# Bisphenol A as degradation product of monomers used in resin-based dental materials

Research article

# BISPHENOL A AS DEGRADATION PRODUCT OF MONOMERS USED IN RESIN-BASED DENTAL MATERIALS

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## ABSTRACT

**Objectives:** There is still much debate about the release of bisphenol-A (BPA) from dental materials. Therefore, this study aimed to quantify BPA present as an impurity in both BPA-based and non-BPA-based monomers and to evaluate whether these monomers may degrade to BPA upon salivary, bacterial, and chemical challenges.

**Methods:** BPA was determined in three different amounts (1, 2, and 3 μmol) of each monomer (TEGDMA, UDMA, mUDMA, BisGMA, BisEMA-3, -6, -10, -30, BisPMA, EBPADMA urethane, BADGE, and BisDMA). Next, the monomers (3 μmol) were immersed in whole human pooled saliva collected from adults, *Streptococcus mutans* ($2x10^7$ CFU/mL), and acidic (0.1 M HCl), alkaline (0.1 M NaOH), and control media. The amount of BPA was quantified using a specific and highly sensitive UPLC-MS/MS method including derivatization of BPA by pyridine-3-sulfonyl chloride.

**Results:** The raw monomers BisGMA (0.0006%) and BisEMA (0.0025%) contained trace amounts of BPA as impurities. The monomers BisGMA, BisEMA-3, BisEMA-10, BisPMA and BADGE were degraded into BPA in saliva and by *S. mutans*. In addition, BisPMA and BADGE were degraded under alkaline conditions.

**Significance:** Impurities and degradation of monomers could account for the release of BPA from BPA-based resin-based dental materials. Manufacturers of dental materials can reduce the BPA content by using only monomers of the highest purity. Furthermore, it is recommendable to exclude BPA-based monomers in composites in order to minimize human exposure to BPA, especially when intended for use in children.

## KEYWORDS

Key words: Bisphenol A, dental monomer, impurity, degradation

# 1. INTRODUCTION

Resin-based materials are nowadays standardly used in multiple domains of dentistry and have a wide range of applications. Most frequently, they are used in restorative dentistry, where tooth-colored composite materials bonded to tooth tissue by adhesives have replaced amalgam fillings as the first choice material to restore worn, traumatized or decayed teeth. Furthermore, they are also used as pit and fissure sealant (pediatric dentistry), as luting cement or temporary material (prosthetic dentistry), and as adhesive for brackets (orthodontics) and splints (periodontology). Nevertheless, despite their successful use for several decades, there are still concerns about the safety and biocompatibility of these materials.

Resin-based composites are a mixture of methacrylate monomers with reinforcing inorganic filler particles. The first resin-based composite was based on a derivative of bisphenol A (BPA), namely bisphenol A glycidylmethacrylate (BisGMA), which is still the main monomer in contemporary resin materials (1). For clinical applications, the raw uncured materials are converted into polymers, mostly initiated by light-curing. However, this conversion is never complete and the degree of conversion ranges between 53 and 86 % (2–4). As a result, patients are exposed to non-polymerized 'residual' BPA-based monomers and their potential degradation products that can leach out of composite restorations into the mouth (5–7), a process that may be enhanced by biodegradation (8), and may results in toxic effects (9–11).

Concerns regarding the release of hormonally active compounds from methacrylate-based resins arose already in 1996 when elevated amounts of BPA, a well-known endocrine disruptor, were detected in saliva collected from patients after treatment with a resin-based dental sealant. Furthermore, stimulated proliferation of estrogen-

sensitive MCF-7 cells due to estrogenic activity, was ascribed to BPA present in these eluates (12). Although these results were controversial and resulted in much debate (13,14), this research has raised awareness about the presence of endocrine disruptors, such as BPA, in resin-based materials. Although BPA is never used as an intentional ingredient in composite materials, it may be present as an impurity in the production process of BPA-based monomers.

To date, only two studies assessed the possible degradation of monomers into BPA (15,16). BisGMA and BisDMA, monomers previously used in the fissure sealant (12), were subjected to degradation tests. It was found that BisGMA is stable in terms of formation of BPA, but that BisDMA can easily be converted into BPA upon exposure to esterase enzymes, unstimulated human pooled saliva and extreme alkaline media. Furthermore, BPA could only be detected as an impurity in batches of BisDMA, not in BisGMA. It was concluded that BPA release should not be expected from materials based on BisGMA, if monomers of high purity are used (15). In contrast, other studies were able to detect trace amounts of BPA as an impurity in batches of BisGMA monomers (17), which to a certain extent explains the presence of BPA in eluates from composites in several studies. Other studies on monomer/material degradation used bisphenol A bis(2,3-dihydroxypropyl) either (BisHPPP) as a marker for biodegradation of resin and monomers. BisHPPP was formed upon hydrolysis of the esterbonds of BisGMA by *S. mutans in vitro*, and *in vivo* by esterases present in saliva (5,18,19). Recently, it was shown that also BisPMA could be metabolized *in vitro* into BPA using human liver microsomes (20).

