA and total BPA in human saliva. Although in low concentrations, eight out of twenty samples taken from patients having RBMs contained BPA. In the control group, BPA was detected only in three out of 20 samples [42].

In a study by Michelsen et al., monomers were present in saliva up to 24 hours after placement of RBCs. The detected quantities ranged from 0.028 to 9.65 µg ml (-1) for Bis-GMA, 0.015 to 0.19 µg ml (-1) for

HEMA and 0.004 to 1.2 μg ml (-1) for UDMA. These monomers could not be detected one week after placement [33].

Lee et al., measured the changes of salivary BPA before, five minutes and seven days after placement of RBCs. Before RBCs placement no significant difference between the existing fillings and the saliva BPA level was found [45]. However, after RBC restoration placement, BPA in saliva levels five minutes after placement increased to 3.64 μg/L from 0.15 μgL before placement. Seven days after placement the BPA saliva levels decreased to 0.59 μg/L. The authors concluded that BPA levels increased in proportion to the number of filled surfaces, but below the accepted TDI.

Downs et al., detected salivary BPA in the range of 0.07-6.00 ng/ml before RBS placement. After sealant placement, Salivary BPA levels spiked at 3.98 ng/ml to 9.08 ng/ml over the first three hours and returned to baseline level after 24 hours [58]. This phenomenon was supported by Kingman et al., who found out that salivary concentration of BPA peaked in the first hour after placement from 0.43ng/ml before RBS placement to 0.64ng/ml after placement [74]. The use of rubber dam resulted in lower salivary BPA concentration of 0.60 ng/ml compared to 0.71 ng/ ml when no rubber dam was placed.

Release into urine

Studies about the metabolic pathway of orally ingested monomers revealed that they metabolize in vivo and within 24 hours after ingestion monomers are biologically converted to CO_2, in a proportion of up to 80%, and approximately 12% to 15% is excreted via urine and the rest through faeces [75].

In the study of Kingman et al., contrary to salivary BPA, BPA levels in urine decreased in the first hour after RBCs placement, from 1.75ng/ml to 1.05ng/ml. A 43% increase in the concentration BPA levels was detected in urine from 9 to 30 hours after placement, from 1.96ng/ml – 1.67ng/ml to 2.24ng/ml-2.38ng/ml [74]. Urinary BPA levels were not influenced by the application of rubber dam.

RBC may produce temporary increases in urinary BPA levels that may not be detected 14 days or 6 months after treatment. The highest increase in concentrations is seen in the first few days [43].

McKinney et al., did not find a statistically significant association between the number of sealants or restorations and urinary BPA levels in children treated with RBS although children with seven or more sealants had a mean BPA concentration 25% higher than the children without sealants [44].

Chung et al., obtained urine samples from 495 children aged between 8 to 9 years [60]. The medium creatinine-adjusted urinary BPA level was 2.08 ± 3.81 μg⁄g. No urinary BPA was detected in children with ten or less RBSs. BPA urinary levels increased drastically, up to 9.13 μg⁄g, when more than eleven RBSs were placed.

Factors affecting the elution of BPA from dental restorative materials

Temperature

Increased temperature and alterations in acid or base conditions increase the leaching of BPA [76]. Atabek et al., stored samples of RBCs in 2 ml of water at an initial temperature of 37°C [77]. Water with a temperature of 59°C was added to the samples, at 1, 6, 24 hours, 2,3,4,5, and six days. All samples, excluding the control group, eluted BPA which peaked at 13.9 μg/ml after two days. All amounts eluted were below the reference dose for daily intake. RBSs eluted more BPA compared to other RBCs. A possible explanation may be because of the presence of the oxygen inhibition layer which occurs on the surface of RBCs and RBSs [78].

Degree of conversion

One of the fundamental conditions for obtaining a high degree of cure is to provide to the material that is being cured with an adequate light energy density to facilitate free radical polymerisation through the C=C double bonds from monomers into polymers [79].

A short curing time of 5 to 10 seconds results in a low DC and a higher amount of monomer elution. Curing for 20 seconds and 40 seconds resulted in no significant differences in the DC or the amount of eluted substances [12, 80].

Increase in a tip to sample surface distance resulted in a concomitant decrease in DC [66, 67]. One study reported an increase in DC with a LED LCU compared to a Halogen LCU [67].

A strong inverse correlation exists between the DC and the amount of elutable components from RBMs [12, 67, 80].

