Treatment with Dental Polymer-based Restorative Materials

Exposure to Bisphenol A
Effects on Pregnancy Outcomes

Trine Lise Lundekvam Berge

Thesis for the degree of Philosophiae Doctor (PhD)
University of Bergen, Norway
2019

UNIVERSITY OF BERGEN

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Print: Skipnes Kommunikasjon / University of Bergen

In memory of my parents, Lilly and Nord Lundekvam,
for their endless love and support

"Wisdom is not a product of schooling but of the lifelong attempt to acquire it"
Albert Einstein (1879-1955)

Contents

Scientific environment

The scientific work of this thesis was initiated and carried out at the Dental Biomaterials Adverse Reaction Unit, NORCE Norwegian Research Centre AS. The Principal Researcher, Lars Björkman (PhD), who also is an Adjunct Professor at the Department of Clinical Dentistry, University of Bergen, has been my main supervisor. Gunvor Bentung Lygre (Dr. Odont), Senior Researcher (Forsker II) at NORCE Norwegian Research Centre AS, has been my co-supervisor.

The clinical work including the dental treatment was carried out at Årstad Dental Clinic, The Public Dental Health Care Services, Hordaland County Council, Norway.

During my PhD period, I have had a position as researcher/dentist at the Norwegian Dental Biomaterial Adverse Reaction Unit, NORCE Norwegian Research Centre AS and have been a Doctoral Research Fellow at The Oral Health Centre of Expertise in Western Norway, Hordaland, Hordaland County Council. As a PhD candidate, I have been affiliated with the PhD program at the Department of Clinical Dentistry, Faculty of Medicine, University of Bergen.

The Dental Biomaterial Adverse Reaction Unit and the Oral Health Centre of Expertise in Western Norway, Hordaland, have supported me financially. The Dental Biomaterials Adverse Reaction Unit is funded by the Norwegian Directorate of Health and covered the cost of the research projects in Study I and in Study III. Study II was funded by a grant from the Norwegian Directorate of Health.

Acknowledgements

First, I want to thank the participants in the studies comprising this thesis. Without your cooperation and willingness to spend your time, the studies could not have been performed. I am grateful to the Department of Clinical Dentistry, Faculty of Medicine, for giving me the opportunity to participate in the PhD program at the University of Bergen. I would like to thank The Dental Biomaterial Adverse Reaction Unit, NORCE AS and the Oral Health Centre of Expertise in Western Norway, The Public Dental Health Care Services, Hordaland County Council for financial support.

During the PhD period, I have received help and support from a large number of people. In particular, I want to thank:

My main supervisor, Principal Researcher and Adjunct Professor **Lars Björkman**, for guiding me throughout these years with extensive research experience, wide knowledge, and never-ending enthusiasm. Thank you for encouraging me to dive into the scientific field and for sharing your knowledge with me. Your door has always been open, and my suggestions and questions have been met with positivity and respect.

My supervisor, Senior Researcher **Gunvor Bentung Lygre**, for excellent scientific guidance and unlimited support, encouragement, and availability. I am truly grateful for your special skill to simplify things, your meaningful, constructive contribution to my work, and for having you by my side throughout the work for this thesis.

Professor **Stein Atle Lie**, for invaluable support concerning statistical analyses and understanding. Thank you for the good conversations and for your sense of humor. I am grateful to have you as a coauthor.

Senior university lecturer **Christian Lindh**, Department of Occupational and Environmental medicine, Lund University, Sweden, for developing the analytical

methods, being responsible for the analyses of bisphenol A, and for important contributions as a coauthor.

Hilde Kopperud, Nordic Institute of Dental Materials (NIOM AS), for kindly sharing your expertise in the field of dental biomaterials and analytical methods.

Arne Åsan, **Ellen Berggreen**, and **Bente Holmgren**, of the Public Dental Health Care Services, Hordaland County Council, for your support and for acknowledging this study.

My friends and colleagues at **Årstad**, **Nesttun**, and **Student Welfare Organization dental clinics** for helping me recruiting participants to Study I and II and for always being interested and positive. **Martha Sørensen** and **Hazel Rødseth** for being supportive and providing clinical facilities. Special warm thanks to **everyone working at Årstad Dental Clinic**. I am forever grateful for our professional cooperation and friendship. Especially warm thanks to **Karen-Ruth Hofland** for assisting me during the clinical work with neatness and professionalism, sharing joys, sorrows, and life lessons.

My friends and colleagues at **Oral Health Centre of Expertise**. Warm thanks for your support, encouragement, and for including me in a stimulating working environment.

June-Vibecke Knudtsen Indrevik, **Elina Troscenko** and Professor **Asgeir Bårdsen**. Thank you for your encouragement, availability, and excellent administrative guidance during the PhD program. **Christine Kronenberger** – thank you for your administrative support and kindness.

Siren Hammer Østvold for always helping me out with shipping of biological samples and for initiating social gatherings on the 4th floor. All my **PhD colleagues** – thank you for sharing interesting seminars, scientific experiences, joy and frustration during these years. Appreciation is also extended to **everyone working on the 4th**

floor. Thank you for including me in your generous and skilled working environment, for morning coffee, and for inspiring discussions about life and research.

Professor **Kristin Klock** for kindly sharing your great knowledge and for always being positive and helpful.

My caring friends and colleagues at the Adverse Reaction Unit – **Birgitte**, **Johanna**, **Kåre**, **Merete**, and **Randi**. Thank you for all the support and encouragement, for professional discussions, as well as talks about life and for sharing laughs and sorrows. I feel privileged to work with you.

My precious and loving **friends**, thank you for your support, encouragement, and understanding. I look forward to having more time with every one of you.

My large and amazing **family**, thank you for our enjoyable time spent together and for your understanding and encouragement. Special warm thanks to my three caring brothers **Inge Otto**, **Per Arne**, and **Stein Erik**, who always have been there for me.

Last, but not least, the key stones of my life – **Morten**, thank you for all your caring love, extensive patience, support, and encouragement through these years. **Joachim**, **Henrik**, and **Håkon**, thank you for making me happy and proud every day and for reminding me that the most important thing in life is not a PhD.

Abbreviations

Bis-DMA	Bisphenol A dimethacrylate
Bis-EMA	Bisphenol A ethoxylate dimethacrylate
Bis-GMA	Bisphenol A glycidylmethacrylate
BMI	Body mass index
BPA	Bisphenol A
CAS	Chemical Abstract Service
CE	"Conformité Européene" ("European Conformity")
CQ	Camphoroquinone
EFSA	European Food Safety Agency
HEMA	2-Hydroxyethyl methacrylate
ISO	The International Organization for Standardization
LC	Liquid chromatography
LOD	Limit of detection
MBRN	Medical Birth Registry of Norway
MDR	Medical Devices Regulation
MoBa	The Norwegian Mother and Child Cohort Study
MS	Mass spectrometry
N/A	Not available
NIPH	Norwegian Institute of Public Health
OR	Odds ratio
SD	Standard deviation
SPSS	Statistical Package for the Social Science
TEGDMA	Triethyleneglycol dimethacrylate
UDMA	Urethane dimethacrylate
WHO	World Health Organization

Abstract

Dental polymer-based materials have become the first choice for restorative treatment in many countries, and the increased use of these materials over the last decades has raised questions about their biological safety. It has been shown that monomers as well as contaminants can leak from dental polymer-based restorations. Due to its estrogenic effect, bisphenol A (BPA) has been considered as a compound of specific interest. Exposure to BPA during early developmental stages of life is of particular concern. Large epidemiological studies exploring whether placement of dental polymer-based restorative materials in pregnant women is associated with increased risk for the fetus are warranted.

The overall aim of the present work was to gain knowledge about the exposure to BPA from dental polymer-based restorations in humans and to investigate whether placement of polymer-based dental fillings during pregnancy is associated with increased risk for adverse birth outcomes.

The thesis comprises three studies. In the first study, 20 individuals with six or more tooth surfaces filled with polymer-based materials (composite group) and 20 individuals without dental polymer-based materials (comparison group) were enrolled. Saliva was collected to assess if presence of dental polymer-based fillings is associated with increased salivary BPA level. In the second study, 20 patients who were scheduled for treatment of at least two tooth surfaces with dental polymer-based restorative material were included. Saliva and urine were collected before and up to one week after treatment to assess if placement of dental polymer-based material is associated with increased BPA concentrations in saliva and urine. The BPA concentration in the biological samples was determined using liquid chromatography/mass spectrometry. Presence of dental polymer-based fillings was associated with slightly higher concentration of BPA in saliva. Directly after treatment with dental polymer-based material, there was a considerable increase in the concentration of BPA in saliva. After the initial increase, the concentration

decreased exponentially over time. One week after treatment, the salivary BPA level was only marginally higher compared to the pretreatment level. In urine, no statistically significant change of BPA concentration after placement was observed.

In the third study, data from the large Norwegian Mother and Child Cohort Study was used to investigate if placement of white fillings during pregnancy was associated with increased risk for adverse birth outcomes. The results indicated that there was no statistically significant increased risk for adverse birth outcomes for participants who had white fillings placed during pregnancy compared with women who did not consult a dentist during pregnancy.

In conclusion, dental polymer-based restorative materials might contribute to BPA exposure in humans. However, the exposure appears to be relatively short and transient. Women participating in the Norwegian Mother and Child Cohort Study who received dental polymer-based restorations (white fillings) during pregnancy had no increased risk for adverse birth outcomes including stillbirth, malformations, preterm birth, and low or high birth weight.

List of Publications:

This thesis is based on the following papers:

Paper I

Berge TLL, Lygre GB, Jönsson BAG, Lindh CH, Björkman L. Bisphenol A concentration in human saliva related to dental polymer-based fillings. *Clinical Oral Investigations.* 2017;21(8):2561-8.

Paper II

Berge TLL, Lygre GB, Lie SA, Lindh CH, Björkman L. Bisphenol A in human saliva and urine before and after treatment with dental polymer-based restorative materials. Accepted for publication in *European Journal of Oral Sciences, 29 May 2019*.

Paper III

Berge TLL, Lygre GB, Lie SA, Björkman L. Polymer-based dental filling materials placed during pregnancy and risk to the foetus. *BMC Oral Health.* 2018;18(144).

1. Introduction

Today tooth-colored dental polymer-based materials are available for various clinical applications and have become the first choice for restorative treatment in several countries (1). The focus on minimally invasive and aesthetic dentistry, as well as the decision for a global phase-down of amalgam (2) have likely contributed to this trend. The term dental polymer-based restorative material generally refers to a reinforced polymer (resin) matrix used to replace or restore missing portions of tooth structure directly on the tooth (3, 4). The increased use of these materials in recent decades has raised questions about their safety and biocompatibility. The materials are not inert in the oral environment, and they release components with potential adverse effects (5).

Over the years, dental polymer-based restorative materials, also known as resin-based restorative materials, dental composites, or tooth-colored filling materials, have been subject to continuous and extensive research in an effort to improve their mechanical properties, their bonding to the tooth structure, and their biocompatibility.

Major improvements in clinical performance of tooth-colored dental materials took place in the late 1950s and early 1960s (6). In 1955, Buonocore introduced the acid-etch technique as a method to increase the adhesion of the polymeric material to enamel (7). In the early 1960s, silica powder was combined with bisphenol A glycidylmethacrylate (bis-GMA, also called “Bowen’s resin”), and this new product exhibited increased strength, increased hardness, and decreased polymerization shrinkage compared to the previous resin-based materials (8, 9).

1.1 Dental polymer-based restorative materials

Dental polymer-based restorative materials in use today generally consist of different types of inorganic filler particles embedded in an organic polymer-based matrix (4, 10). The main organic ingredients are methacrylate monomers, which during

polymerization are cross-linked to create a rigid polymer network. Furthermore, the matrix also consists of photo initiators and other additives (4, 10).

