Long-term elution of bisphenol A from dental composites

Research article

LONG-TERM ELUTION OF BISPHENOL A FROM RESIN-BASED DENTAL COMPOSITES

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ABSTRACT

Introduction: BPA release from resin-based composites on the short term has been reported in several *in-vitro* and *in*-vivo studies. However, it remains unclear whether these materials also leach BPA on the long term. Despite,the one year elution of various BPA-based methacrylate monomers from resin-based dental composites was previously described, quantitative data have not been reported due to the lack of a sensitive method to accurately quantify the low levels that might be released.

Materials and methods: Composite disks (n = 6, 6 mm diameter and 2 mm height) from four commercial materials (G-ænial Posterior, Venus, Ceram.x mono and Filtek Supreme XTE) were immersed in 1 mL of water or ethanol as extraction solvent and stored in the dark at 37 °C. The extraction solvent was renewed weekly. Samples were derivatized with pyridine-3-sulfonyl chloride before analysis with UPLC-MS/MS.

Results: Derivatizing BPA increased the sensitivity of the analytical method. BPA eluted continuously in ethanol from all four tested composites over a period of one year when a weekly refreshing protocol was followed. BPA elution was clearly higher when ethanol was used as extraction solution. In water, BPA elution persisted for the entire period, but levels could not be accurately quantified anymore after several weeks.

Significance: Resin-based composites can be considered as a potential long-term source of BPA, and thus should not be neglected when assessing the overall exposure to endocrine disrupting chemicals.

KEYWORDS

BPA, elution, resin-based dental composite, monomers, endocrine disruptor

1. INTRODUCTION

Dental composites, of which the great majority contain bisphenol A (BPA)-based methacrylate monomers, have replaced amalgam as the golden standard material in restorative dentistry. They are increasingly used in daily practice because of their superior esthetics and ease of handling, although shortcomings such as a limited lifetime compared to amalgam have manifested (1). Despite the successful use of these materials, there is still some uncertainty about the safety and biocompatibility of composites, especially in scientific literature (2). This is mainly attributed to the release of several components in the oral cavity after light curing (3).

From all leached and detected ingredients, BPA has led to the most controversy. Hence, despite numerous controversial discussions and a lack of consensus about its safety (4–6), BPA was classified by the European Chemicals Agency (ECHA) as a 'substance of very high concern' since it was identified as an endocrine disruptor for human health (toxic for human reproduction based on Article 57c) and environment (Article 57f), as determined in Regulation (EC) No 1907/2006 (7). Moreover, besides its well-known estrogenic activity, growing evidence indicates that exposure to BPA is also associated with an increased risk of developing type 2 diabetes (8,9), obesity (10), adverse immune effects (5), and altered neuroendocrine development (11,12).

BPA itself, however, is not added as an intentional ingredient in resin-based composites, but is present as an impurity since it is used in the synthesis of monomers used in resin-based composites (13). In addition, increased amounts of BPA were recently quantified upon salivary and bacterial challenge of BPA-based monomers (14). Thus, monomer degradation may play a role in the BPA release in the oral cavity.

Since composite materials are expected to have a service life of several years in the mouth, extended storage periods in *in-vitro* studies are necessary to investigate the long-term release of various ingredients from composites. It was already shown *in vitro* that (BPA-based) monomers can elute from resin-based composites over a period up to one year following a weekly refreshing protocol of the extraction solution (i.e. water, ethanol and artificial saliva) (15). However, BPA levels could not be accurately quantified since they were lower than the method's lower limit of quantification (i.e. 50 ng/mL or 219 pmol). Furthermore, good knowledge on the long-term release of BPA from resin-based materials is primordial to evaluate if these materials can be considered as a potential relevant long-term source that contributes to the overall human exposure of BPA.

The aim of the present study was to evaluate the long-term *in-vitro* release of BPA from four resin-based commercial composites over a period of one year. Composite disks were immersed in either water or ethanol during a period of 52 weeks, while the extraction solutions were refreshed weekly. The release of BPA was quantified using a sensitive ultra-performance liquid chromatography–tandem mass spectrometry (UPLC–MS/MS) quantification method, that allows accurate quantification of low levels of BPA (16).

2. MATERIALS AND METHODS

2.1. Investigated materials

Four resin-based dental composites were selected based on their resin composition and contain at least one BPA-based monomer, as mentioned in the safety data sheets provided by the manufacturer (Table 1).