Santini et al., reported that the DC of OAs was between 45% and 60%. Although Lucerin TPO was not identified in the tested RBCs, a dual-wave LCU resulted in a higher DC, and the DC was more material dependent than LCU dependent [81].

In supporting the mentioned affirmations regarding the polymerization time and its direct influence on the degree of cure, is the study by Aldossary et al., in which the total light energy transmission through bulk-fill RBCs using two curing regimes (800mW/cm^2 for 20 seconds and 1,600mW/cm^2 for 10 seconds) was compared [82]. The authors concluded that the samples that were polymerized for 20 seconds had a better degree of cure than the samples that were polymerized for 10 seconds even if the irradiance on the last ones was higher. Exposure time is more important than irradiance for a better degree of conversion.

Pongprueksa et al., concluded that bulk-filling techniques resulted in lower DCs and resulted in the release of 0.053 μg of BPA after one week of storage in absolute ethanol at 37°C [83].

Storage conditions

Tsitrou et al., stored four dental RBCs in different storage mediums for 24 hours and seven days [84]. In the first 24 hours, eluted BPA peaked at 11 μg/ml., with quantities of 5-8 μg/ml detected at seven days [84].

The highest amount of leached BPA and monomers have been shown to occur immediately after placement, one to three hours, and in the first 24

hours in several *in vitro* and *in vivo* studies [2, 33, 34, 45, 58, 61, 66, 71, 74].

Malkiewicz detected BPA at 32.1 µg/ml., one hour after curing OA samples in vitro [61]. Fleisch et al., reported the detection of salivary BPA up to three hours after the placement of RBCs and Van Landuyt et al., observed 132.36 µmol of salivary BPA in the first twenty-four hours after a full molar RBC crown [2, 34].

Sevkusic et al., stored RBC samples in deuterated methanol and deuterated water for up to 180 days [85]. BPA was detected in almost all tested RBC samples, and the highest quantity recorded at 90 to 180 days after immersion. In another study by Malkiewicz et al., the highest concentration of BPA (91.809 µg-cm3) was detected twenty-four hours after storing the RBC samples in water solution, whereas BPA was detected at 1.469 mmol/l from deuterated methanol and at 0.031 mmol/l from the deuterated water [85, 86].

Storage medium

The most popular storage mediums are ethanol 75%, artificial saliva, acetone, methanol Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS).

Studies indicate that BPA and other monomers are more readily detected when RBCs samples are stored in organic solutions, like methanol, ethanol 75% and ethanol/water, than in other media [41, 49, 50, 65, 68]. This can be attributed to the organic solubility parameter of ethanol which is similar to that of the monomers from RBCs.

According to American Dental Association, acetonitrile and methanol are strong solvents of organic compounds and are preferred over ethanol for maximizing the number of eluted monomers [87].

One aspect of culture media storage solutions is that they may contain unbound monomers which may lead to false-negative results [84].

Deviot et al., BPA was detected by GC/MS at 57 ng/ml., from samples that were light cured for 20 seconds and immersed in saliva and at 1684 ng/ml, from samples that were light cured for two seconds and immersed in acetonitrile [88]. The same authors detected BPA by LC/MS from sample immersed in methanol, 0.1 ng/ml. All three determinations were done seven days after light curing.

Militec et al., concluded that the storage medium influences the DC of RBC samples [49]. It may be assumed that elution of monomers and BPA also is affected by the storage medium and may not always reflect the elution kinetics in the oral environment.

Polymerization time

In a study by Kwon et al., a longer polymerisation time resulted in a higher DC with a lower release of some TEGDMA, and UDMA, with no significant effect on Bis-GMA but an increase in elution of BPA [40]. The authors concluded that polymerisation conditions are not identical for all monomers. Contrary to the findings of Kwon, Purushothaman et al., stated that the highest amount of BPA was released when the curing time was less than 20 to 10 seconds [67].

Polymerization distance

An increased elution of BPA results when the tip to RBC material surface distance is increased, possibly explained by the photolysis of BPA based resins under high light intensity. Also, a shorter curing distance can result in lower release rates for TEGDMA and UDMA [40].

When OA samples were cured at different LCU tip to surface distances, from 0 mm and 10 mm, Purushothaman et al., reported that the least amount of BPA was released when the samples were polymerised at 0 mm tip to surface distance when using a LED LCU and the most released BPA was detected from the samples that were polymerised at a 10mm tip distance [67]. In a similar study, OA samples polymerised at varying tip to surface distances released varying amounts of BPA. Samples cured at 0 mm released the smallest amount of BPA from day one to day 35, compared to samples cured at 10 mm [66].