Legal aspects

Because the main intended function of dental materials is to replace lost tissue, these materials are defined as medical devices (11). In the European Union they have to meet general requirements of the European Medical Devices Regulation (MDR/2017/745) in order to obtain the CE marking (12) that is mandatory for the product to be marketed in the European Economic Area (including Norway). By CE marking, the manufacturer demonstrates that the product is in compliance with the essential requirements (e.g. "Requirements regarding design and manufacture") of the MDR and other applicable directives from the European Commission (EC directives). According to the MDR, medical devices should not compromise the safety of patients or operators (11). Potential hazardous constituents should be labeled and supplied with an information sheet (e.g. safety data sheets) (13). The MDR does not describe in detail how the requirements have to be fulfilled. One possibility is compliance with appropriate standards, such as those of the International Organization for Standardization (ISO). Currently, for dental polymer-based restorative materials, ISO 4049-2019 is the standard that specifies the requirements (https://www.iso.org/standard/67596.html). However, even if standards are used, the requirements in the MDR are always legally decisive.

1.1.1 Composition

1.1.1.1 *The organic polymer matrix*

Monomers

The main ingredients in the organic matrix are most commonly a blend of aromatic and aliphatic dimethacrylate monomers with two reactive groups (Figure 1). The matrix monomers are fluids with different viscosities and molecular weights. The most common monomer used is the highly viscous bis-GMA (14). Other monomers

used in combination with or instead of bis-GMA include urethane dimethacrylate (UDMA) and bisphenol A dimethacrylate (bis-DMA). To reduce the viscosity and obtain optimal clinical consistency of the material, low molecular-weight co-monomers, for example, triethyleneglycol dimethacrylate (TEGDMA) or bisphenol A ethoxylate dimethacrylate (bis-EMA), are added as diluents by the manufactures (4, 15). In order to simplify the application procedures, attempts have been made to use a variety of other matrix monomers over the years (16). Moreover, materials relying on silorane and ormocer (organically modified ceramic) technologies have also been proposed by the manufacturers (17).

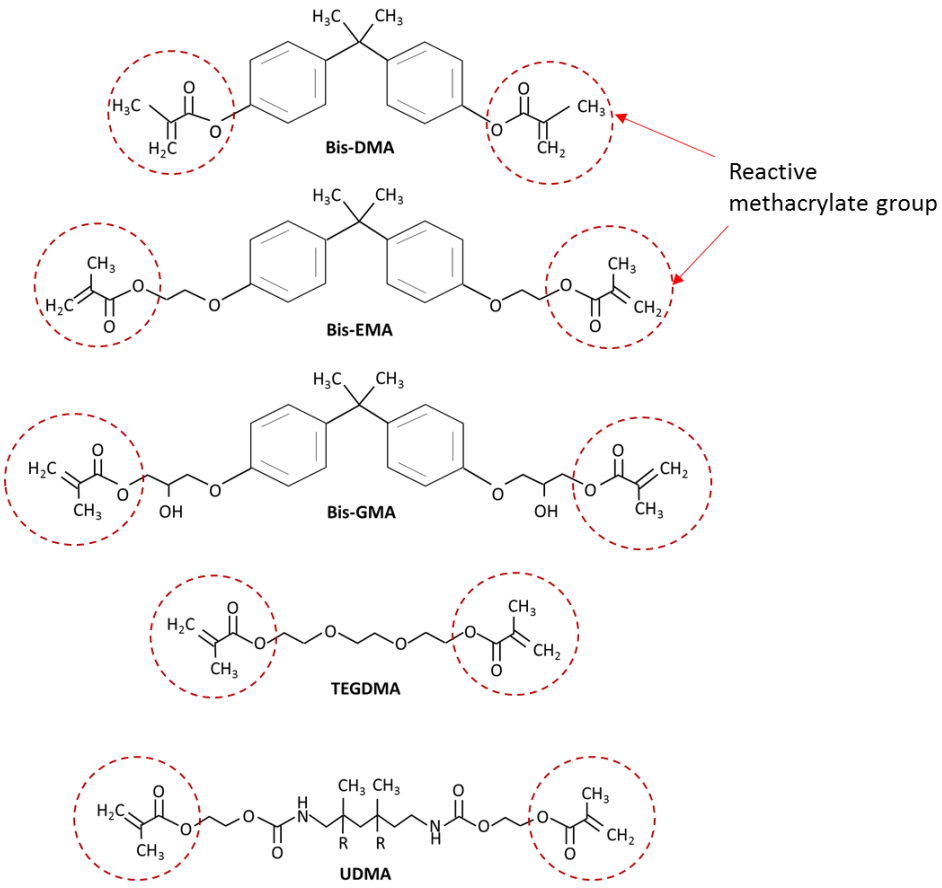


Figure 1. Structures of the monomers bis-GMA, bis-EMA, bis-DMA, TEGDMA, and UDMA.

Initiators and inhibitors/stabilizers

To promote the polymerization reaction, initiators are added to the matrix. In most materials, the polymerization is activated by light (18). Camphorquinone (CQ) is a commonly used photo initiator and absorbs light with wavelength from 400–500 nm (visible light). In two-component, chemical-activated materials, an organic amide is

incorporated in the catalyst paste and an organic peroxide is incorporated in the universal paste. The polymerization starts as soon as the catalyst paste and the universal paste are mixed. Dual-cured materials use a combination of chemical and light activation and contain initiators and accelerators that allow light activation followed by self-curing. To prevent premature activation of the polymerization, and thereby extend the materials' storage life and to ensure sufficient working time, inhibitors or stabilizers are added (4). Components that can enhance color stability are additional ingredients (4).

1.1.1.2 *Filler particles*

To improve the physical and mechanical properties of the polymer-based materials, different sizes and types of filler particles are incorporated in the polymer matrix. Traditionally these particles have been minerals such as quartz, glass, or ceramic, as well as organic pre-polymerized resin particles. Benefits of the fillers include reinforcement of the polymer matrix, increased wear resistance, reduction of the volume shrinkage and thermal expansion, and optimization of the workability as well as imparting radiopacity and a degree of translucency. Polymer-based restorative materials are generally classified based on the size of the particles, namely macro-filled, micro-filled, and nano-filled composites and combinations of these (hybrids) (18).

1.1.1.3 *Coupling agents*

The filler particles are coated with coupling agents (e.g. silane) that have reactive groups that can ensure a strong bond between the filler particles and the polymer matrix (19) (Figure 2).

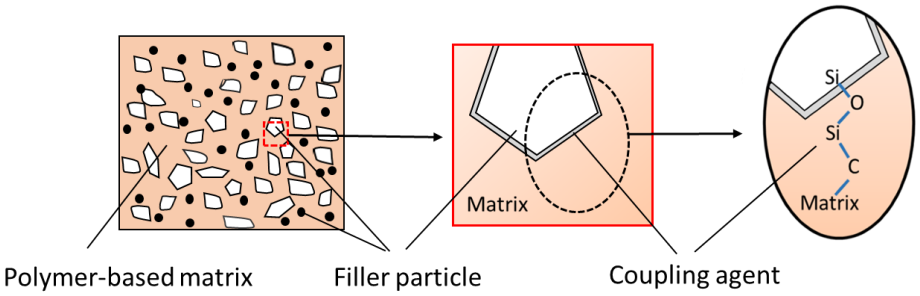
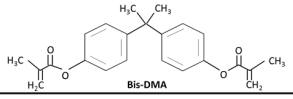
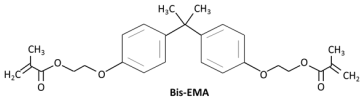
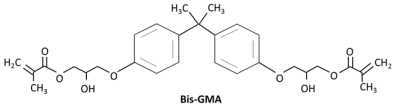
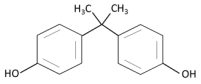
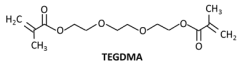
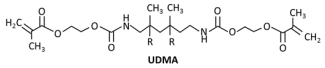
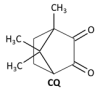
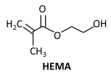


Figure 2. Schematic illustration of filler particles coated with a coupling agent (silane) and embedded in a polymer-based matrix. Inspiration for the figure (18, 19).

1.1.2 Adhesive systems

To bond the polymer-based restorative material to the tooth substance, an adhesive system is needed. Although enamel and dentin differ in structure and composition, resin-based systems available today can bond to both the enamel and dentin of the tooth. The systems are classified according to the etching strategy, such as total etch (etch-and-rinse) or self-etch (20). Total etch require a separate acid step to etch the enamel and dentin, a subsequent rinse, and application of primer and adhesive. Phosphoric acid gel (30–40%) is used to demineralize the tooth structure. Total etch can be presented as a three or two-step system depending on whether primer and adhesive are separate or combined in a single bottle. In self-etch systems, acidic monomers etch and prime the tooth simultaneously before application of the adhesive (two-step). Self-etch systems are also available as a one-step alternative with the acidic monomer and the adhesive combined in the same bottle (20).

Table 1. Characteristics of some potential eluates from dental polymer-based materials.

Compound and CAS number	Structural formula	Molecular formula	Molecular weight (g/mol)	Properties of concern[a]
Bis-DMA 3253-39-2	 Bis-DMA	$C_{23}H_{24}O_4$	364.43	Not classified
Bis-EMA 41637-38-1	 Bis-EMA	$C_{27}H_{32}O_6$	452.55	Not classified[b]
Bis-GMA 1565-94-2	 Bis-GMA	$C_{29}H_{36}O_8$	512.60	Ss
BPA 80-05-7	 BPA	$C_{15}H_{16}O_2$	228.29	R, Ss
TEGDMA 109-16-0	 TEGDMA	$C_{14}H_{22}O_6$	286.32	Ss
UDMA 72869-86-4	 UDMA	$C_{23}H_{38}N_2O_8$	470.56	Ss
CQ 10373-78-1	 CQ	$C_{10}H_{14}O_2$	166.22	Not classified
HEMA 868-77-9	 HEMA	$C_6H_{10}O_3$	130.14	Ss

a) Source: European Chemical Agency, https://echa.europa.eu/. Info Card data generated from information based on industry data. R – Toxic to reproduction; Ss – Skin sensitizer; Updated June 05, 2019.

b) To be evaluated; substance included in Community Rolling Action Plan (CoRAP) (21).

1.1.3 Polymerization

Originally, dental composites were two-component and the polymerization was chemically activated. However, during the 1970s light curing and more operator-friendly materials became available. Today, visible blue-light-activated materials with increased depth of cure, controllable working time, and other advantages are available (10).

Dimethacrylate monomers undergo free radical addition polymerization by opening the carbon double bonds and forming single bonds (Figure 3). During this process, which requires a source of free radicals to be initiated, most of the monomers are converted into polymers and cross-linked to form a three-dimensional network system.

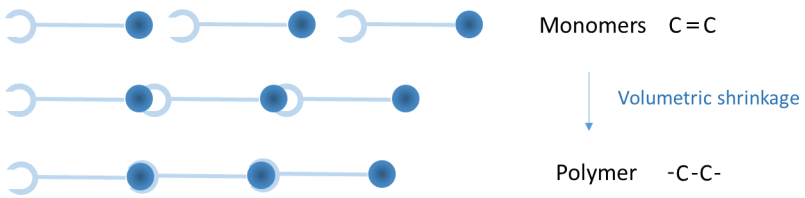


Figure 3. Schematic illustration of the polymerization of methacrylates and resulting volumetric shrinkage. Figure modified from (4).

Highly viscous monomers (e.g. bis-GMA) and crosslinking of polymer chains reduce the monomers' ability to move and hence, to participate efficiently in the polymerization within the bulk of the material. Thus, the conversion of monomers to polymers, either chemical or light initiated, is never complete (22-24). The degree of conversion, defined as the percentage of carbon double bonds that converts into single bonds during polymerization, might vary between 50% and 70% (10). Most of the monomers will polymerize and have at least one of their reactive methacrylate groups bound to the network. However, the cured material might also contain

completely unreacted monomers (residual monomers) that can migrate out of the network (24) (Figure 4).