However, more research on the biodegradation and conversion of BPA based-monomers into BPA is necessary. First, most available data on degradation result from studies from almost 20 years ago. Technology has evolved immensely since then resulting in highly sensitive measuring (analytical) methods (21). Second, various other BPA-based monomers, such as BisEMA and BisPMA, are frequently used nowadays in composites and have not sufficiently been tested with regard to stability and biodegradation. Therefore, the aim of this study was to determine whether BPA-based monomers can be degraded into BPA. The hypothesis tested was that BPA-based monomers, such as BisGMA, BisPMA and BisEMA, currently used in commercially available composites, cannot be degraded into BPA.

## 2. MATERIALS AND METHODS

### 2.1. Investigated monomers

An overview of all dental monomers, both BPA-based and non-BPA-based monomers, is given in Table 1. These monomers were selected based on the composition of current commercially available resin-based materials, as mentioned in the safety data sheets provided by the manufacturer, covering various fields of dentistry.

The following monomers were obtained from Esstech (Essington, PA, United States): BisGMA, BisEMA-3, BisEMA-6, BisEMA-10, BisEMA-30, TEGDMA, and UDMA. The following monomers were purchased from Sigma-Aldrich (Saint Louis, MO, USA): BisGMA, BisDMA, BADGE, TEGDMA, and UDMA. EBAPDMA urethane and mUDMA were obtained from Dentsply (Konstanz, Germany). BisPMA was purchased from Sagechem (Hangzhou, China).

## 2.2. Chemicals and reagents

BPA (≥99%), the internal standard (IS) deuterated BPA-$d_{16}$ (analytical standard), the derivatization reagent pyridine-3-sulfonyl (PS) chloride (≥95%), formic acid (FA, LC-MS grade), methanol (MeOH, LC-MS grade), and acetonitrile (ACN, LC-MS grade) were purchased from Sigma-Aldrich (Saint Louis, MO, USA). $H_2O$ (LC-MS grade) was purchased from Biosolve (Valkenswaard, The Netherlands). Dimethylsulfoxide (DMSO), hydrochloric acid (HCl), sodium hydroxide (NaOH), and ammonium fluoride ($NH_4F$) were purchased from Merck KGaA (Darmstadt, Germany). Oasis PRiME HLB (3 mL; 60 mg) extraction cartridges were purchased from Waters Corporation (Milford, MA, USA). AFFINIMIP SPE Bisphenols extraction cartridges were purchased from AFFINISEP (Petit-Couronne, France).

## 2.3. BPA impurities in stock monomers

To determine the presence of BPA in the raw monomers (i.e. the amount of impurities under the form of BPA), dental monomers were dissolved in DMSO at a concentration of respectively 100, 200, and 300 mM. 10 µL aliquots (to obtain respectively 1, 2 and 3 µmol of dental monomers) were diluted in 1 mL MeOH and were analyzed for the presence of BPA after derivatization as described below (n=5). 40 pmol BPA-$d_{16}$ was added as internal standard.

## 2.4. Salivary degradation

Stimulated whole human saliva from healthy adult volunteers (n=10) was collected in sterile polypropylene tubes immediately after waking up and before breakfast by chewing on a wax gum for saliva stimulation (available from Saliva-Check Buffer, GC Europe, Haasrode, Belgium) on 5 separate days. Volunteers were instructed to first rinse their mouth with water before collecting saliva and to avoid consumption of food that has been in contact with plastic packaging materials the day before collecting.

Each respective day, all tubes were pooled together and used as degradation medium for respectively one sample replicate, for a total of 5 replicates, and 3 negative control replicates, for a total of 15 replicates. Volunteers were selected based on low salivary BPA levels (tested *a priori*) among researchers from the University of Leuven and gave written informed consent. The study was approved by the Ethical Committee Onderzoek UZ/KU Leuven (reference number: S57170). Three µmol of each monomer was dissolved in 5 mL whole human pooled saliva and incubated for 24 hours at 37 °C in a rotary shaker (230 rpm). 40 pmol BPA-$d_{16}$ was added as internal standard. Samples were centrifuged to remove cell debris and solid-phase extraction (SPE) was performed as described below.

### 2.5. Bacterial degradation

*Streptococcus mutans* (ATCC 25175) stored at −80 °C was first cultured on blood agar plates (37 °C, 5 % $CO_2$). Colonies from these blood agar plates were then cultivated overnight in Brain Heart Infusion (BHI) broth (Becton Dickinson and Company, Franklin Lakes, NJ, USA), and used for the experiments. The concentration of bacteria was adjusted spectrophotometrically at 600 nm (GeneQuant 100, GE Healthcare, UK) to $2x10^7$ CFU/mL ($OD_{600}$ = 0.025). Three µmol of each monomer was added to 5 mL of the bacterial suspensions (n=5). All samples contained 1% DMSO to increase the solubility in aqueous media. Samples were incubated (37 °C, 5% $CO_2$) in a rotary shaker (230 rpm) under aerobic conditions for a period of 48 hours. 40 pmol BPA-$d_{16}$ was added as internal standard. Samples were centrifuged to remove bacteria and solid-phase extraction was performed as described below.

### 2.6. Chemical degradation

Three µmol of each monomer was dissolved in 5 mL of respectively 0.1 M HCl (‘pH 1’) and 0.1 M NaOH (‘pH 13’) as degradation medium, and LC-MS grade water (‘pH 7’)

as control (n=5). All samples contained 1% DMSO to increase the solubility in aqueous media. After 24 hours of rotary incubation (230 rpm) at 37 °C, samples were neutralized with 500 µL of respectively 1 M NaOH and 1 M HCl. 500 µL of LC-MS grade water was added to control samples. 40 pmol BPA-$d_{16}$ was added as internal standard. Next, solid-phase extraction was performed to remove salts as described below.