2.2. Elution experiment

Samples were prepared and light-cured as described in Putzeys *et al* (15,17). After polymerization, the specimens (n = 6 for each solvent) were immediately immersed in 1 mL of H_2O or EtOH in glass vials that were firmly closed with aluminum crimp caps with molded septa of butyl/PTFE. The ratio between the sample and the extraction solution volume was greater than 1:10 and the samples were fully immersed, which is in line with the requirements of ISO10993-13. The samples were stored in the dark at 37 °C and the extraction solution was renewed every week during a period of one year. Samples were stored at −80 °C until analysis. To avoid contamination, care was taken to use only glass pipettes and glass containers. An aliquot of 500 µL was taken for the analysis of BPA after which 40 pmol stable isotope labeled BPA-d_{16} was added as internal standard. Appropriate negative controls containing only H_2O or EtOH were also processed as real samples. These values were subtracted from sample values.

2.3. BPA detection

Although the extraction solutions were refreshed every week during a period of one year, only the samples of week 1, 2, 3, 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44, 48 and 52 were analyzed using UPLC–MS/MS.

2.3.1. BPA derivatization

BPA derivatization was done according to Regueiro et al (18), 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, 5 µmol PS chloride in 200 µL acetonitrile was added 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 the

addition of 100 µL formic acid (1 M). The reaction mixture was passed through a 0.2 µm regenerated cellulose syringe filter and analyzed by UPLC-MS/MS.

2.3.2. UPLC-MS/MS analysis of BPA

BPA was detected using an UPLC-MS/MS method as previously described (16), with minor adjustments. Sample run time was prolonged until 4 minutes to ensure the clearance from all components.

2.4. Statistical analysis

R software (version 3.6.1, R Foundation for Statistical Computing, Vienna, Austria) was used for statistical analysis. The differences in cumulative BPA elution after 52 weeks between the different extraction media were compared using paired t-test, respectively for each material. The differences in cumulative BPA elution after 52 weeks between the four different materials were compared using one-way ANOVA, respectively for each extraction solution. In order to calculate the total BPA release in one year, the release on the non-analyzed time points was estimated using a nonparametric regression model (locally weighted scatterplot smoothing, LOWESS method). Level of significance was set at $p<0.05$.

3. RESULTS

3.1. Validation results

The instrumental limit of detection was 0.20 pmol BPA. A calibration curve of BPA was obtained using BPA-d_{16} as internal standard and was linear within the calibration range. The lower limit of quantification (LLOQ) was 0.78 pmol BPA. The correlation coefficient R^2 of the regression equation was > 0.99. If the values of 60% or less of the

replicates were below LLOQ, these values were replaced by LLOQ/2 (i.e. 0.39 pmol BPA) for statistical comparison. When the values of more than 60% of the replicates were below LLOQ, these values were replaced by LOD (i.e. 0.20 pmol BPA) (19).

3.2. Elution of BPA

Depending on the composite and the extraction solution, BPA continuously eluted from the materials, up until a period of 52 weeks after polymerization and initial immersion into the solvent (Table 2 and Figure 1). Cumulative BPA release was significantly lower when water was used as extraction solution compared to pure EtOH for all materials ($p<0.001$), except Filtek Supreme XTE ($p=0.36$).

In water, the BPA elution from G-ænial Posterior, Venus, Ceram.x mono and Filtek Supreme XTE could not be accurately quantified anymore in all replicates after a period of respectively 12, 2, 4 and 4 weeks. However, BPA levels increased again above LOQ between week 16 and 24, and after 16 weeks until the end of the tested period for Venus and Filtek Supreme XTE, respectively. Nonetheless, BPA elution from all materials persisted until the end of the tested period in levels above the limit of detection. G-ænial Posterior (54.6 ± 1.9 pmol) released significantly more BPA than both Venus (23.7 ± 4.6 pmol) and Ceram.x mono (31.4 ± 1.6 pmol), but also released significantly less BPA compared to Filtek Supreme XTE (78.7 ± 6.9 pmol) after 52 weeks ($p<0.0001$). No significant difference in cumulative BPA elution was observed between Venus and Ceram.x mono ($p=0.31$).

In ethanol, the BPA elution from all materials persisted until the end of the tested period in levels above the limit of quantification. Both Venus (98.4 ± 4.7 pmol) and Ceram.x mono (86.9 ± 2.2 pmol) released significantly more BPA than Filtek Supreme XTE (71.6 ± 7.9 pmol), but also released significantly less BPA compared to G-ænial

Posterior (313.9 ± 8.5 pmol) after 52 weeks ($p<0.0001$). No significant difference in cumulative BPA elution was observed between Venus and Ceram.x mono ($p=0.09$).