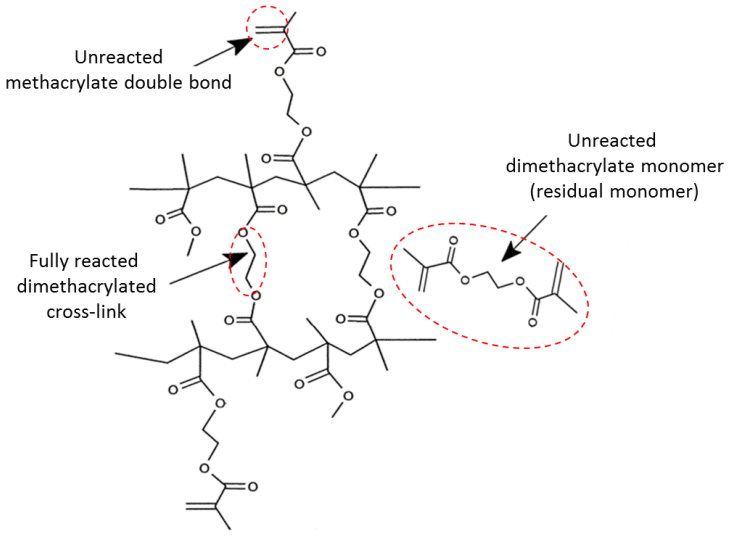


Figure 4. Polymer network and dimethacrylate groups with zero, one, or two unreacted double bond. Completely unreacted monomers (residual monomer) can migrate out of the polymerized material. Figure modified from (10).

The extent to which monomers are converted might be influenced by several factors including the composition of the polymer-based material, the curing time, the light intensity, the distance to the light curing tip, and the thickness of the incremental layer (25). Oxygen reacts rapidly with free radicals and thus inhibits the polymerization reaction on the matrix surface (the oxygen-inhibited layer) (26). Thus, on the free (exposed) surfaces of the dental polymer-based restorations the degree of conversion might decrease up to 20% resulting in higher amounts of potentially elutable residual monomers (10, 27).

1.1.4 Degradation and release of substances

Results from numerous in vitro and in vivo studies have shown that unpolymerized monomers, additives, and filler components might leak from dental polymer-based materials (5, 28-31). In addition, impurities from the production process and degradation products formed during and after curing might be present in the polymerized material and might migrate out of the material (32, 33).

In the oral cavity, the leakage might initially be due to the incomplete polymerization on the surfaces and later to mechanical, enzymatic, and hydrolytic degradation of the material (27, 34-37). Aging and wear of the materials might result in porosities leading to increased release of residual monomers originally trapped in the polymer network.

Substances released from polymer-based restorations might reach the biological environment by diffusion through the pulp via dentinal tubuli, directly through the oral mucosa, by absorption via volatile components in the lungs and by ingestion of released substances in the gastrointestinal tract (27).

1.1.5 Biological effects

Studies have shown that dental polymer-based materials and their constituents potentially have allergic (38, 39), cytotoxic (40-42), genotoxic (42), or estrogenic effects (43, 44). The potential release and estrogenic effect of bisphenol A (BPA), has been of particular concern (45).

1.2 Bisphenol A (BPA)

1.2.1 What is BPA?

Bisphenol A (Cas nr: 80-05-7) is a white, solid organic synthetic compound prepared by the combination of two equivalents of phenol with one equivalent of acetone (hence the suffix A in the name) (Figure 5). Some specifications of BPA are shown in Table 2.

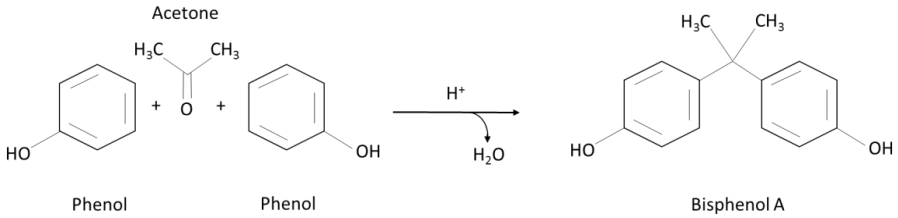


Figure 5. Synthesis of BPA from two phenol molecules and one acetone molecule in the presence of a catalyst (e.g. hydrogen chloride). Figure adapted from (https://commons.wikimedia.org/wiki/).

Table 2. Substance identifications and physico-chemical characteristics of BPA (46).

Chemical name	Bisphenol A (BPA)
Physical state at normal	White solid flakes or powder
CAS number	80-05-7
Formula	$C_{15}H_{16}O_2$
Molecular weight	228.29 g/mol
Melting point	~160 °C
Solubility in water	~300 mg/l (low)

BPA was first reported as a synthetic chemical in the 1890s by the Russian chemist Alexander P. Dianin (47). During the 1930s, when there was a global scientific effort to develop pharmaceutical estrogens, BPA was identified to have estrogenic properties (48). However, diethylstilbestrol (DES) was found to be more potent and

BPA was temporarily dismissed (49). BPA's commercial value was reassessed during the 1950s when chemists discovered BPA as a very valuable chemical in the plastics industry. Since then BPA has been widely used in commercial production.

Today, BPA is a high-volume production chemical, mainly used in the manufacture of polycarbonate plastics and epoxy resins. Based on data from the European Chemical Agency, 1–10 million tons of BPA is manufactured and/or imported in the European Economic Area every year (50). Polycarbonate plastics are used in numerous everyday items such as food and beverage containers, plastic tableware, children's toys, mobile phones, and many other consumer products. Epoxy resins are often used inside metal cans as protective linings. BPA might also be present in thermal paper, medical devices, and a wide range of other products (51). Given the large amount of BPA containing products, exposure to BPA in developed countries is ubiquitous (52).

1.2.2 Potential sources of exposure

In humans, the major BPA exposure is assumed to be through the diet. BPA can migrate in small amounts into food and beverages stored in materials made of polycarbonate or in cans coated with epoxy linings (51, 53, 54). However, BPA levels in human matrices (e.g. urine) cannot be explained by dietary exposure alone (54, 55). Non-dietary sources and pathways have received increased attention, and recent studies have indicated human exposure also from several non-dietary sources such as dust and indoor air, thermal paper, cosmetics, and medical devices including dental materials (51, 54-57).

1.2.3 BPA in dental materials

BPA is not intentionally added to dental polymer-based materials. However, it is a raw material in the synthesis of several of the widely used methacrylate monomers (Figure 6) and might be found as an impurity in the organic matrix (5, 58).

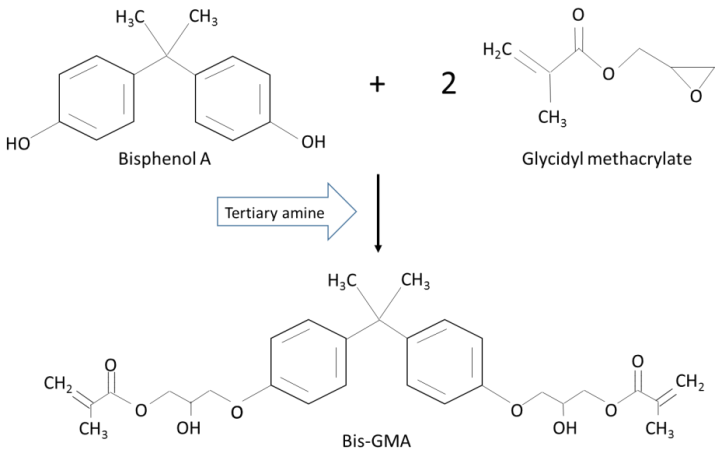


Figure 6. Synthesis of bis-GMA from BPA and glycidyl methacrylate.

Human saliva contains enzymes (e.g. proteases and esterases) that might have an impact on dental materials and the released compounds (34). In 1996, Olea et al. conducted the first in vivo study examining released components after placement of a dental sealant and detected BPA in saliva within 24 hours after placement of a bis-DMA-based sealant (44). Studies have shown that there is a considerable conversion of bis-DMA to BPA due to hydrolysis at the ester bond (-O-CO-) (59-61). However, BPA derivatives with an ether bond (-O-), such as bis-GMA and bis-EMA, do not hydrolyze to BPA (58-61) (Figure 7).

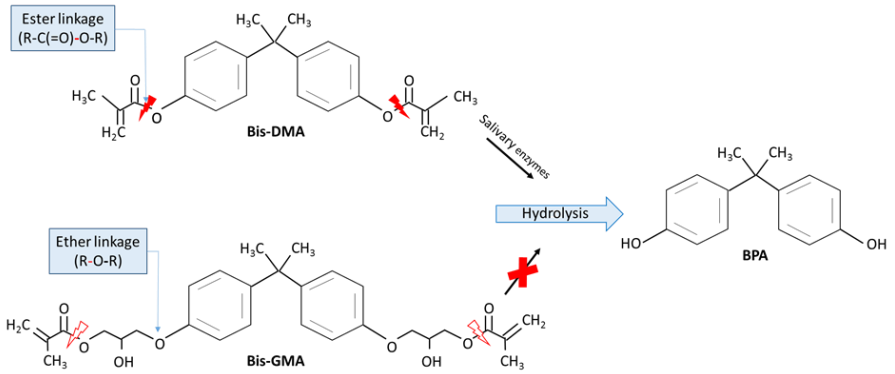


Figure 7. Hydrolysis of bis-DMA into BPA at the ester linkage. Bis-GMA does not hydrolyze to BPA because the ether linkage is resistant to hydrolysis. Figure modified from (62).

1.2.4 Metabolism of BPA

BPA can enter the human body via different routes. The major route is considered to be through the gastrointestinal tract after ingestion (54). After investigating anesthetized dogs, Gayrard et al. suggested that BPA exposure could occur through the oral mucosa (sublingual), leading to detectable levels of unconjugated BPA in the blood (63). In contrast, Teeguarden et al. reported evidence against sublingual BPA absorption in humans ingesting soup containing deuterated BPA (D_6-BPA) (64). Toxicokinetic studies have indicated that BPA can enter the body through the skin (e.g. via contact with cosmetics or thermal paper) or through the respiratory mucosa via dust (54, 65, 66) (Figure 8). In addition, other parenteral exposure routes, such as subcutaneous and intravenous routes (e.g. relevant for medical devices), might be important, especially for prematurely born infants (57).

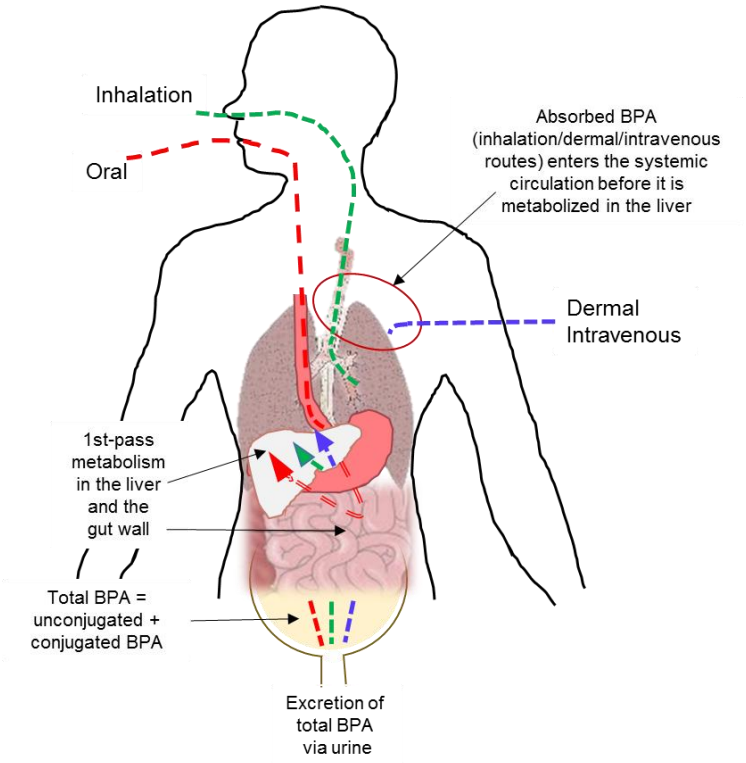


Figure 8. Illustration of different routes of exposure to BPA. Inspiration for the figure (56).