## 2.7. BPA detection method

### *2.7.1. Solid-phase extraction*

In order to purify BPA and remove as much as possible impurities from the samples that could interfere with MS analysis, solid phase extraction was performed. An Oasis PRiME HLB cartridge (60 mg/3 mL) was pre-washed with 2 x 3 mL ACN and subsequently conditioned with 1 x 3 mL $H_2O$. Samples were acidified with 1% FA (1:1) before loaded into the cartridge and subsequently washed with 1 x 3 mL $H_2O$. Next, the cartridges were dried for 5 minutes under vacuum (10 mmHg) and BPA was eluted with 2 x 2 mL ACN.

For bacterial and salivary degradation, an additional purification step was introduced as these media contain more salts, proteins and other potentially interfering compounds. First, 6 mL $H_2O$ was added to the eluate. AFFINIMIP SPE Bisphenols cartridges were pre-washed with 1 x 3 mL MeOH (containing 2% formic acid) and 1 x 3 mL ACN and conditioned with 1 x 3 mL $H_2O$. Samples were loaded into the cartridge, which were subsequently washed with 3 x 3 mL $H_2O$ and 2 x 3 mL $H_2O$/ACN (60:40). Next, the cartridges were dried for 3 minutes under vacuum and BPA was eluted dropwise with 2 x 2 mL MeOH.

### *2.7.2. BPA derivatization*

BPA derivatization was performed according to Regueiro et al (22), with minor changes. Samples were evaporated to dryness under a nitrogen flow and reconstituted in 200 µL of sodium carbonate buffer (50 mM, pH 9.8). Then, 15 µmol PS chloride in 200 µL acetonitrile was added, in order to obtain the derivatization reagent in excess, and the vial was cap-sealed. After vortex-shaking for 10 s, the reaction mixture was placed in a dry block heater at 70 °C for 20 min. The reaction was stopped by cooling down on ice and 100 µL formic acid (1 M) was added. The reaction mixture was subsequently passed through a 0.20 µm regenerated cellulose syringe filter and analyzed by UPLC-MS/MS.

### *2.7.3. UPLC-MS/MS analysis of BPA*

BPA was detected using an UPLC-MS/MS method as previously described (23), with minor adjustments. 1 mM $NH_4F$ was used as additive in both mobile phases. In addition, analysis run time was prolonged to 5 minutes to ensure the clearance from all components. Separate calibration standards were made for respectively BPA impurity and chemical degradation, and for bacterial and salivary degradation experiments.

Values from the negative controls were subtracted from sample values to correct for possible background contamination originating from all used materials (e.g. SPE cartridges) and from BPA already present in the saliva from the volunteers, even though great care was taken to use only glass and polypropylene tubes, and volunteers were instructed to minimize their BPA exposure.

### 2.8. Statistical analysis

To assess if monomers were degraded into BPA, each degradation condition was compared with one-way ANOVA with post-hoc Dunnett's test (BPA impurity levels served as control). If BPA was not detected as an impurity, values were substituted by LOQ/2 for statistical comparison. R3.6.1 software (R Foundation for Statistical Computing, Vienna, Austria) was used for statistical analysis. Level of significance was set at $\alpha<0.05$.

## 3. RESULTS

### 3.1. Validation results

The limit of quantification (LOQ) was 0.32 pmol BPA (corresponds to 0.11 pmol BPA/µmol monomer) for the impurity and chemical degradation experiments. LOQ was 6.33 pmol BPA (corresponds to 2.11 pmol BPA/µmol monomer) for both salivary and bacterial degradation experiments.

### 3.2. BPA impurities in stock monomers

To be qualified as an impurity, BPA had to be quantified in levels above the LOQ and a linear increase had to be observed with increased amount of monomer. BisGMA (Esstech), BisGMA (Sigma-Aldrich), and BisEMA-3 contained respectively 5.99 ± 0.18, 6.21 ± 0.10, and 24.61 ± 0.83 pmol BPA/µmol monomer as an impurity. BPA was not detected in all other monomers (TEGDMA, UDMA, mUDMA, BisEMA-10, BisEMA-30, and EBAPDMA urethane) in levels above the LOQ, or no linear increase was observed (BisEMA-6, BisPMA, and BADGE). Therefore, these values were not qualified as BPA impurity. Furthermore, BPA impurities in BisDMA could not be reported since it was decomposed completely into BPA upon derivatization.

### 3.3. Salivary degradation

Compared to the amount of BPA as impurity present in the monomers, statistically significantly increased BPA levels were observed upon exposure to human pooled saliva for BisGMA (Esstech) (9.12 ± 1.63 pmol/µmol 'monomer'), BisGMA (Sigma-Aldrich) (10.42 ± 0.90 pmol BPA/µmol 'monomer'), BisEMA-3 (41.74 ± 1.50 pmol/µmol 'monomer'), BisEMA-10 (4.20 ± 1.68 pmol/µmol 'monomer'), BisPMA (14.32 ± 1.94 pmol/µmol 'monomer'), and BADGE (6.22 ± 1.69 pmol/µmol 'monomer'). BPA was not detected in all other monomers in levels above the LOQ, or no statistically significantly difference was observed between BPA impurities.