BPA was not always quantified upon weekly renewal of the extraction solution. Therefore, the cumulative release (as shown in Table 2 and Figure 1) does not give a complete view of the total release in 52 weeks. By interpolating our results using LOESS regression, the total amount of BPA released in one year from G-ænial Posterior, Venus, Ceram.x mono and Filtek Supreme XTE was estimated to be 91.7 pmol, 67.0 pmol, 48.9 pmol, and 253.1 pmol in water, and 603.4 pmol, 262.4 pmol, 255.6 pmol, and 196.4 pmol in ethanol, respectively (Table 3 and Figure 2).

3.3. Estimation of the potential exposure to BPA from dental composite

With the highest elution results in water during the first four weeks from G-ænial Posterior, the estimated daily intake (EDI) of BPA released from total crown restorations was calculated based on the average total crown surface areas (3) and the assumption that the body weight of adults and children was 70 kg and 20 kg, respectively (Table 4). Patients with total wear (tooth loss due to attrition, abrasion and erosion) typically require full crown restorations of all teeth. In this worst-case scenario, the total exposed surface area (including all 32 teeth) would be 7372 mm^2.

4. DISCUSSION

Currrently, dental composites are not considered as a potential relevant (long-term) source of BPA. Furthermore, due to a lack of appropriate experimental setups designed for long-term elution on one hand and a lack of sensitive analytical methods able to accurately quantify low levels of BPA on the other hand, little information is available about the long-term release. Typically, samples are incubated for long periods without renewal of the incubation solution (20,21). In contrast, in our previous

study, the long-term release of (BPA-based) monomers was determined in a setup with equal-interval solvent change (17). However, the eluates were analyzed with an analytical method that simultaneously quantified up to 11 compounds, including BPA, in one run. The major disadvantage of this non-specific approach is a loss in sensitivity. Consequently, the released amounts of BPA were all under the methods LLOQ (i.e. 219 pmol BPA/mL).

An accurate quantification of low BPA levels requires a more specific and sensitive analytical method. We were able to lower our methods LLOQ (i.e. 0.72 pmol BPA) by using a derivatization reagent (i.e. pyridine-3-sulfonyl chloride), which allowed an accurate quantification of low levels of BPA. Previously, we already characterized the daily BPA-release in artificial saliva from dental composites over a period of one week using this procedure (16). This is the first time that the long-term elution of BPA was determined following a weekly refreshing protocol over a period of one year.

In water, all materials released BPA in detectable amounts during the entire testing period although BPA could not be detected anymore in levels above the LLOQ after several weeks. However, BPA elution from Filtek Supreme XTE increased again after 12 weeks, which might suggest that hydrolytic degradation of the polymer enhances the leaching process in later phases. Since only 500 µL eluate was used for analysis, it might be possible that for some samples of later time points, BPA could have been detected if the complete sample was used. In contrast, BPA was still released in quantifiable amounts from all materials after a storage period of 52 weeks in ethanol. Therefore, dental composites could be considered as a potential long-term source of BPA.

Nonetheless, questions about the relevance of the exposure from dental materials remain. In a worst-case scenario, children are estimated to be exposed to approximately 2 ng/kg bw/day during the first week based on our results. In epidemiological studies, however, increased salivary BPA levels are only reported during the first hours directly after dental treatment (22–25), which contribute to the total oral exposure of BPA. The reported *in-vivo* amounts are lower and possibly neglectable when compared to exposure from other sources such as the diet and remain therefore undetected.

Compared to the current temporary (t-)TDI of 4000 ng/kg bw/day set by the European Food Safety Authority (EFSA), one can thus conclude that dental composites pose no health risks. However, this t-TDI is based on adverse effects on the kidney observed in multigeneration reproductive toxicity studies in mice (26) and concerns have been expressed about possible BPA effects observed at low doses on mammary gland, reproductive, neurological, immune and/or metabolic systems. In addition, mixtures of endocrine disruptors can produce adverse effects, even when each chemical is present at low doses that individually do not induce observable effects, known as the cocktail-effect (27,28). Therefore, a re-evaluation of potential BPA hazards is currently on-going by EFSA considering these recent publications.