Type of Light Curing Unit

In the study by Purushothaman et al., eluted BPA was detected from RBCs cured with LED or Halogen LCUs (HLCs) [67]. RBCs cured for ten seconds by HLCs released the highest concentrations of BPA at 17.73 ppm on day one, 23.77 ppm and on day twenty-one. The least amount of BPA was released from samples cured for twenty seconds by a LED LCU, at 5.3 ppm to 10.62 ppm on day one. Even when cured for 40 seconds with an HLC LCU, samples released more BPA at from 12.52 ppm to 17.71 ppm on day one, than when cured for 20 seconds with a LED LCU.

Polydorou et al. [48] used LED LCUs and HLCUs to cure samples, could not obtain consistent results for the LCUs and reported that each RBC had a unique elution kinetic.

According to Carvalho et al., less residual monomers remained in polymerized RBCs when cured by LED LCUs compared to HLCUs [89]. Santini states that to obtain proper polymerisation the LCU must produce sufficient light power and, importantly, the wavelength output of the LCU must match the absorption spectrum of the RBC photoinitiator [90]. This will facilitate an optimum DC with low monomer leakage.

Methods of detection

BPA was detected from RBC samples immersed in saliva and acetonitrile using GC-MS but was only identified by LC-MS when samples were immersed in methanol. A disadvantage of GC/MS is that heat is used in the detection process, and this may affect the structure of Bis-GMA monomer and cleave it into BPA, leading to a false positive result. It has been suggested that it is unsuitable to use GC-MS to detect high molecular-weight monomers like BisGMA, BisEMA and UDMA GC/MS as this may overestimate the released amount of BPA from RBCs [33]. Instead of GC-MS, LC-MS together with a clean-up pretreatment is a better choice for BPA detection, and LC/MS-MS is considered the better technique because of its accuracy and its detection limit, which is lower than that of HPLC [88, 91].

Medical issues related to Bisphenol-A

A large number of studies indicate that BPA has adverse effects on living organisms. Although it was first synthesized in 1891, its estrogenic stimulating capabilities were first evidenced in 1930 [3]. BPA can act as an endocrine disruptor by binding to estrogen receptors in addition to blocking the estrogenic response by competing with endogenous E2 [92, 93]. BPA can bind to thyroid receptors and influence thyroid functions by its agonistic and antagonistic effects. It can interact with the immune system and the developing central nervous system [94].

The hypothesis that BPA could have a non-monotonic dose-response curve has raised even more concerns, and in 2012 it was decided to lower the TDI from 50 ug/kg bw/day, established in 2006 by EFSA, temporarily to 5 ug/kg bw/day [23].

BPA can be detected in urine saliva, fetal livers, breast milk, in both adults and children [35, 42-45, 54, 57, 58, 60, 71-744, 95, 96]. BPA can have adverse effects on human health affecting the reproduction system, influencing the development of the fetus or newborn, can cause different metabolic affections and other health effects.

In infertility studies in women, BPA is incriminated for a poor ovarian response, reduced maturation of oocytes, lower numbers of normally fertilized oocytes and elevated androgen concentrations [97-99]. Bloom et al., obtained results contrary to these and the authors attributed this to them measuring unconjugated BPA rather than total BPA [100]. In another study, higher serum BPA levels in males were associated with reduced sperm quality and reduced embryo quality [101]. Higher urinary BPA levels in women were associated with implantation failure in IVF protocols [102]. High urinary BPA levels were associated with a lower self-reported sexual function, erectile function and sexual desire, in male Chinese occupational workers working in epoxy resin manufacturing facilities [103]. Another group environmentally exposed to BPA also exhibited a reduced male low sexual desire and overall sexual satisfaction [104]. Li et al., reported levels of urinary BPA up to 38.7 µg/L which correlated with lower sperm qualities, in occupational workers [105]. In the control group, the BPA urine levels were 1.4 µg/L, and there was a negative correlation between urinary BPA and sperm concentration and count. The conclusion was that BPA might be detrimental at lower doses than the accepted TDI. High urinary BPA levels are also associated with elevated follicle stimulating hormone and a lower ratio of estradiol: testosterone [106]. Occupationally exposed workers (male and female) to BPA may have children who at birth have a much lower birth weight than children of non-exposed workers [107]. Maternal urinary BPA levels are also associated with increased head circumference [108].