### 3.4. Bacterial degradation

Statistically significantly increased BPA levels were observed upon exposure to *S. mutans* for respectively BisGMA (Esstech) (11.77 ± 0.66 pmol/µmol 'monomer'), BisGMA (Sigma-Aldrich) (12.56 ± 0.49 pmol BPA/µmol 'monomer'), BisEMA-3 (49.75 ± 2.14 pmol/µmol 'monomer'), BisEMA-6 (9.16 ± 5.37 pmol/µmol 'monomer'), BisEMA-10 (5.39 ± 1.54 pmol/µmol 'monomer'), BisEMA-30 (2.37 ± 0.23 pmol/µmol 'monomer'), BisPMA (14.32 ± 1.94 pmol/µmol 'monomer'), and BADGE (6.22 ± 1.69 pmol/µmol 'monomer'). BPA was not detected in all other monomers in levels above the LOQ.

### 3.5. Chemical degradation

#### *3.5.1. Acidic degradation ('pH 1')*

Exposure to acidic media did not result in statistically significantly increased BPA levels for respectively BisGMA (Esstech) (5.16 ± 1.13 pmol BPA/µmol 'monomer'), and BisEMA-3 (23.48 ± 2.66 pmol BPA/µmol 'monomer'). Statistically significantly decreased BPA levels were observed for BisGMA (Sigma-Aldrich) (4.37 ± 0.74 pmol BPA/µmol 'monomer') compared to BPA impurity in this monomer batch. Statistically

significantly increased BPA levels were observed for BADGE (3.16 ± 1.24 pmol BPA/µmol 'monomer').

*3.5.2. Alkaline degradation ('pH 13')*

Exposure to alkaline media did not result in statistically significantly increased BPA levels for BisGMA (Esstech) (5.76 ± 0.22 pmol BPA/µmol 'monomer'), BisGMA (Sigma-Aldrich) (5.94 ± 0.57 pmol BPA/µmol 'monomer') and BisEMA-3 (26.04 ± 0.97 pmol BPA/µmol 'monomer'), respectively. Statistically significantly increased BPA levels were, however, observed for BisEMA-30 (1.03 ± 0.40 pmol BPA/µmol 'monomer'), BisPMA (8.49 ± 0.79 pmol BPA/µmol 'monomer') and BADGE (5.70 ± 0.54 pmol BPA/µmol 'monomer').

*3.5.3. Neutral degradation ('pH 7')*

No statistically significantly increased BPA levels were observed for respectively BisGMA (Esstech) (6.54 ± 0.26 pmol BPA/µmol 'monomer'), BisGMA (Sigma-Aldrich) (6.43 ± 0.47 pmol BPA/µmol 'monomer') and BisEMA-3 (25.91 ± 0.60 pmol BPA/µmol 'monomer'). Statistically significantly increased BPA levels were, however, observed for BADGE (2.42 ± 1.17 pmol BPA/µmol 'monomer').

## 4. DISCUSSION

The release of the endocrine disruptor BPA from resin-based dental materials is nowadays mainly ascribed to its presence as impurity in the monomers that make up the ingredients of dental composites, since BPA itself is not an intentional ingredient. However, it has also been speculated that BPA may be released upon (bio)degradation of BPA-based monomers over time. Until now, however, there was no direct evidence

available proving that, besides BisDMA, other BPA-based monomers can degrade into BPA under *in-vitro* conditions (15,24). In this study, however, we demonstrated that BisGMA, BisEMA, BisPMA and BADGE, all monomers frequently used in commercial composites, may be degraded to BPA when they are in contact to human saliva or to *S. mutans in vitro*. The null hypothesis must thus be rejected.

Our study consisted of two parts: in the first part, the presence of BPA in commercially purchased monomers was tested; in the second part, the presence of BPA due to decomposition was evaluated after salivary, bacterial and chemical challenge of the monomers. Nowadays, UHPLC-MS/MS is largely used for an accurate quantification of low-level impurities, such as BPA. To further decrease the detection limit, we included a derivatization step with PS chloride, which allowed the identification and quantification of low levels of BPA as impurities from dental monomers.

First, the purity of the BPA-based monomers included in this study was tested by dissolving three different amounts of each monomer in methanol. Non-BPA-based monomers were also tested to exclude potential contamination with BPA. Methanol was used due to its suitability to dissolve hydrophobic compounds. Only in BisGMA (both from Esstech and Sigma Aldrich), and in BisEMA-3, small amounts of BPA could be detected. BisEMA-3 contained the highest amount of BPA impurities (i.e. 0.0024%). In contrast, BPA impurities were not detected in BisEMA-10 and -30. In BisEMA-6, only small amounts of BPA were detected, although no increase was observed with increasing amount of monomer. Therefore, we concluded that the amount of BPA in BisEMA-6 was very low and could not be qualified as impurity under the given experimental conditions. Previous research showed that each respective standard BisEMA monomer was actually a mixture of several BisEMA molecules with differences in epoxy chain spacer length (25–27). For instance, BisEMA-3 used in this