As mentioned before, our previous study failed to report the detection of BPA in these samples. The highest level of BPA (i.e. 150 pmol BPA or 34.2 ng/mL), released from G-ænial Posterior after week 1 in ethanol was still considerably below the methods quantification limit (i.e. 50 ng/mL). Also other studies failed to detect the elution of BPA as well (29,30). In addition, the amounts of BPA present as impurities are small, and it is therefore extremely difficult to follow the release of trace amounts of BPA from

commercial materials when sensitive quantification methods are not available. This highlights the importance of a sensitive analytical method.

Only few other studies focused on long-term elution of BPA. In a study by Mourouzis *et al*., BPA was not detected upon immersion of resin-modified cements and composite resin CAD-CAM blocks in both water and ethanol for a period up to 60 days (20). In a study by Polydorou *et al*., BPA could still be detected up to an immersion period of 28 days in 75% EtOH, but not after 1 year of storage (21). Furthermore, no BPA was eluted from Filtek Supreme XTE, which is in contrast to our findings. This study also points out the need for a weekly refreshment protocol of the extraction medium, since similar amounts of BisGMA were found after each immersion/storage period (1 day, 1 week, 4 weeks and 1 year). Cokic *et al*. showed in a recent study that elution kinetics in *in-vitro* experiments are also influenced by saturation of the extraction solvent by the leached monomers and compounds, which may result in reduced elution (3). Therefore, following a weekly refreshing protocol is recommended when assessing long-term monomer elution.

Predictions about BPA release from a specific material are very difficult to make. First, the exact composition of commercially available resin-based composites is disclosed as a trademark secret. Furthermore, information about the long-term release of BPA-based monomers is not indicative of the BPA release. The long-term release of BPA-based monomers from Filtek Supreme XTE is significantly higher in ethanol compared to G-ænial Posterior (15). In contrast, significantly more BPA was released from the latter material. Differences in purity in the batches of monomers used to produce composite materials could possibly explain this discrepancy between manufacturers. Nevertheless, we also showed that the material Filtek Supreme XTE (1.53 ± 0.06 µg BPA/g material) contains significantly more BPA impurities compared to G-ænial

Posterior (1.11 ± 0.06 µg BPA/g material) (paper under review). Thus, one could hypothesize that the BPA release from Filtek Supreme XTE should have been greater compared to G-ænial Posterior. However, our results show that significantly more BPA was released in ethanol from G-ænial Posterior, but not in water. The differences between solvents indicate that also other factors than merely resin composition should be considered when evaluating monomer elution.

Immersion in water was used to simulate human saliva and is therefore more relevant for human exposure than ethanol, although saliva contains proteins and enzymes with esterase activity. It is possible that BPA elution wanes earlier in saliva compared to water and ethanol, possibly due to the formation of a salivary pellicle, as already suggested by Putzeys *et al.* (15). Ethanol is an organic solvent and represents a worst-case scenario for total elution.

To obtain more insights in the characteristics/kinetics of BPA release, it could be interesting on one hand to control the resin composition of experimental composites, which allows assessing how the release of BPA is influenced by the resin matrix. On the other hand, intentionally adding (low levels of) BPA to experimental composites will allow better characterize the BPA release on long-term.

5. CONCLUSION

Resin-based composites continue to release BPA in water and ethanol over a period of minimum one year when a weekly refreshing protocol is followed. Although no evidence has been reported of long-term release *in vivo*, dental materials should not

be neglected when describing human exposure to BPA and assessing possible associated health effects.

ACKNOWLEDGMENTS

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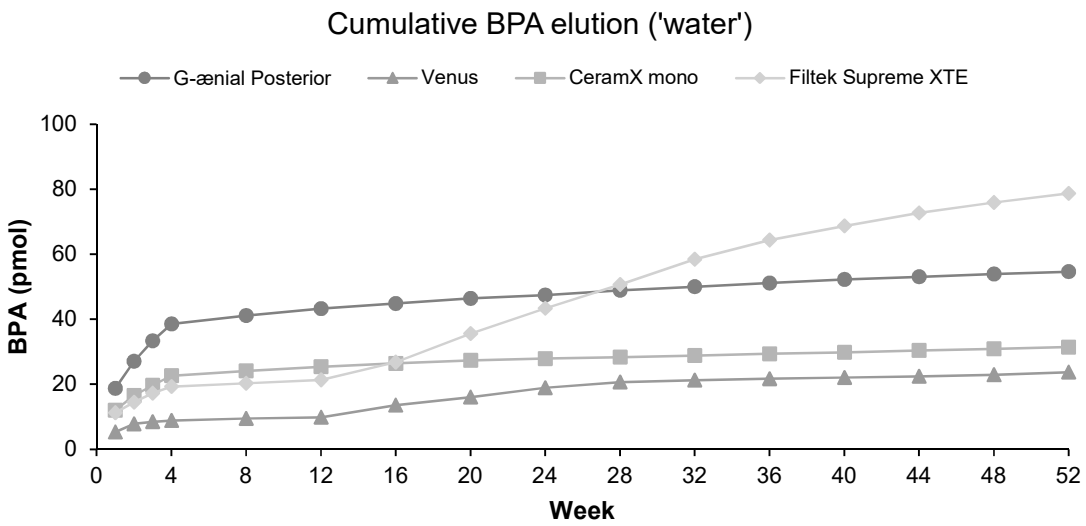
FIGURES