The estrogenicity of BPA in humans is dependent on the routes of exposure. Ingested BPA is rapidly absorbed in the gastrointestinal tract and inactivated through the first-pass metabolism in the gut wall and in the liver (67-69). In this metabolic process, most of the BPA is converted from a bioactive, estrogenic form (unconjugated/free BPA) via enzyme activity to water-soluble non-estrogenic forms (conjugated BPA). The conjugated metabolites, mainly BPA glucuronide and, to a lesser extent, BPA sulfate, have no estrogen receptor affinity and are therefore of less toxicological concern (70, 71) (Figure 9).

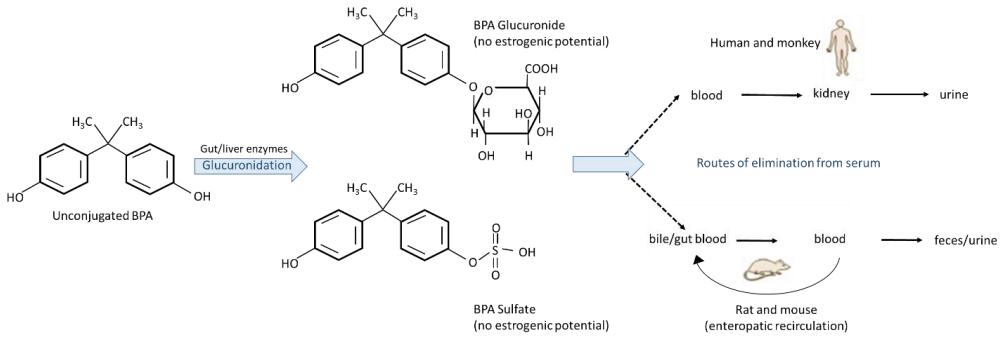


Figure 9. Schematic illustration of BPA glucuronidation in the gut and liver and routes of elimination of BPA conjugates in humans and rodents. Inspiration for the figure (47, 72, 73).

In contrast, if BPA enters the body via the parenteral routes (e.g. dermal or inhalation routes), results from animal studies have concluded that the absorbed BPA enters the systemic circulation before it is metabolized in the liver (74-76). Hence, unconjugated BPA (the bioactive form) might circulate in the body for a longer period of time.

In rodents (i.e. rats and mice) BPA is also mainly glucuronidated, although it is suggested that the BPA glucuronide can be deconjugated in the gut and be re-circulated back to the liver ("enterohepatic recirculation") resulting in slower elimination of BPA (Figure 9) (75). Thus, ingestion of an equivalent BPA dose might result in higher blood levels of unconjugated BPA in rodents compared to humans.

Pharmacokinetics studies using controlled doses of isotopically labeled BPA suggest that following oral administration in adult humans, 84–97% of ingested BPA is absorbed and excreted as BPA or BPA conjugates in urine within 5–7 hours (64, 73, 77). This is consistent with a short elimination half-life of BPA in urine of approximately 2 hours (64). After 24 hours, recovery from urine is almost complete (64, 73, 77).

1.2.5 Potential health effects

Endocrine-disrupting chemical

BPA is now well known to be an endocrine-disrupting chemical, with the ability to interfere with and mimic estrogenic hormones (78, 79). An endocrine disruptor is defined as "an exogenous substance or mixture that alters the function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations" (80, 81).

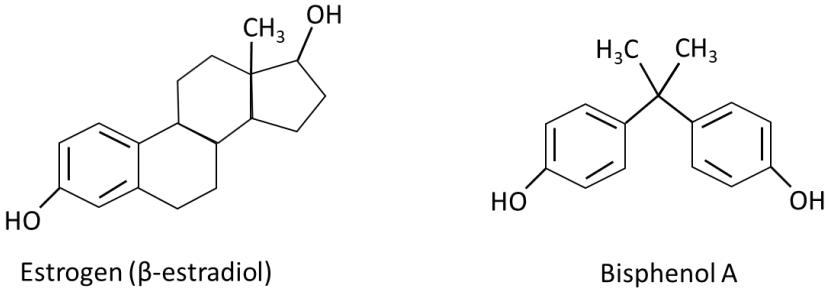


Figure 10. The structures of BPA and estradiol have some structural similarities, thus BPA has the ability to bind to estrogen receptors.

Due to its structural analogy to estradiol (two phenol functional groups and two benzene rings) (Figure 10), BPA can bind to estrogen receptors (ERα and ERβ) and act as a weak estrogen as well as an antiestrogen, blocking the estrogenic response (78, 79) (Figure 11). Further, BPA has been shown to bind to thyroid receptors and might interfere with thyroid function (82). BPA can also interact with the immune system and the developing nervous system (83).

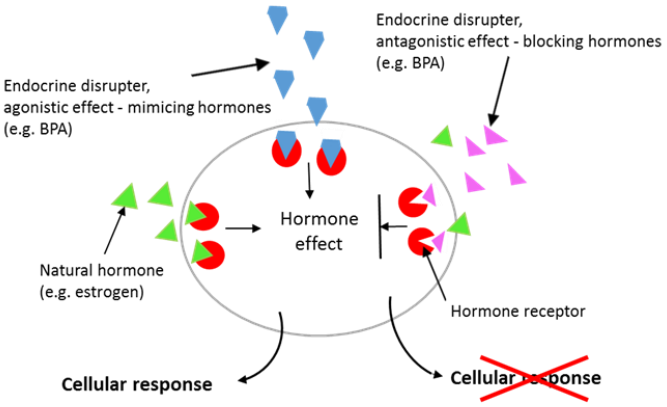


Figure 11. Mechanism through which endocrine disruptors can influence the endocrine system after being absorbed by the body. Figure modified from (84).

Concern has been raised about the potential human health risk of low-level exposure to BPA. It has been reported that the biological effects of BPA show a nonmonotonic dose-response curve, a pattern that is characterized by intense reactivity at low levels and no or less response at high levels (83, 85).

The results of numerous in vitro and animal studies have demonstrated an association between low-dose exposure to BPA and a variety of negative outcomes (86, 87). In fact, some studies show effects from doses that are comparable to calculated human exposures (i.e. doses <10 microgram per kilogram bodyweight (µg/kg bw) per day) (86-88). In several epidemiological studies, BPA levels in human populations have been linked to reproductive abnormalities, adverse developmental effects, metabolic disease, and breast cancer among other health conditions (89-91). The mechanisms of action behind these possible effects are not fully understood.

1.2.6 Risk assessment

Assessment of low-dose effects from BPA has been disputed (92), and represents a challenge for the traditional regulatory health risk assessment (93, 94). To assess the risk of serious negative effects to human health, it is important to consider the concentration of unconjugated BPA in the circulation.

The current opinion of risk assessment agencies, such as the European Food Safety Authority (EFSA), is that negative health effects from BPA exposure cannot be excluded (95). In 2015 the EFSA reduced the recommended Tolerable Daily Intake of BPA from 50 to 4 µg/kg bw per day (95). However, there is still some debate regarding this estimated safe level of BPA exposure. Although unconjugated BPA has been detected in blood in several studies (94), other authors have claimed that the observed unconjugated BPA concentration resulted from contamination of the samples during storage or sample preparation (67, 96). Nevertheless, in 2017, based on the scientific evidence of probable serious effects to human health, the Member State Committee of the European Chemical Agency (ECHA) decided to label BPA as a substance of very high concern (80). Currently, since November 2018, a working group from the EFSA is re-evaluating the potential hazards of BPA in food based on studies and data published after 2012. The updated assessment is expected to be completed in 2020 (97).

Human intake of BPA has generally been estimated in two different ways (56). In the biomonitoring approach, concentrations of total BPA (unconjugated + conjugated) measured directly in human tissues (e.g. urine, blood) are used to estimate (back calculate) the total exposure from all possible sources. Studies using this approach have estimated exposures in a range from 0.02 to 0.03 µg/kg bw per day among adults in the United States and Japan (98-100), to 0.04 µg/kg bw per day among 20–29-year-old students in Germany (101), and to 0.07 µg/kg bw per day among 6–8-year-old girls in the United States (102). In the aggregating approach (forward method), the researchers add the amounts of BPA detected in all known sources

through different exposure pathways. This method requires sources of exposure to be identified and measured. Using this method, the estimated exposure ranges from 0.04 to 0.06 µg/kg bw per day (103) to 1.4 µg/kg bw per day for adults consuming canned food (high exposure group) (104). Calculations based on biomonitoring are preferred for estimating total intake because all sources of exposure are accounted for. However, estimates based on sources of exposure are useful for calculating the relative contributions of various exposure pathways to total intake.

Biomonitoring data show that human exposure to BPA is widespread, and more than 90% of the populations in the United States and Canada have measurable BPA concentrations in their urine (98, 105). Of particular concern has been exposure during vulnerable periods of life, such as during fetal and early postnatal development (106-108) because fetuses and neonates might have reduced capability to metabolize and excrete BPA from the body compared with older individuals (109). Moreover, studies have reported that BPA might cross the placental barrier (110, 111). Thus, exposure to BPA during pregnancy might pose a potential risk to the vulnerable fetus, and there is a need to identify sources of BPA and their relative contributions to the total exposure.

The recommendation in the National Clinical Guideline for dental restorative treatment, published by the Norwegian Directorate for Health and Social Affairs in 2003, was to avoid placement of dental fillings during pregnancy (112). According to the Directorate, this recommendation is based on the precautionary principle, and thus further research on the topic is needed.

1.3 The Norwegian Mother and Child Cohort Study (MoBa)

The Norwegian Mother and Child Cohort Study is an ongoing prospective population-based cohort study conducted by the Norwegian Institute of Public Health (NIPH) (113, 114). From 1999 to the end of 2008, pregnant women in Norway were

invited to MoBa through a postal invitation in connection with their first routine ultrasound examination. Approximately 41% of pregnant women attended the study, and the cohort currently comprises more than 108,000 pregnancies, 114,000 children, 95,000 mothers and 75,000 fathers (113). Approximately 16,400 women participate with more than one pregnancy.

The main aim of MoBa is to find causes of serious diseases, with a focus on the interplay between early exposures and genetic factors. The NIPH intends to follow the families for years to come and to create a Norwegian research database of high quality.

MoBa is mainly based upon self-reporting through questionnaires. Written informed consent was obtained from each participant upon recruitment. During pregnancy, the mother provided answers to three questionnaires that focused on different exposures as well as health-related history, whereas the father responded to one questionnaire on medication, health, and occupational exposure. Additional questionnaires on the development of the child, the health of the mother and the child and lifestyle exposures are sent out at regular intervals (Figure 12). Apart from this, blood and urine samples were collected from both parents during pregnancy and from the mother and child after birth. All biological samples are stored in a biobank. At the age of 6–7 years, children participating in MoBa were asked to supply one or more primary teeth (115). Additional data and biological materials have also been collected in numerous sub-studies. Moreover, MoBa is linked to several national health registries such as the Medical Birth Registry of Norway (MBRN) (116), the National Patient Registry, the Norwegian Prescription Database, and the Cancer Registry among others (113).