study was previously found to contain monomers ranging from BisEMA-2 to BisEMA-7 (25). It is plausible that the presence of BPA impurities in BisGMA and BisEMA-3 must be attributed to an inefficient purification step during the production of these monomers. We also hypothesize that it is easier to remove BPA when it is aimed to only retain higher-molecular-weight monomers after separation, signifying that it is easier to produce Bis-EMA-6, -10 and -30 which are free of BPA. Anyhow, these results confirm the assumption that BPA may leach from composite restorations due to impurities in the ingredients used to mix the composite. It is therefore advisable that dental manufacturers always use monomers of the highest purity to minimize the release of BPA from resin-based dental materials. In addition, the purity of BisDMA was also tested in this study. However, BisDMA decomposed completely into BPA upon derivatization, and was therefore not included in the degradation experiments.

Schmalz *et al.* could not identify the presence of BPA in two commercial pure BisGMA-monomers, most likely due to a too low amount of tested monomer in combination with a less sensitive detection HPLC-UV/Vis analytical method (15). Only by using a more sensitive fluorescence detection method, Imai *et al.* was able to confirm the presence of BPA as an impurity in three commercial BisGMA monomers (17). In his study, however, the observed concentrations of BPA impurities ranged between 0.010% and 0.029% (42.6 – 130.0 µg BPA/g BisGMA), which is at least 16 times higher than the values observed in our study (i.e. 0.0006%). This difference might be attributed to differences in the purity of the batches of monomers. The amount of monomer used to detect BPA also proved to be important. In a pilot study, we noticed that a high enough amount of monomers needed to be tested to allow correct detection and quantification of BPA. By analogy, also the amount of derivatization reagent must be sufficiently high

to ensure an accurate detection. The highest amount with 3 µmol of monomer was also used for the following monomer degradation experiments in aqueous media.

When monomers elute from dental materials in the oral cavity, they are first exposed to saliva, but information on whether BPA-based monomers degrade into BPA upon to salivary exposure is missing. Therefore, we assessed the influence of whole human pooled saliva on monomer degradation. Previous research showed that enzymes, both from human and bacterial origin, present in saliva can degrade methacrylates, leading to the formation of methacrylic acids (8,28). However, these studies did not focus on the detection of BPA, except for the study of Schmalz (15).

The absence of BPA in the collected saliva could not be guaranteed, as BPA is ubiquitously present in our environment, but several measures were taken to ensure that BPA levels were low. As such, the presence of resin-based restorations was also not an absolute exclusion criterion for volunteers. In order to keep the background BPA levels as low as possible, volunteers were also selected based on low salivary BPA levels (tested *a priori*, data not shown). Furthermore, volunteers were instructed to avoid plastic food packaging materials the day before sampling. The amount of BPA in the collected saliva used for the experiment was also tested to confirm a low amount of BPA, and this amount was deducted from the quantity of BPA detected in the experimental samples. The fact that stimulated saliva has been used, could have had an effect on the outcome due to dilution of the bacterial and enzyme activity. (8) However, previous research failed to show statistical differences between stimulated and unstimulated saliva with regard to decomposition of TEGDMA. (29). This indicates that our results may also be representable for monomer degradation in unstimulated saliva.

We observed increased levels of BPA upon exposure to human pooled saliva for the monomers BisGMA, BisEMA-3, BisEMA-10, BisPMA and BADGE, which can only be attributed to partial decomposition of the monomers into BPA and other decomposition products. Only small portions of the monomers (0.0017%, 0.0003% and 0.0004% for respectively BisEMA-3, BisGMA (Esstech) and BisGMA (Sigma-Aldrich)) were degraded in 24 hours. To assess whether this activity should be described to bacterial degradation present in saliva, monomers were also exposed to *Streptococcus mutans*.

*S. mutans* is a well-known primary acidogenic and pathogenic species in dental plaque, and thus also present in saliva (30). It metabolizes dietary carbohydrates, such as sucrose, and generates lactic acid as a by-product. Ultimately, the accumulation of lactic acid in the plaque biofilm results in a localized drop in pH and subsequent demineralization of tooth enamel (the critical pH for enamel dissolution is around 5.5), marking the onset of dental decay (31,32). Recently, an enzyme with esterase activity has been isolated from *S. mutans*, known to degrade the resin part of the restoration-tooth interface, which facilitates the formation of secondary caries (19,33). Mutans streptococci tend to be more prevalent on restored surfaces than on sound tooth surfaces (34). Moreover, it was shown that the virulence of cariogenic bacteria is up-regulated by resin biodegradation by-products, creating a positive feedback loop that stimulates biodegradation (35). This abbreviates the lifespan of a composite restoration and ultimately leads to its replacement. The bacterial strain used in this study (*S. mutans* ATCC 25175) was derived from carious dentine, and an exposure time of 48h was chosen based on previous experiments (36). Exposure to *S. mutans* indeed resulted in increased levels of BPA, which must be attributed to bacterial degradation of the monomers.

We observed degradation of the following monomers into BPA upon exposure to *S. mutans*: BisGMA, BisEMA-3, BisEMA-6, BisEMA-10, BisEMA-30, BisPMA, and BADGE. However, the results for BisEMA-30 should be interpreted with caution, since BPA levels were close to LOQ and BPA was not found in all sample replicates in levels above the LOQ. Nevertheless, only small portions (0.0025%, 0.0006% and 0.0006% for respectively BisEMA-3, BisGMA (Esstech) and BisGMA (Sigma-Aldrich)) of the tested monomers were degraded.