Figure 1: Cumulative elution

A



B

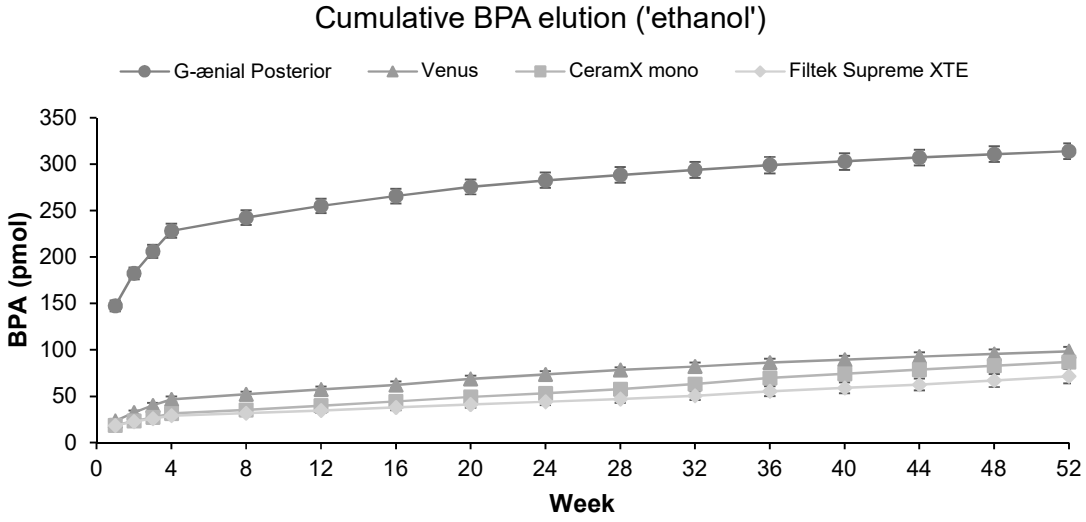
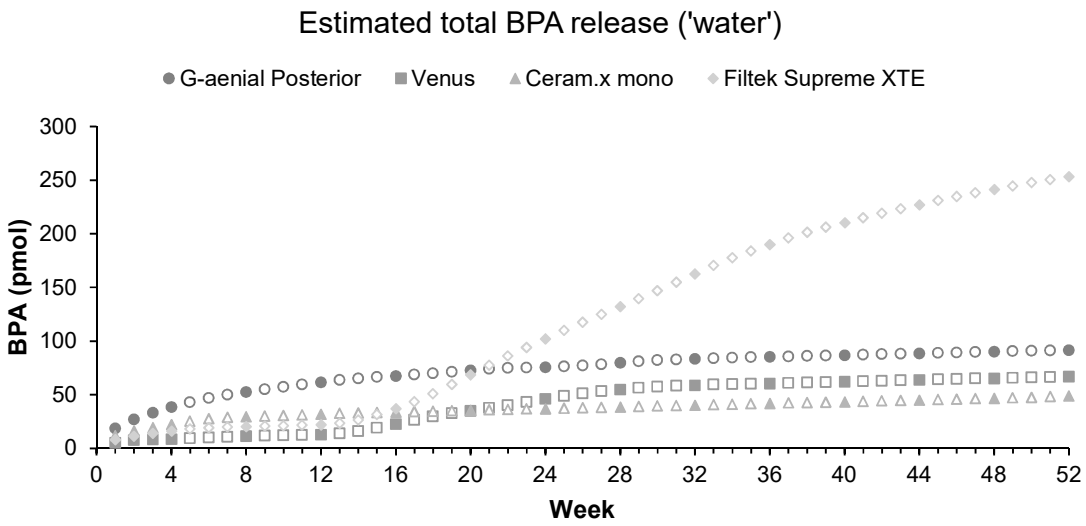