Tang et al., concluded that children born near the BPA polluted SY River in China, had lower 17-beta-estradiol and total testosterone than children from born in control areas [109].

Although BPA cannot be associated directly with polycystic ovary syndrome higher serum BPA levels were found in women diagnosed with PCOS [99, 110]. In one study, serum BPA was detected in 52.7% of the women diagnosed with endometriosis [111]. Elevated serum BPA levels are also associated with miscarriages resulting from a BPA induced chromosomal abnormality of the oocytes [112].

Studies on primates suggest that prenatal exposure to BPA may increase the susceptibility of tissues to carcinogens, though in humans, a correlation between BPA and breast cancer could not be established [113, 114].

In a study by Cantonwine et al., BPA has been associated with shorter pregnancies and premature births [115]. Non-monotonic effects of maternal BPA are believed to cause negative birth outcomes such as small birth weight and a shorter gestational period [116]. There are also earlier studies which could not find an association between BPA exposure in the prenatal period and birth defects [117, 118]. It was believed that children from parents exposed to BPA might suffer from genital abnormalities such as a shorter anogenital distance, cryptorchidism and hypospadias. For cryptorchidism and hypospadias, no studies were found in which BPA was the causing factor [119]. However, there are studies which prove that BPA plays an important role due to its antiandrogenic effects in utero. Shorter anogenital distances were seen in boys when the mother was exposed o BPA [120].

Exposure to EDC, and implicitly BPA, during gestation or early postnatal years, can affect human health and predispose the individual to certain diseases later in life [121, 122]. Brain exposure to BPA, during in utero development, may cause altered behaviour in children such as aggressive behaviour, hyperactivity, depression, poor emotional control, which are more evident in girls than boys [122]. In a prospective cohort study prenatal exposure to BPA was associated with emotionally reactivity and sleep problems, aggressive behaviour in boys and girls and with anxiety, depression, and aggressive [123]. The results of studies from New England Children's Amalgam Trial, which assessed the psychosocial status of children that received RBC restorations, supported these findings [124].

Higher total urinary BPA is associated with insulin resistance, type-2 diabetes in normal weight and overweight individuals, cardiovascular disease, hypertension, peripheral arterial disease and high cholesterol levels [125-131]. Liver function with elevated liver enzymes was modified in adults with high urinary BPA [126]. Urinary BPA was found to be high in both children and adults with higher BMI and waist circumference [132-136] and BPA altered the release of adiponectin from adipose tissue can contribute to an increased BMI [137].

Total urinary BPA was found to have an inverse relationship to some thyroid stimulating hormones and a direct relationship with others, resulting in the possible disruption of the thyroid functioning in adults [138, 139] and newborns [140].

An increased antibody titer to cytomegalovirus (CMV) indicates a depressed immune system and can be an early sign of immune dysfunction. Clayton et al., associated a high BPA level with an increased CMV antibody titer [141].

BPA was also associated with albuminuria, which is an indicator of endothelial dysfunction in kidneys and some authors state that it is also a predictor of cardiovascular disease and type-2 diabetes [142-144].

BPA is associated with chronic inflammation and oxidative stress [110]. Trasande et al., concluded that BPA could induce oxidative stress within the renal parenchyma affecting of the kidney endothelium [144].

Correlations between BPA exposure and demographic parameters have been reported with black patients and non-Hispanics having a higher BPA exposure than other ethnic groups. Mexican Americans had the lowest exposure with children exhibiting higher levels of exposure [126, 131, 132, 141].

Conclusion

All tested dental restorative materials release BPA *in vitro* and *in vivo* though BPA released by contemporary materials is low. Nevertheless, it follows that the effects of BPA, as reported in the literature, might occur with the clinical use of these resin-based materials.

The significant increase in the mean BPA levels occurs usually about 30 minutes after the placement of restoratives for saliva and 24 hours for urine and therefore are considered to be related to the clinical procedure.

Most studies report that BPA levels detected *in vitro* and *in vivo* are lower than recommended TDI, though stated TDIs have been modified over the years and may be downgraded in the future.

Dentists must be attentive to the possibility of BPA being released following their clinical procedures and must modify their procedures to reduce if not eliminate elution of BPA.

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