To better gain insights whether these observations are rather enzyme- or pH-based, we also tested the effect of acidic and alkaline pH. Upon consumption of glucose-rich nutrition, bacteria are stimulated to produce lactic acid, which leads to a transient decrease in pH. Moreover, dental materials are also exposed to acids and alkaline nutrients when consuming food and beverages. Therefore, we assessed the influence of alkaline ('pH 13') and acidic ('pH 1') media on the degradation of monomers, using a neutral medium ('pH 7') as control. Only in alkaline medium, increased levels of BPA for BisEMA-30, BisPMA and BADGE were observed, which can be attributed to degradation to BPA. Furthermore, low levels of BPA were found for BisEMA-6, BisEMA-10, and BisEMA-30. However, these results should be interpreted with caution, since BPA was not found in all sample replicates in levels above the LOQ, and the analytical signal was weak. In contrast, decreased levels of BPA for BisGMA (Sigma-Aldrich) in acidic medium were observed, although the difference was minor.

Previous research showed that resin-based materials may be *in-vitro* degraded in a situation of high cariogenic challenge (i.e. acidic environment) (37), but in this study, we found no evidence that monomers degrade to BPA under acidic circumstances. Nonetheless, several studies reported that pendant methacrylate groups could be hydrolyzed in polymerized materials (18,19). It has been proposed that this structural

damage could facilitate the release of BPA from cured materials. However, more research is warranted.

Even though these *in-vitro* tests are not capable of reproducing all complex processes (such as pH fluctuations, bacterial contamination, salivary flow rate, …) in the oral cavity, the results of this study may give an insight of what might be anticipated *in vivo*. Until now, it was not known that BPA-based monomers, except for BisDMA, could be decomposed into BPA. The clinical relevance of the used amounts of monomers is arguable, since the exact amount of monomers that could be released from composite restorations *in vivo* is still not known (38). Michelsen *et al.* quantified BisGMA levels (ranging from 0.028 to 9.651 µg/mL) in saliva samples from 10 patients collected 10 minutes after treatment with a BPA-based adhesive and composite, while BisEMA was not detected. After 24 hours, no monomers could be detected anymore (6). Another study only reported the detection of degradation products of BPA-based monomers (5). However, it was recently shown that monomers are released *in vitro* even after one year, which may make dental restorations a potential long-term source for exposure to endocrine disrupting chemicals. In clinical conditions, composite surfaces are almost immediately covered with a salivary pellicle, followed by a biofilm that ultimately forms dental plaque. This could influence the surface monomer degradation and elution of monomers (2). Anyhow, more research is necessary to assess whether material degradation of polymerized materials also leads to an increase in BPA elution.

There is currently no conclusive evidence concerning the short-term and long-term effect of BPA on human health after the application of a resin-based dental material. Significant increased salivary and urinary BPA levels were found shortly after the restoration placement (39–41), although these levels are still low compared to exposure through food intake. Nevertheless, one should always consider the fact that

BPA is a substance that has been shown to exert effects by low-dose exposure (42). Furthermore, since BPA is already present as an impurity in dental monomers used to produce resin-based dental materials, it would be recommendable that future composites should not contain BPA-based monomers, especially when they are intended for use in children.

## 5. CONCLUSIONS

Accurate knowledge of monomers that contain BPA as impurities and that can be decomposed into BPA will help to develop BPA-free composites, thereby limiting human exposure. An increase in BPA levels was observed upon salivary and bacterial degradation of the monomers BisGMA, BisEMA, BisPMA, and BADGE. Furthermore, BisPMA and BADGE were also degraded into BPA under alkaline conditions. Impurities and degradation of these monomers may result in BPA release from dental materials.