Figure 2: Total elution

A



B

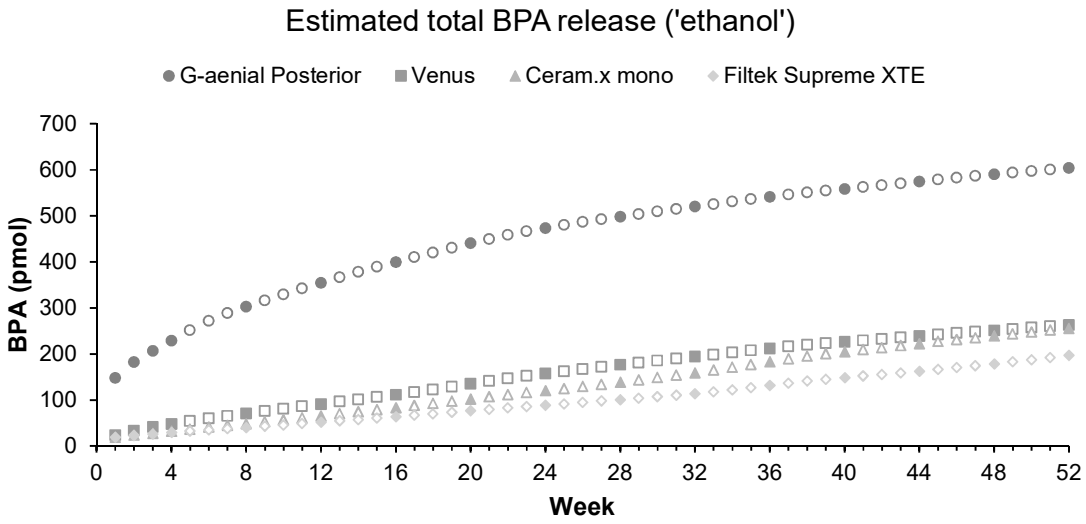


FIGURE LEGENDS

Figure 1. Cumulative BPA elution in water (A) and ethanol (B).

Figure 2. Estimated total BPA elution in water (A) and ethanol (B). Filled and unfilled markers represent respectively quantified and non-quantified time points.

TABLES

Table 1. Overview of the materials used in this study

Material	Shade	Manufacturer	Resin composition
G-ænial Posterior	P-A3	GC Europe, Leuven, Belgium	UDMA, TEGDMA, BisEMA*
Venus	A3	Kulzer, Hanau, Germany	BisGMA*, TEGDMA
Ceram.x mono	A3	Dentsly, Konstanz, Germany	BisGMA*, TEGDMA, UDMA
Filtek Supreme XTE	A3	3M ESPE, Seefeld, Germany	BisGMA*, UDMA, TEGDMA, BisEMA(6)*, PEGDMA

Asterisk (*) indicates BPA-based monomers.

Abbreviations: BisGMA: bisphenol A diglycidyl methacrylate; BisEMA: ethoxylated bisphenol A dimethacrylate; PEGDMA: polyethylene glycol dimethacrylate; TEGDMA: triethylene glycol dimethacrylate; UDMA: urethane dimethacrylate