	During pregnancy			Birth	After birth			
	Week 18 Ultrasound	Week 22	Week 30	Birth	0.5, 1.5, 3 and 5 years	6-7 years	8 years	13 and 14 years
Child				Umbilical cord blood	**6 MONTHS** Questionnaire (Q4) to mother about environmental factors and lifestyle **18 MONTHS** Questionnaire (Q5) about health and lifestyle **36 MONTHS** Questionnaire (Q6) with focus on child's health, behaviour, autism and ADHD **5 YEARS** Questionnaire (Q-5year) with focus on child's language development and skills	Collection of teeth 	Questionnaire (Q-8year) with focus on child's mental health and development	Questionnaire to 13-year-olds about mental health Questionnaire to 14-year-olds about diet
Mother	Questionnaire (Q1) to mother (general)  Blood from mother	Questionnaire (Q2) to mother about diet 	Questionnaire (Q3) to mother about environmenental factors and lifestyle 	Blood from mother		Questionnaire (Q-7year) with focus on asthma, allergy and diet		Questionnaire about health and lifestyle
Father	Questionnaire to father (general) Blood from father							Questionnaire about health and lifestyle
Others					Questionnaire to childcare centres		Questionnaire to school teacher	

Figure 12. Data collection in MoBa. Figure modified from the leaflet «MoBa» in short.

2. Aims

The overall aim of this thesis was to gain knowledge about the exposure to BPA from dental polymer-based restorations in humans and to investigate whether placement of polymer-based dental fillings during pregnancy is associated with increased risk for adverse birth outcomes.

The specific aims were:

- To quantify salivary concentrations of BPA and to assess if presence of dental polymer-based filling materials is associated with increased BPA levels in saliva (Paper I).

- To quantify BPA concentrations in saliva and urine before and after treatment with dental polymer-based restorative materials to assess if placement of these materials is associated with increased BPA levels in saliva and urine (Paper II).

- To investigate whether the placement of polymer-based dental fillings during pregnancy is associated with adverse birth outcomes including stillbirth, preterm birth, malformations, and low or high birth weight (Paper III).

3. Material and methods

The material and methods used in the included studies are described in the respective papers. Here follows a brief summary.

3.1 Paper I

Individuals between 20 and 35 years of age were recruited to the study from three public dental clinics during their routine dental check-ups. Patients fulfilling the study inclusion criteria were included from January 2013 to March 2014. Twenty individuals who had six or more tooth surfaces previously filled with dental polymer-based materials were enrolled in the composite group. The fillings had to be 1 week or older. Twenty patients without dental polymer-based materials were included in the comparison group. Subjects with chronic disease and/or current medications were not included. Exclusion criteria were smoking, use of snuff, drug abuse, use of dental splints, dental prostheses, and previous or current orthodontic treatment. In addition, dental students and dental health workers were not included. All participants provided written informed consent.

Clinical examination

Before clinical examination of each participant, intraoral radiographs taken during the routine check-up were reviewed in order to detect and verify existing fillings. The total number of teeth, pre-filled surfaces, and, if available, type of restorative materials used, and time of last filling placed were recorded during a detailed dental examination. Each filling surface was assigned a score from 1 to 3 depending on the extent of the surface area, yielding the variable "filling points" (117). Small filling surfaces were designated the lowest score of 1. The score of 2 was typically given to surfaces of intermediate size (e.g. approximal or occlusal surfaces of premolars). Restorations in molars extending over the total occlusal fissure system or over the approximal surface were typically given the highest score of 3.

Collection and analysis of saliva samples

All participants were instructed to refrain from eating, drinking, brushing their teeth, using lipstick, etc., for at least 2 hours before sampling. On the day of sampling, the participants were asked (see Appendix I) about exposures that potentially could contribute to salivary BPA concentration. Potential variables were dichotomized into groups: Intake of breakfast before sampling (yes/no), intake of canned food in the last week (yes/no), job that involved handling of receipts (yes/no), and use of chewing gum daily (yes/no).

Saliva was collected at the dental clinic while the participant was seated in a dental chair (Figure 13). The individuals were encouraged to make active cheek and tongue movements for one minute, and the accumulated saliva was collected in a polypropylene tube until 5 ml were sampled. The sampling time was recorded. Each sample, marked with an ID code and date, was immediately placed in a refrigerator (4°C) and then stored at −80°C until they were sent for analysis. The saliva specimens were analyzed at the Laboratory of the Division of Occupational and Environmental Medicine at Lund University, Sweden, using liquid chromatography-triple quadrupole mass spectrometry (LC/MS/MS; QTRAP 5500; AB Sciex, Foster City, CA, USA). The laboratory is a European reference laboratory for BPA in urine (http://www.eu-bm.info/democophes) and a reference laboratory for BPA in urine in the Erlangen round robin inter-laboratory control program. For determination of total BPA in saliva, aliquots were digested with glucuronidase and an isotopically labeled internal standard for BPA (D_{16}-BPA) was added. Proteins were precipitated using acetonitrile. Unconjugated BPA was determined without the use of glucuronidase. The difference between total and unconjugated BPA represented the concentration of conjugated BPA. The limit of detection (LOD) was determined to be 0.1 ng/ml. For a detailed description of the analytical method, see Appendix II.

Figure 13. Collection of saliva using a polypropylene tube. Photos: TLL Berge

3.2 Paper II

Twenty healthy patients 16–40 years of age who were provided a treatment plan for at least one dental restoration (two or more surfaces) with polymer-based filling material were recruited to the study from two public dental clinics during their routine dental check-ups. Smokers, snuff users, drug abusers, dental students, dental health workers, subjects with removable dentures or dental splints, who were undergoing orthodontic treatment, or who had polymer-based fillings placed during the previous 3 months were not included. Individuals fulfilling the selection criteria were consecutively included from January 2016 to November 2017. All participants provided written informed consent.

Clinical examination and dental treatment

Each participant underwent a detailed dental examination where the number of previously filled tooth surfaces and, if available, the type of preexisting restorative materials was recorded by one dentist. The same dentist performed the dental treatment at one public dental clinic. Tooth preparation, restoration, and polishing were performed in an ordinary clinical setting and according to standardized procedures and materials used at the clinic. High-volume evacuator equipment was used during cavity preparations, etching, bonding, and finishing procedures. Rubber

dam isolation was not used. A three-step etch-and-rinse procedure (ANA Etching Gel 37%, Directa, Upplands Väsby, Sweden, and OptiBond FL, Kerr, USA) was performed. The same batch of a widely used bis-GMA-based material (Tetric EvoCeram, 0.2 g compules, Color A2, LOT 014504, Ivoclar Vivadent AG, Liechtenstein) was used throughout the study. Material compositions based on the manufacturer's user manual and safety data sheet are provided in Table 3. For each patient, filling material from a discrete compule was inserted in incremental layers. Each layer was cured with the light intensity and exposure time recommended by the manufacturer. Care was taken to reduce excess material, and any surplus was removed from the filling surface and put back into the compule. The need for adjustment of the fillings was marginal. The amount (weight in grams) of material used in each participant was estimated by weighing each compule before and after treatment. The dental curing lamp was controlled for acceptable light intensity prior to each treatment session using a single dental radiometer. The extent of each filling surface was estimated by giving the area scores from 1 to 3 ("filling points") as described for Paper I (117).

Table 3. Composition of Tetric EvoCeram based on the manufacturer's "Instructions for Use" and the safety data sheet.

Composition	Ingredients	Content, weight (%)
Polymer matrix	Bis-GMA	3–<10
	Bis-EMA	3–<10
	UDMA	3–<10
	Additives, initiators, stabilizers, pigments	<1
Inorganic fillers (particle size range between 40 nm and 3 μm)	Barium glass	82–83
	Ytterbium trifluoride	
	Mixed oxides and copolymers	

Collection and analysis of saliva and urine samples

The participants were instructed to refrain from eating, drinking, brushing their teeth, using lipstick, etc., for 10 hours prior to sampling. All treatment and sampling sessions were scheduled for the morning.

Information about the participants' dental hygiene habits and if they handled receipts at work was obtained. In addition, on each day of sampling the individual's consumption of canned and microwaved food during the previous 24 hours and previous week was recorded (see Appendix III). The participants were instructed to empty their bladder during the early morning at home. Each participant provided a total of five saliva samples and four urine samples. The first saliva and urine samples were collected immediately before treatment. Sampling of a second saliva sample was started 10 minutes after placement of the polymer-based fillings, and subsequent saliva and urine samples were collected 1 hour, 24 hours, and 1 week after placement of the fillings (Figure 14). Field blanks, samples from the cooling water, and water from the dental unit were also collected, treated, stored, and analyzed in the same manner as the biological samples.

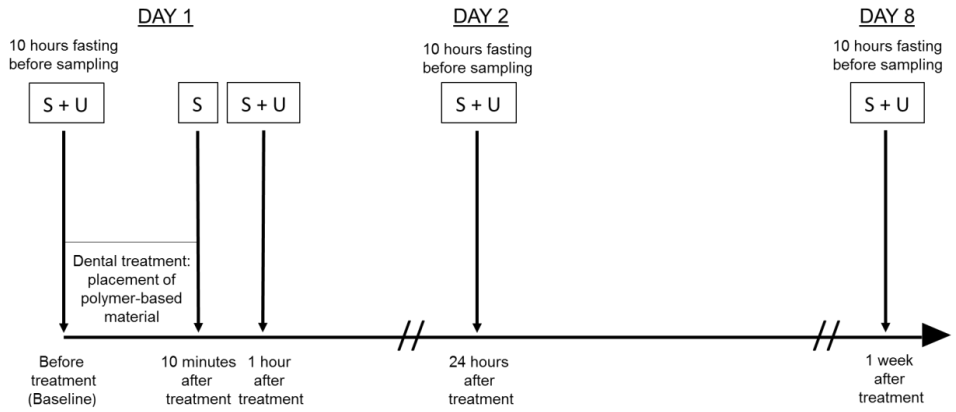


Figure 14. Time schedule of saliva (S) and urine (U) sampling in Study II.

Saliva specimens (2 ml) were collected using the same procedures and instructions as described in Paper I. No gargling was allowed in the time period between placement of the fillings and the saliva sampling 10 minutes after treatment. Urine specimens were collected in 100 ml polypropylene cups. Immediately after collection, the samples were refrigerated at 4°C and within the same day transferred into 15 ml polypropylene tubes and stored at −80°C until they were sent to the laboratory for analysis.

Determination of BPA in saliva and urine

Urine and saliva samples were analyzed at the reference laboratory in Lund, Sweden, using LC/MS/MS as described in Paper II and Appendix IV. The LOD was determined to be 0.1 ng/ml. Field blanks, laboratory blanks, and two different in-house-prepared quality control samples were analyzed in the analytical batches. The within-run and between-day precision was acceptable. The concentration of BPA in urine was adjusted for urinary density (118).

For quality control, some of the saliva samples, representing the full range of salivary BPA concentrations detected in Lund, were analyzed at an independent laboratory (Nordic Institute of Dental Materials, Oslo, Norway) using LC/MS/MS.

3.3 Paper III

Study III was based on data from MoBa (version 8 of the quality-assured MoBa data files) (113, 114) and from the Medical Birth Registry of Norway (MBRN) (116). Self-reported information from the participating women was obtained from two of the questionnaires (Q1 and Q3) responded to during pregnancy week 17 and 30, respectively (Figure 12 and Appendix V). Only singleton births were included in the present study (Figure 15).