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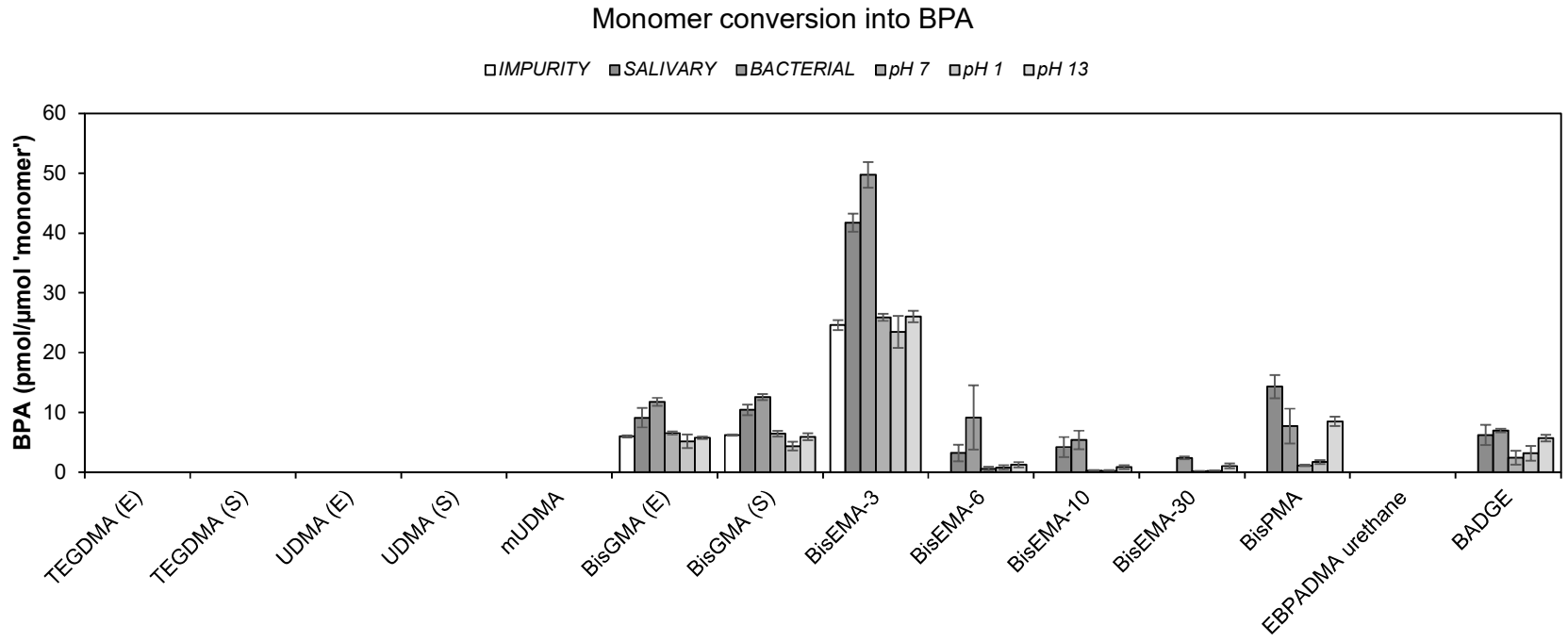
## FIGURES

*Figure 1*

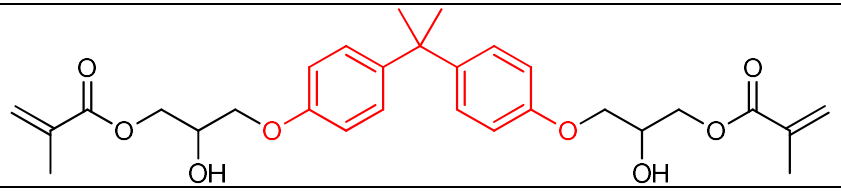
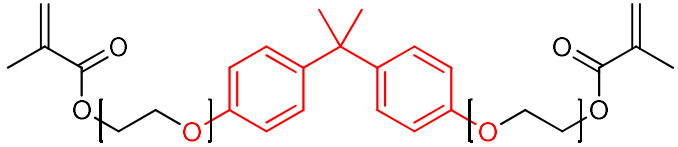
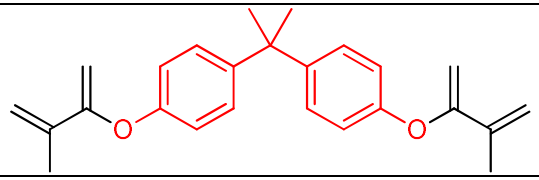
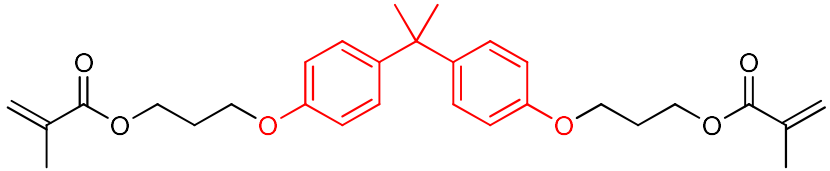


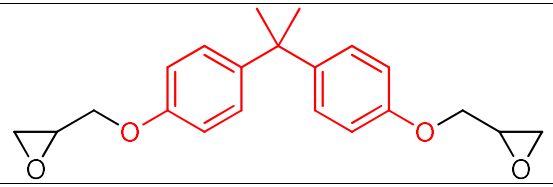
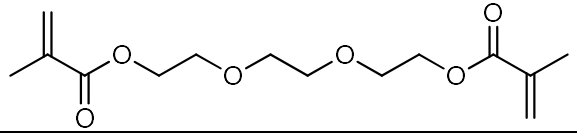
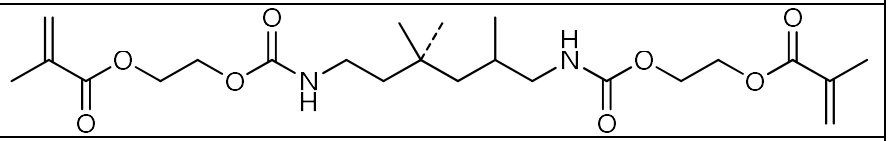
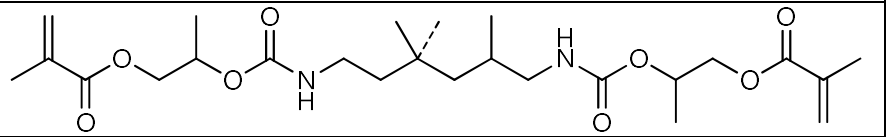
## FIGURE LEGENDS