Table 2. BPA elution

Week	G-ænial Posterior*,b,A		Venus*,c,B		Ceram.x mono*,c,B		Filtek Supreme XTE*,a,C	
	Water	Ethanol	Water	Ethanol	Water	Ethanol	Water	Ethanol
1	18.7 ± 1.1	147.3 ± 5.9	5.3 ± 0.6	23.4 ± 2.2	11.8 ± 0.4	18.7 ± 0.7	11.2 ± 7.3	18.5 ± 1.3
2	8.4 ± 0.8	35.0 ± 1.0	2.5 ± 0.3	9.9 ± 1.5	4.5 ± 0.2	4.6 ± 0.2	3.3 ± 0.5	4.1 ± 0.3
3	6.3 ± 0.6	23.8 ± 0.9	0.6 ± 0.5	7.7 ± 0.7	3.2 ± 0.5	4.0 ± 0.1	2.7 ± 0.4	3.4 ± 0.3
4	5.2 ± 0.4	22.1 ± 0.6	0.4 ± 0.0	6.6 ± 0.5	2.9 ± 0.6	4.2 ± 0.3	2.0 ± 0.2	3.1 ± 0.3
8	2.6 ± 0.2	14.2 ± 0.6	0.6 ± 0.6	5.3 ± 0.4	1.5 ± 0.2	3.9 ± 0.3	1.1 ± 0.1	2.6 ± 0.3
12	2.1 ± 0.2	12.5 ± 0.5	0.4 ± 0.0	5.2 ± 0.2	1.3 ± 0.2	4.5 ± 0.2	1.0 ± 0.2	3.0 ± 0.3
16	1.6 ± 0.1	10.1 ± 0.5	3.7 ± 1.4	5.1 ± 0.4	1.0 ± 0.2	4.4 ± 0.2	5.5 ± 1.4	3.1 ± 0.3
20	1.6 ± 0.3	9.9 ± 0.4	2.5 ± 1.3	6.3 ± 0.3	0.9 ± 0.1	4.9 ± 0.2	8.7 ± 1.0	3.2 ± 0.5
24	1.1 ± 0.1	7.2 ± 0.5	2.9 ± 1.4	5.0 ± 0.4	0.5 ± 0.2	4.3 ± 0.2	7.8 ± 1.0	2.9 ± 0.4
28	1.5 ± 0.8	5.8 ± 0.4	1.7 ± 2.7	4.7 ± 0.5	0.4 ± 0.2	4.7 ± 0.2	7.3 ± 1.0	3.0 ± 0.5
32	1.1 ± 0.2	5.4 ± 0.3	0.6 ± 0.5	4.4 ± 0.4	0.4 ± 0.2	5.4 ± 0.2	7.8 ± 1.2	3.5 ± 0.6
36	1.2 ± 0.2	5.1 ± 0.3	0.4 ± 0.0	4.5 ± 0.3	0.4 ± 0.2	6.3 ± 0.2	5.9 ± 0.8	5.0 ± 0.8
40	1.1 ± 0.1	3.9 ± 0.2	0.4 ± 0.0	3.0 ± 0.2	0.4 ± 0.2	4.7 ± 0.3	4.3 ± 0.7	3.6 ± 0.6
44	0.8 ± 0.1	4.3 ± 0.9	0.4 ± 0.0	3.3 ± 1.2	0.4 ± 0.4	4.4 ± 0.3	4.0 ± 0.7	3.6 ± 0.5
48	0.8 ± 0.1	3.6 ± 0.1	0.4 ± 0.0	2.9 ± 0.3	0.4 ± 0.2	4.1 ± 0.2	3.2 ± 0.6	4.3 ± 0.7
52	0.7 ± 0.1	3.2 ± 0.2	0.4 ± 0.0	2.9 ± 0.2	0.6 ± 0.5	4.2 ± 0.1	2.8 ± 0.5	4.6 ± 0.9
Cumulative	54.6 ± 1.9	313.9 ± 8.5	23.7 ± 4.6	98.4 ± 4.7	31.4 ± 1.6	86.9 ± 2.2	78.7 ± 6.9	71.6 ± 7.9

Results are expressed as pmol BPA (mean ± standard deviation). Asterisk (*) indicate a significant difference in cumulative BPA elution between water and ethanol for each respective material (paired t-test, $p<0.05$). Different small and capital letters indicate significant differences in cumulative BPA elution in respectively water and ethanol between the four materials (one-way ANOVA + Tukey post-hoc test, $p<0.05$).