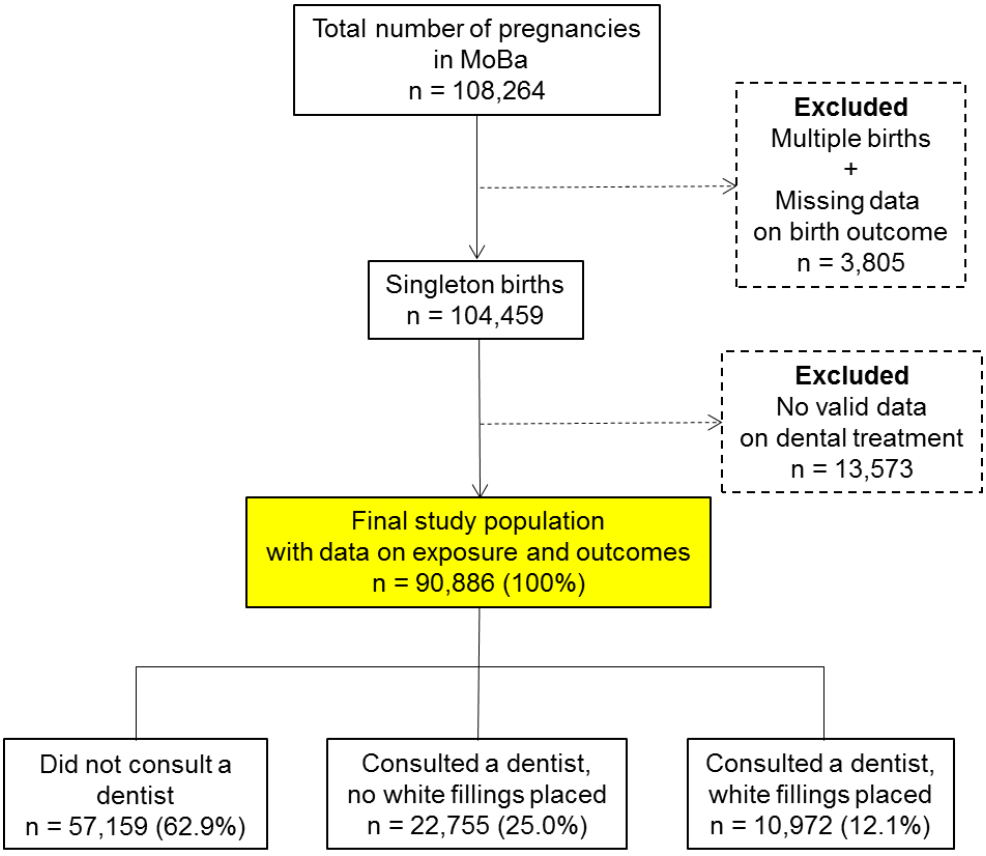


Figure 15. Flowchart showing the number of participants included in Study III and the groups available for analyses.

Exposure variable

White fillings placed during pregnancy was the exposure variable, and data on dental treatment during pregnancy were obtained from Q3. The women reported if they had consulted a dentist during pregnancy (Q3c, in the English version, Question 34: "Have you been to the dentist during this pregnancy? Yes/No") and if so, whether they had received white dental fillings (Question 35c: "If, yes, did the dentist perform any of the following treatment? New white fillings placed? (Yes/No") (See Appendix V). Dental treatments during pregnancy were categorized as participants who did not

consult a dentist during pregnancy (reference category), participants who consulted a dentist but had no white fillings placed, and participants who consulted a dentist and had white fillings placed (Figure 15). Participants with missing data on birth outcomes or with unacceptable information about dental treatment during pregnancy were excluded, leaving a study population that included 90,886 pregnancies (Figure 15).

Outcome variables

Information about pregnancy outcomes (stillbirth, preterm delivery, malformations, birth weight) was gathered from the MBRN (116). Stillbirth was defined as the death of a fetus with a gestational age of 22 weeks or more or with a birth weight of 425 g or more. Gestational age was estimated from the ultrasound examination in the 17th week of pregnancy. Information on last menstrual period was used if an ultrasound investigation had not been performed. Infants born prior to or during gestational week 32 were classified as very preterm, and if they were born between gestational week 33 and 37 they were classified as late preterm (119, 120). All diagnoses from the MBRN records are based on the International Statistical Classification of Diseases, 10th Revision (ICD-10) (121). Malformations were defined as any birth defects registered in the MBRN. Infants were classified as low- and high-birth weight infants according to the World Health Organization's (WHO's) recommended definitions (122). They were defined as small for gestational age if they were less than the 10th percentile and large for gestational age if they were larger than the 90th percentile (119, 120). Newborns with weight below the 2.5th percentile were defined as very small for gestational age (122).

Potential confounding variables

The following potential confounders were included in the analyses: parity, mother's age at delivery, maternal body mass index (BMI; kg/m^2), education, smoking habits, and alcohol consumption during pregnancy. Information about parity was based on data reported by the mothers in MoBa and from the MBRN. Parity was defined as the

number of former births (with a gestational age of 12 weeks or more) and divided into two categories (none and one or more). Information about the mother's age at delivery was gathered from the MBRN and divided into six categories (≤19, 20–24, 25–29, 30–34, 35–39 and 40+ years). Data on maternal height, pre-pregnancy weight, education years, smoking habits, and alcohol consumption during pregnancy were obtained from Q1. Maternal BMI was calculated and categorized according to the WHO classification (123). Maternal education and maternal smoking were divided into three categories (≤12, 13–16, and ≥17 years) and (never, occasionally, and daily), respectively. Maternal alcohol consumption was divided into four categories (never, less than once a week, once a week, and more than once a week).

3.4 Statistical methods

Analyses were performed using the statistical software program SPSS (IBM SPSS, New York, USA) version 21 in Paper I, version 25 in Paper II, and version 24 in Paper III. For the power calculation, IBM-SPSS Sample Power (release 3.0.1) was used. For the mixed effect analyses in Paper II Stata (Stata corp., Texas, USA) version 15 was used. P-values less than 0.05 were considered statistically significant.

Power and sample size considerations

At the time Study I and Study II were planned, we found no published data that could be used for power calculations. Thus, the following assumptions were made.

For Study I, it was assumed that 75% of the individuals in the group with polymer-based dental fillings had detectable concentrations in saliva and that 25% of the individuals in the group without such restorations had detectable concentrations. If such were the case, a study with 20 individuals in each group would have 96% power to detect a significant result (with alpha set to 0.05 and using a one-sided Chi-square test).

For Study II, it was assumed that 70% of the individuals had values below the LOD in saliva prior to the treatment and above the LOD after the treatment and that 10% of the individuals had values similar to or above the LOD prior to the treatment and values below the LOD after the treatment. It was also assumed that 20% of the individuals showed no changes and thus either had values below the LOD prior to and after the treatment or they had values above the LOD prior to and after the treatment. Using these assumptions, a study including 20 individuals would have 97% power to detect a significant result (with alpha set to 0.05 and using a one-sided McNemar test).

Numerical variables were presented as means, minimum and maximum values, and standard deviations (SDs). Categorical variables were presented (summarized) as numbers and percentages.

In Study I and II, values below the LOD were set to one half of the LOD in the statistical analysis (124).

3.4.1 Paper I

The main hypotheses to test were H_0: There is no difference in BPA concentration in saliva between the composite group and the comparison group, and H_1: The BPA concentration in saliva is higher in the composite group compared to the comparison group.

Differences in salivary BPA concentrations (the dependent variable) between exposure groups (composite group vs. comparison group) and other groups based on background characteristics that potentially could contribute to salivary BPA concentration (see Table 2 in Paper I) were assessed using the Mann–Whitney U-test (125). Spearman rank correlation was used to test the correlation between salivary BPA concentrations and the continuous variables of participant's age, number of

polymer-based filled surfaces, number of polymer-based points, time since last filling placed, time of day of saliva sampling, saliva collection duration, and saliva secretion rate. The chi-square test was used to assess differences in proportions of detectable concentrations between groups, and logistic regression analysis was performed to calculate odds ratios (ORs) for having detectable concentrations of BPA in saliva.

3.4.2 Paper II

The primary hypotheses to test were: H_0: There is no difference in BPA concentration in saliva before and after treatment with dental polymer-based restorative material and H_1: The BPA concentration in saliva increases after treatment with dental polymer-based restorative material. In addition, changes in BPA concentrations in urine before and after treatment with dental polymer-based restorative material were analyzed.

Linear mixed effects regression models were applied for the analyses of repeated measures of the BPA concentration in saliva and urine. In the models, the repeated nature of the measurements of the data were accounted for using the participant's ID, entered as a random factor, with an additional factor for time to account for the difference in variation over time.

The time point for the measurements was considered a categorical variable comparing the succeeding measurements with the pretreatment value. All available data, including data for participants with missing observations at some time points, were used in the model. Furthermore, the analyses were extended to explore whether other variables were significant in addition to time point (see Table 1-3 in Paper II).

3.4.3 Paper III

Logistic regression analyses were performed to evaluate whether dental treatment with polymer-based material (white fillings) during pregnancy was associated with risk for adverse birth outcomes (stillbirth, preterm birth, malformation, and low or high birth weight). The outcomes (dependent variables) were dichotomized as present (1) or absent (0).

Odds ratios with 95% confidence intervals (CIs) were calculated. The OR was adjusted for maternal age, education, pre-pregnancy BMI, parity, smoking during pregnancy, and alcohol consumption during pregnancy. The OR expresses differences in the risk of presenting with the outcomes between categories of the independent variables (Did not consult a dentist during pregnancy; Consulted a dentist, no white fillings placed; Consulted a dentist, white fillings placed). An OR >1 indicated an increased risk, whereas OR <1 indicated a decreased risk. A non-significant outcome was understood if 1 was included in the 95% CI.

3.5 Ethics

The ethical considerations were in accordance with the Declaration of Helsinki. Participation was voluntary and based on written informed consent, and all participants were allowed to withdraw from the studies without giving any reason and without any negative impact for the individual. Study protocols were reviewed and approved by The Regional Committees for Medical Research Ethics (REK), Norway (Approval numbers for Study I: REC South-East B, 2012/602; for Study II: REC South-East B, 2014/1529; for Study III: REC South-East D, 2011/727).

Study II was registered at ClinicalTrials.gov, number NCT02575118.

Search for literature ended 19th June 2019

4. Summary of results

This section gives a brief summary of the results presented in the three papers that constitute this thesis.

4.1 Paper I

The participants had a mean age of 24.2 years ranging from 20 to 34 years in the composite group and from 20 to 35 years in the comparison group. Women predominated in both groups. The participants in the composite group had a mean of 12.3 (SD = 4.7) surfaces previously filled with dental polymer-based material, which corresponded to a mean sum-score of 32.2 (SD = 15.2) filling points. The fillings had been placed from 1 week to several years before inclusion in the study. The mean salivary flow rate was similar in the two groups. The salivary BPA concentration was very low in both groups, and most values were below the LOD of 0.1 ng/ml. In the composite group, 8 of 20 (40%) had detectable concentrations of BPA in their saliva compared to 3 of 20 (15%) in the comparison group.

The composite group had a marginally higher concentration of BPA in their saliva (0.12 ng/ml) compared with the comparison group (below the LOD) ($p = 0.044$, Mann–Whitney U-test; one-sided exact test). Practically all BPA in the saliva samples was unconjugated, and conjugated BPA was generally not detected. When examining other potential variables that might contribute to the variation of unconjugated BPA in saliva, only intake of breakfast showed a statistically significant effect ($p = 0.003$, Mann–Whitney U-test). The correlation between BPA level in saliva and the number of surfaces filled with polymer-based material was not statistically significant (Spearman correlation coefficient 0.209; $p = 0.195$). Neither was the correlation with the surface area of the polymer-based fillings (as measured by the number of filling points) (Spearman correlation coefficient 0.245; $p = 0.299$).

4.2 Paper II

The majority of the participants were women and the mean age was 23.4 years with a range of 17 to 36 years. The participants had a mean of 11.8 (SD = 9.6) pre-existing tooth-colored filling surfaces, corresponding to 25.7 (SD = 21.7) pre-existing filling points. In the present study, the mean number of tooth-surfaces restored with polymer-based filling material was 2.7 (SD = 1.9), corresponding to 7.7 (SD = 0.7) filling points. The mean weight of polymer-based material placed in each participant was 0.158 g (SD = 0.067).