**Figure 1.** BPA levels upon monomer degradation. (Values are expressed in pmol BPA/μmol monomer as mean ± standard deviation)

# TABLES

*Table 1. Overview of the monomers used in this study*

| Monomer | Manufacturer | CAS | MW (g/mol) | Mol. formula | Structure |
|---|---|---|---|---|---|
| BisGMA(E)<br>BisGMA(S) | Esstech, Inc.<br>Sigma-Aldrich | 1565-94-2 | 512.6 | $C_{29}H_{36}O_8$ |  |
| BisEMA(3)<br>BisEMA(6)<br>BisEMA(10)<br>BisEMA(30) | Esstech, Inc. | 41637-38-1 | 496.6<br>628.7<br>805<br>1686 | $C_{29}H_{36}O_{10}$<br>$C_{35}H_{48}O_{10}$<br>$C_{43}H_{64}O_{14}$<br>$C_{83}H_{144}O_{34}$ |  |
| BisDMA | Sigma-Aldrich | 3253-39-2 | 364.4 | $C_{23}H_{24}O_4$ |  |
| BisPMA | Sagechem | 27689-12-9 | 480.6 | $C_{29}H_{36}O_6$ |  |
| EBPADMA urethane | Dentsply | 216646-17-1 | 1361.1 | $C_{74}H_{96}N_4O_{20}$ | Proprietary monomer |

| | | | | | |
|---|---|---|---|---|---|
| BADGE | Sigma-Aldrich | 1675-54-3 | 340.4 | $C_{21}H_{24}O_4$ |  |
| TEGDMA (E)<br>TEGDMA (S) | Esstech<br>Sigma-Aldrich | 109-16-0 | 286.3 | $C_{14}H_{22}O_6$ |  |
| UDMA (E)<br>UDMA (S) | Esstech<br>Sigma-Aldrich | 72869-86-4 | 470.6 | $C_{23}H_{38}N_2O_8$ |  |
| mUDMA | Dentsply | 105883-40-7 | 498.6 | $C_{25}H_{42}N_2O_8$ |  |

**Abbreviations**: BisGMA: bisphenol A diglycidyl methacrylate; BisEMA: ethoxylated bisphenol A dimethacrylate; BisDMA: bisphenol A dimethacrylate; BisPMA: 2,2-Bis(4-methacryloxypropoxyphenyl)propane; EBPADMA urethane: ethoxylated bisphenol A dimethacrylate urethane; BADGE: bisphenol A diglycidyl ether; TEGDMA: triethylene glycol dimethacrylate; UDMA: urethane dimethacrylate; mUDMA: methylurethane dimethacrylate

*Table 2. Overview of the results*

| **Monomer** | **BPA impurity** (n=15) | **Degradation experiment** (n=5) | | | | |
|---|---|---|---|---|---|---|
| | | Salivary | Bacterial | Chemical | | |
| | | | | pH 7 | pH 1 | pH 13 |
| TEGDMA (E) | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ |
| TEGDMA (S) | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ |
| UDMA (E) | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ |
| UDMA (S) | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ |
| mUDMA | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ |
| BisGMA (E) | 5.99 ± 0.18 | 9.12* ± 1.63 | 11.77* ± 0.66 | 6.54 ± 0.26 | 5.16 ± 1.13 | 5.76 ± 0.22 |
| BisGMA (S) | 6.21 ± 0.10 | 10.42* ± 0.90 | 12.56* ± 0.49 | 6.43 ± 0.47 | 4.37* ± 0.74 | 5.94 ± 0.57 |
| BisEMA-3 | 24.61 ± 0.83 | 41.74* ± 1.50* | 49.75* ± 2.14* | 25.91* ± 0.60 | 23.48 ± 2.66 | 26.04 ± 0.97 |
| BisEMA-6 | <LOQ | 3.20 ± 1.40 | 9.16* ± 5.37 | 0.56 ± 0.33 | 0.77 ± 0.38 | 1.25 ± 0.44 |
| BisEMA-10 | <LOQ | 4.20* ± 1.68 | 5.39* ± 1.54 | 0.26 ± 0.09 | 0.22 ± 0.11 | 0.82 ± 0.34 |
| BisEMA-30 | <LOQ | <LOQ | 2.37* ± 0.23 | 0.16 ± 0.04 | 0.21 ± 0.07 | 1.03* ± 0.40 |
| BisPMA | <LOQ | 14.32* ± 1.94 | 7.71* ± 2.91 | 1.13 ± 0.13 | 1.68 ± 0.33 | 8.49* ± 0.79 |
| EBPADMA urethane | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ |
| BADGE | <LOQ | 6.22* ± 1.69 | 6.95* ± 0.32 | 2.42* ± 1.17 | 3.16* ± 1.24 | 5.70* ± 0.54 |
| BisDMA | N.A. | N.I. | N.I. | N.I. | N.I. | N.I. |

Results are expressed as pmol BPA/µmol 'monomer' (mean ± standard deviation). Degradation experiments were compared against BPA impurity using post-hoc Dunnett (*p<0.05). *(<LOQ: below limit of quantification; N.A.: BPA impurities in BisDMA could not be reported due to complete decomposition upon derivatization and was therefore not included (N.I.) in degradation experiments)*