Table 3. Estimated weekly BPA elution

Week	G-ænial Posterior		Venus		Ceram.x mono		Filtek Supreme XTE	
	Water	Ethanol	Water	Ethanol	Water	Ethanol	Water	Ethanol
1	18.7	147.3	5.3	23.4	11.9	18.7	8.3	18.5
2	8.4	35.0	2.4	9.9	4.6	4.6	3.2	4.1
3	6.3	23.8	0.7	7.7	3.1	4.0	2.7	3.4
4	5.2	22.1	0.4	6.6	2.9	4.2	2.1	3.1
5	4.5	22.9	0.6	6.0	2.9	4.5	1.8	2.8
6	3.8	20.4	0.7	5.7	2.2	4.3	1.2	2.7
7	3.1	16.7	0.7	5.5	1.3	4.0	0.7	2.6
8	2.6	14.2	0.6	5.3	0.7	3.9	0.4	2.6
9	2.4	13.4	0.5	5.2	0.5	4.0	0.4	2.7
10	2.3	13.0	0.3	5.2	0.5	4.2	0.4	2.8
11	2.3	12.8	0.2	5.2	0.6	4.4	0.4	2.9
12	2.1	12.5	0.4	5.2	0.7	4.5	0.4	3.0
13	1.9	12.0	1.1	5.1	0.6	4.5	1.3	3.0
14	1.6	11.5	2.1	5.1	0.5	4.5	3.1	3.0
15	1.3	11.0	3.1	5.0	0.5	4.4	5.0	3.1
16	1.1	10.6	3.7	5.1	0.4	4.4	5.8	3.1
17	1.1	10.4	3.7	5.4	0.4	4.5	6.3	3.1
18	1.3	10.3	3.3	5.8	0.4	4.7	7.4	3.2
19	1.4	10.2	2.8	6.3	0.4	4.9	8.6	3.2
20	1.4	9.9	2.5	6.3	0.4	4.9	9.1	3.2
21	1.2	9.4	2.5	6.1	0.4	4.8	8.9	3.2
22	0.8	8.6	2.7	5.8	0.4	4.6	8.5	3.1
23	0.5	7.8	2.9	5.3	0.4	4.4	8.1	3.0
24	0.4	7.2	2.9	5.0	0.4	4.3	8.0	3.0
25	0.5	6.7	2.7	4.9	0.4	4.4	7.9	2.9
26	0.9	6.4	2.4	4.8	0.4	4.5	7.6	2.9
27	1.2	6.0	2.1	4.8	0.4	4.6	7.3	3.0
28	1.4	5.8	1.7	4.7	0.4	4.7	7.2	3.0
29	1.3	5.6	1.4	4.6	0.4	4.9	7.3	3.1
30	1.1	5.5	1.1	4.5	0.4	5.0	7.6	3.2
31	0.8	5.5	0.8	4.4	0.4	5.2	7.8	3.3
32	0.6	5.4	0.6	4.4	0.4	5.4	8.0	3.5
33	0.5	5.3	0.5	4.4	0.4	5.7	7.7	3.9
34	0.5	5.3	0.4	4.5	0.4	6.0	7.0	4.4
35	0.4	5.2	0.4	4.5	0.4	6.3	6.4	4.8
36	0.4	5.1	0.4	4.5	0.4	6.3	6.1	5.0
37	0.4	4.8	0.4	4.2	0.4	6.1	5.9	4.8
38	0.4	4.4	0.4	3.7	0.4	5.6	5.3	4.4
39	0.4	4.1	0.4	3.3	0.4	5.1	4.7	3.9
40	0.4	3.9	0.4	3.0	0.4	4.7	4.4	3.6
41	0.4	3.9	0.4	3.0	0.4	4.5	4.4	3.5
42	0.4	4.1	0.4	3.1	0.4	4.5	4.2	3.5
43	0.4	4.2	0.4	3.3	0.4	4.5	4.1	3.5
44	0.4	4.3	0.4	3.3	0.4	4.4	4.0	3.6
45	0.4	4.2	0.4	3.3	0.4	4.3	3.9	3.8
46	0.4	4.0	0.4	3.2	0.4	4.2	3.6	4.0
47	0.4	3.8	0.4	3.0	0.4	4.2	3.4	4.2
48	0.4	3.6	0.4	2.9	0.4	4.1	3.3	4.3
49	0.4	3.5	0.4	2.9	0.4	4.1	3.2	4.4
50	0.4	3.4	0.4	2.9	0.5	4.1	3.0	4.5
51	0.4	3.3	0.4	2.9	0.6	4.2	2.9	4.6
52	0.4	3.2	0.4	2.9	0.7	4.2	2.8	4.6
Total	91.7	603.4	67.0	262.4	48.9	255.6	253.1	196.4

Results are expressed as pmol BPA.

Table 4. Estimated daily intake

Typical restorations		**SA (mm^2)**	**Estimated daily intake (pg/kg bw/day)**			
			Acute exposure (week 1)		**Chronic exposure (week 2-4)**	
			Adults (70 kg)	Children (20 kg)	Adults (70 kg)	Children (20 kg)
Front teeth	**Central incisor**	223	20.6	57.7	7.3	25.4
	Lateral incisor	178	16.5	46.1	5.8	20.3
	Canine	210	19.4	54.4	6.8	24.0
Premolars	**First premolar**	203	18.8	52.6	6.6	23.2
	Second premolar	191	17.7	49.5	6.2	21.8
Molars	**First molar**	315	29.1	81.6	10.3	35.9
	Second molar	276	25.5	71.5	9.0	31.5
	Third molar	247	22.8	64.0	8.1	28.2
4 quadrants		7372	681.7	1908.9	240.4	841.2