One saliva sample collected before treatment was excluded from the statistical analysis due to probable contamination. In addition, one saliva sample and one urine sample, collected from one participant at 1 week after treatment, were excluded because the participant had eaten breakfast before sampling.

Concentration of BPA in saliva

The pretreatment (baseline) levels of salivary BPA were very low, and 11 of 20 participants had values below the LOD. The estimated mean value was 0.11 ng/ml. Compared to the pretreatment levels, the salivary BPA concentration in the samples collected 10 minutes after treatment increased significantly (the mean concentration was 385 ng/ml; $p < 0.001$). Following the immediate posttreatment increase, the BPA concentrations in saliva decreased exponentially with time. However, compared to pretreatment levels, the concentration remained significantly elevated at 1 hour, 24 hours, and 1 week after placement (Figure 16).

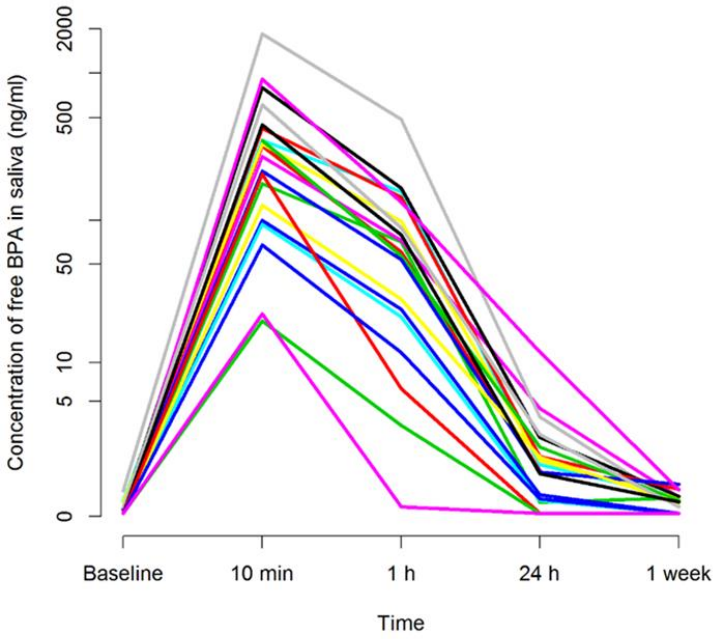


Figure 16. Salivary concentrations (ng/ml) of free (unconjugated) BPA among participants in Study II (individual patterns) before (baseline) and at 10 minutes, 1 hour, 24 hours, and 1 week after treatment with polymer-based filling material (n = 20).

The secondary explorative analyses indicated that the surface area of the new fillings (as expressed by the number of filling points) was associated with the salivary BPA concentrations measured at 24 hours and 1 week after treatment. There were no statistically significant associations between the other variables tested and the BPA levels in saliva at the different time points.

Concentration of BPA in urine

The vast majority of participants (19 of 20; 95%) had detectable BPA concentrations in their urine prior to the placement of polymer-based dental material. The calculated mean concentration was 1.41 ng/ml. There were no statistically significant differences between BPA concentration in urine before (baseline) and after treatment

(Figure 17). The BPA levels in urine samples collected 1 hour after treatment did not show an association with the BPA levels in the saliva samples collected at 10 minutes after treatment.

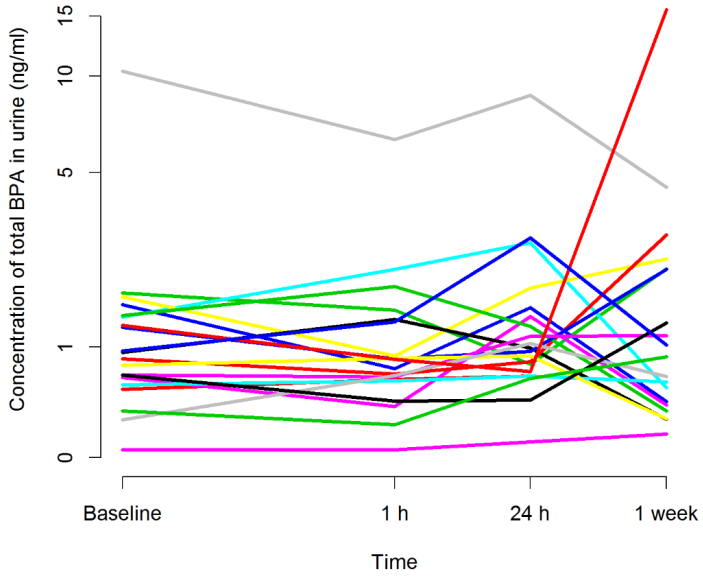


Figure 17. Urine concentrations (ng/ml) of total BPA among participants in Study II (individual patterns) before (baseline) and at 1 hour, 24 hours, and 1 week after treatment with polymer-based filling material (n = 20).

As illustrated in Figure 17, showing BPA concentrations in urine over time, two of the participants had remarkably higher BPA levels compared to the other participants. Both of these were among five participants who reported handling cash register receipts at work. The participant with the highest BPA concentration in urine was also the one with the highest levels of BPA in saliva at all time points. Using handling of receipts as a categorical variable, an overall elevated average level of urinary BPA was found for the group handling receipts ($p = 0.031$, mean difference 0.83 ng/ml; 95% CI: 0.08–1.57).

4.3 Paper III

Among the 90,886 included women, a dentist consultation during pregnancy was reported by 33,727 women. Of these, 10,972 reported having white fillings placed (Figure 14). Of the included pregnancies, 29,387 (51.4%) resulted in the birth of a boy. The proportion of stillbirths, very preterm births, and late preterm births was 0.2%, 0.6%, and 3.8%, respectively. Malformation was registered in 4.8% of the infants.

No associations between the placement of dental polymer-based fillings (white fillings) during pregnancy and adverse birth outcomes were observed.

Gender-specific analysis showed that girls born to mothers who received white fillings during pregnancy had an increased risk of being small for gestational age (below the 10th percentile) compared to the reference group (mothers who did not consult a dentist during pregnancy). The unadjusted OR was 1.14 (95% CI 1.01–1.28; p = 0.029). After adjustment for potential confounders, the OR was reduced and not statistically significant (OR = 1.10, 95% CI 0.97–1.24). Boys born to mothers who had white fillings placed during pregnancy had a marginally increased risk of being born late preterm compared to the boys born in the reference group. The unadjusted OR was 1.16 (95% CI 1.01–1.34; p = 0.041), and the adjusted OR was 1.13 (95% CI 0.98–1.31; p = 0.082).

5. Discussion

This thesis is based on three papers. Paper I and Paper II describe two separate clinical studies focusing on BPA exposure from dental polymer-based filling materials in humans. Paper III presents a study investigating whether placement of polymer-based dental fillings during pregnancy is associated with adverse birth outcomes.

5.1 Methodological considerations

5.1.1 Study design and participants

5.1.1.1 *Paper I*

The study presented in Paper I was designed as a cross-sectional study with one exposed group (composite group) and one unexposed group (comparison group). The aim was to evaluate if the presence of dental polymer-based fillings aged 1 week or older was associated with increased BPA levels in saliva. Patients who were scheduled for a routine dental check-up and who met the inclusion criteria were consecutively invited to the study. Hence, the study participants were recruited using convenience sampling. Because there was no follow up, fewer resources were required to run the study. Thus, the cross-sectional design made the study relatively fast and inexpensive to perform. The inclusion of an unexposed comparison group was advantageous for determining the effect of other potential variables that might contribute to the salivary BPA concentration. However, because the relationship between the presence of dental polymer-based fillings (exposure/dependent variable) and salivary BPA concentration (outcome/independent variable) was measured at a single point in time, only an association and no causation could be inferred from the study. On the other hand, results from this cross-sectional study might be useful to formulate hypotheses and to prepare protocols for other studies with more complex designs.

5.1.1.2 *Paper II*

The study presented in Paper II could be described as a prospective descriptive observational cohort study and was designed to evaluate whether placement of dental polymer-based fillings was associated with increased BPA levels in saliva and urine. The prospective design, observing BPA concentrations at multiple time points, allowed trends in the concentrations over time to be monitored.

The participants were recruited consecutively after routine dental check ups by their own dentist if they were provided a treatment plan for at least one dental restoration (two or more surfaces) of polymer-based filling material and otherwise fulfilled the selection criteria. The dental treatment procedures and the dental filling material used were in line with current clinical practice. Thus, the therapeutic intervention, i.e. placement of polymer-based restorations, was not assigned by the researcher and the study could thus be considered an observational study. The use of only two dental clinics for recruitment of participants and one single operator made it easy to stadardize the assessments and procedures in the study.

In this study, the individuals served as their own controls (i.e., measures before versus after treatment). Thus, the background variation in an unexposed comparison group did not have to be considered. However, including an unexposed comparison group would have strengthened the study design and made it easier to interpret the contribution of BPA from the dental polymer-based material to urinary BPA levels.

5.1.1.3 *Paper III*

In Paper III, the results from an observational cohort study evaluating associations between placement of polymer-based fillings during pregnancy and adverse birth outcomes are presented and discussed. In this study, data from MoBa (113, 114) were used. The main strengths of the study were the large sample size, the large number of women who had white fillings placed during pregnancy, and the linkage to the

MBRN (116). This made it possible to observe rare birth outcomes and to control for potential confounding variables. Moreover, the prospective design of the study reduced the risk of possible recall bias. A limitation of MoBa is the low response rate (41%), which might give rise to selection bias. Potential self-selection of the healthiest women has been discussed previously (126). MoBa participants are older and better educated and comprise a higher percentage of non-smokers compared to non-participants. However, self-selection is not considered to be a validity problem in studies of associations between exposure and outcomes in MoBa (126). Another limitation of the present study is that MoBa is based upon self-reporting. This is discussed in section 5.1.4.

5.1.2 Clinical procedures (Paper I and II)

5.1.2.1 *Choice of dental polymer-based material (Study II)*

Currently there is a wide range of dental polymer-based restorative materials available. Most of them are bis-GMA-based and thus have the potential to release small amounts of BPA (14, 45). Study II was designed to reflect an ordinary clinical situation using a representative bis-GMA-based restorative filling material. The aim of the study was not to compare the release of BPA from different dental polymer-based materials. The material was selected based on the indications specified by the manufacturer, as well as which restorative polymer-based material was reported to be the most commonly sold in Norway during 2013 (Communication by e-mail to LIC Scadenta and Plandent, the two largest dental suppliers in Norway, 08 May 2014). The same batch of a bis-GMA-based material (Tetric EvoCeram, Color A2, LOT 014504, Ivoclar Vivadent AG, Liechtenstein) was used for all participants throughout the study. Using one batch of one dental polymer-based material strengthened the internal validity of the study. However, the external validity of the study is limited. The potential to release BPA might vary between dental polymer-based materials due to differences in monomers used (5). Thus, this study could not be used to predict the

amount of BPA in saliva after placement of other dental polymer-based filling materials.

5.1.2.2 *Sample collection (Study I and II)*

To reduce BPA exposure from other sources, the participants in Study I and II were instructed to avoid food and drink intake prior to sampling. For Study I, it was expected that a 2-hour clearance should be sufficient to avoid influence from the dietary intake. However, the results from Study I suggested that intake of breakfast had a statistically significant effect on the BPA concentrations in saliva and thus indicated that 2 hours of food restriction might not be sufficient. Hence, in Study II the participants were instructed to refrain from food and drink intake 10 hours (overnight) before the scheduled sampling. Furthermore, it cannot be excluded that the time of day of saliva sampling might influence the salivary BPA concentration (Paper I, Table 3, $r_s = 0.275$, $p = 0.086$). The indoor air might be a source of BPA exposure (127, 128), and thus it could be speculated that the concentration of BPA is likely to become higher throughout the day in dental clinics. For this reason, all participants in Study II were scheduled in the morning before 9 a.m. In addition, they were instructed to put the cap back on the tube between each spitting during the saliva sampling.

The participants in Study I and II were asked to provide 5 ml and 2 ml saliva samples, respectively. The duration of the sampling varied between and within participants and could potentially affect the BPA concentration. However, no statistically significant correlation between salivary BPA concentration and sampling time was found.

5.1.2.3 *Choice of biological medium (Study I and II)*

The overall aim of Study I and II was to assess if dental polymer-based fillings have the potential to contribute to human BPA exposure. To evaluate potential exposure, saliva and urine were collected.

Saliva embraces the teeth and is in direct contact with the dental fillings. Therefore, salivary BPA concentrations should be a good indicator of BPA exposure from dental polymer-based restorations and might reflect the highest measurable level of the biologically active form of BPA (unconjugated BPA) released into the oral cavity. Moreover, saliva provides non-invasive, rapid, economical, and easy sampling (129). Although saliva is considered a useful matrix, high inter- and intra-individual variability have been reported in different clinical studies examining saliva concentrations of leachable chemicals from dental polymer-based materials (29, 44, 130) and might pose a challenge. Even though the sampling procedure was standardized, considerable variation between individuals was observed (Figure 16).

Blood and urine have generally been the matrices used to monitor levels of most toxic compounds (131). Urine, like saliva, provides non-invasive, quick, economical, and easy sampling. Ingested BPA is considered to be rapidly absorbed, conjugated, and excreted in the urine within 24 hours after exposure (64, 73, 77), but the validity of studies examining BPA levels in human blood after oral administration has been disputed (52, 132). The controversies have mainly been related to the analytical methods used as well as to concerns regarding possible BPA contamination from the materials/equipment used for the sampling and processing procedures (52, 67, 133-136). Moreover, blood sampling is time-consuming, invasive, and might be associated with anxiety for some individuals.

BPA is mainly present as the conjugated form in human urine. Thus, the potential external contamination (unconjugated form) as suggested regarding blood samples is not an issue.

Furthermore, two previous clinical studies assessing BPA exposure after dental treatment with polymer-based materials were not able to detect any BPA in blood (137, 138). The authors speculated that the lack of detectable values of unconjugated BPA in blood was probably due to relatively low exposure doses or rapid metabolism of BPA.

Because excretion of BPA in the urine is almost complete within 24 hours and because urine offers several advantages compared to blood, urine was the matrix of choice for assessing potential systemic exposure to BPA in Study II.

5.1.3 Determination of BPA concentrations in saliva and urine (Paper I and II)

A European reference laboratory for BPA in urine performed the analysis of the biological samples in Study I and II. To achieve high sensitivity and high specificity, LC/MS/MS with an isotopically labeled internal standard (D_{16}-BPA) was used. The LC/MS/MS is the method of choice for the determination of BPA in biological samples (139). BPA is a widespread chemical, and it might be found ubiquitously. To confirm the validity of the analysis and to identify potential contaminants, quality control samples and field blanks were analyzed together with the samples of saliva and urine. The LOD of the method was low and the analysis had high precision and low variability. In addition, the levels of salivary BPA concentrations in Study II were confirmed by an independent laboratory (Nordic Institute of Dental Materials). Thus, the analysis was considered to be reliable.

5.1.4 Data from the Mother and Child Cohort Study (Paper III)

5.1.4.1 *Exposure indicator*

MoBa is based on self-administered questionnaires designed to be completed by the respondent without the intervention of the researchers collecting the data (114). To obtain reliable answers from the participants, efforts were made to formulate the questions to be as comprehensible as possible. In Norway, the term "white fillings" is practically synonymous with dental polymer-based restoratives or so-called dental composites. Thus, to gain knowledge about treatment with dental polymer-based material during pregnancy, the women were asked if they had received white dental fillings while pregnant (see Appendix V). The use of "white fillings" as an exposure indicator was discussed and elaborated in Paper III. The term may include other tooth-colored dental materials such as resin-modified cements, compomers, and conventional glass-ionomer cements and could potentially give rise to misclassification. However, studies investigating treatment concepts for dental caries in Norway during the MoBa enrolment period showed that the vast majority of responding dentists preferred polymer-based material when restoring permanent teeth (140-142). Although access to dental records with detailed information about dental restorative treatment (e.g. which polymer-based materials were used and number and extension of restorations) would have been preferable, this would be unachievable in such a large epidemiological study as MoBa. Hence, information about possible previous dental restorative treatment, the exact number and extension of the fillings as well as the types and brands of materials placed during pregnancy, is lacking.

5.1.4.2 *Time of exposure*

The participants were asked to report dental treatment during the first 30 weeks of pregnancy. However, they were not asked to specify the exact day or week of pregnancy they consulted the dentist. This restricted the possibility to investigate if

placement of dental polymer-based materials plays important roles at specific time windows over the course of pregnancy. The effects of prenatal exposure to toxic agents might be considerably influenced by the degree and timing of exposure during gestation (143). Some teratogen agents cause adverse effects only during a "critical window" which may be certain days of early development of the fetus when a particular part of the fetus' is developed (143). The Thalidomide tragedy in the late 1950s and the early 1960s is a well known example. Maternal intake of the medication between day 20 and day 36 after fertilization resulted in serious malformations of the fetus (144).

5.2 Comments on the statistical analyses

5.2.1 Paper I

In Study I, the data set was relatively small, and the groups were considered independent. Most of the values were below the LOD and the data were accordingly skewed. Thus, the non-parametric Mann–Whitney U-test, which does not assume a normal distribution, was used to compare the groups (125). Non-parametric tests are generally less powerful than parametric tests with regard to the detection of existing differences. On the other hand, they are less affected by extreme observations. Taking into account that the conditions for using a parametric test were not met and that the data set was small, Spearman rank correlation was the natural choice to test correlations between variables.

There was no reason to believe that the composite group, exposed to dental fillings known to contain impurities of BPA would have lower salivary BPA concentration compared to the comparison group without such fillings. Thus, formulation of a one-sided hypothesis providing more power was appropriate (145). However, this

excluded the possibility to study if the comparison group would have higher concentration of BPA in saliva compared to the composite group.

5.2.2 Paper II

In Study II, multiple values from each individual measured at specific time points were analyzed. Some of the measurements were excluded due to non-compliance and suspicion of contamination. Thus, of the 20 individuals, only 18 of them had complete data. Use of analysis of variance for repeated measures would thus be based only on the 18 individuals. In an effort to keep as much data as possible, the use of linear mixed effects regression models, preventing listwise deletion, was applied.

5.2.3 Paper III

In Study III, the impact of dental treatment with dental polymer-based fillings during pregnancy on negative birth outcomes was investigated. When analyzing the association between a set of predictors and binary outcomes, logistic regression is a standard choice. Analysis with logistic regression allows inclusion of covariates in order to control for possible confounders (i.e. variables associated both with the exposure and the outcome), which could distort the estimated OR for the exposure variable.

5.3 Comments on the results

5.3.1 Paper I

The results from Study I provided weak evidence indicating that existing dental polymer-based fillings (aged one week or older) have a low tendency of BPA

leaching. Although the composite group had marginally elevated levels of unconjugated BPA in saliva compared to the comparison group, the levels were very low and the difference between the groups was small.

Almost all detected BPA in saliva was in the unconjugated form. Conjugated BPA was generally not found. This indicates that the BPA detected in saliva is from local exposure (e.g. released from the polymer-based fillings). It could be hypothesized that salivary BPA could be derived from blood, but this theory is unlikely because BPA from blood most probably would be in the conjugated form (77, 137).

The estimated mean value of unconjugated BPA in the composite group (0.12 ng/ml) corresponded well to the estimated mean salivary BPA concentration detected at baseline (before placement of dental polymer-based fillings) in Study II (0.11 ng/ml). In other studies assessing salivary BPA concentrations prior to placement of dental polymer-based material, slightly higher estimated mean levels were reported (range 0.22–1.0 ng/ml) (28, 130, 137, 138, 146-148). However, in the majority of studies, no restrictions of food or drink intake prior to sampling were described. In the present study, intake of breakfast showed a statistically significant effect on the salivary BPA level. This finding indicates that oral exposure to other sources of BPA, even though ingested more than 2 hours before sampling, might influence the BPA levels in saliva and thus pose potential misclassification bias. However, as discussed in section 5.1.2.2, it cannot be excluded that the time of the day of sampling could be a factor of importance.

5.3.2 Paper II

The main finding in Study II was the significant increase in the BPA concentration in saliva directly after treatment with a dental polymer-based restorative material. The increase was followed by an exponential decrease, but the levels remained significantly elevated at all time points. However, at 1 week after treatment the BPA

concentration was only slightly elevated compared to the baseline levels. This time-course of salivary BPA concentration after placement of dental polymer-based materials is in accordance with other studies (28, 137, 146, 148, 149). The pattern indicates that the main exposure to BPA from dental polymer-based filling materials is limited to a short period after placement. The study revealed no change in urinary BPA levels after placement of polymer-based restorations.

Over the last few decades, several studies have been conducted to assess the amounts of BPA in saliva and urine after placement of dental polymer-based materials (28, 146-148). However, as discussed in Paper II, the types, brands and application modes differ among the materials tested. Moreover, there are wide differences regarding sample size, number and extent of tooth surfaces filled, sampling procedures, and laboratory methods between studies. Another issue is that relevant details in treatment and sampling procedures as well as the LOD, are not always presented by the authors. Thus, quantitative comparison between studies has been considered impossible (150).

The mean BPA concentration detected 10 minutes after treatment was higher than expected. The level was comparable with previously reported salivary BPA concentrations detected after placement of bis-DMA-based dental fissure sealants (137, 149). These studies measured salivary BPA concentrations directly after (149) and 1 to 3 hours after (137) treatment, and the detected values were in the range of 0.3–2.8 ppm and 5.8–105.6 ppb, respectively. The first in vivo study showing the implications of BPA release from dental polymer-based filling materials was published by Olea et al. in 1996 (44). The authors reported cumulative salivary BPA concentrations 1 hour after sealant placement, that were approximately 100 times higher than the concentrations detected directly after treatment in the present study. However, the reliability of the analytical methods used in the Olea study have been questioned (59), and the levels have not been confirmed by later studies. Other researchers, evaluating the release of BPA after placement of bis-GMA-based dental polymer-based materials used as restoratives and in orthodontic treatment (28, 146-

148), have presented salivary BPA concentrations considerably lower than the levels detected in the present study.

It has been reported that use of rubber dam during placement of dental polymer-based materials might reduce the increase of salivary BPA concentration directly after treatment (28). However, although maintaining a "dry field" is important when performing moisture-sensitive techniques, like placements of polymer-based restorations, the results from studies indicate that most dentists in non-academic clinical practice do not use rubber dam isolation during operative dentistry procedures (151, 152). In the present study, the purpose was to perform the dental treatment according to common clinical procedures. Thus, rubber dam was not used. Moreover, in order to detect the maximum BPA exposure after treatment, the participants were not allowed to rinse their mouth with water between filling placement and the subsequent saliva sampling. As reported by Sasaki et al., gargling water for 30 seconds after placement of polymer-based material might decrease salivary BPA concentrations to nearly baseline levels (130). Thus, it is plausible that methodological issues could have influenced the salivary BPA concentrations detected 10 minutes after treatment in the present study.

Further analyses showed statistically significant associations between the surface area of the placed fillings, expressed as number of filling points, and the salivary BPA concentration detected 24 hours and 1 week after treatment. These findings support results from other studies (153, 154) and may indicate a leakage from the free (exposed) surfaces of the fillings due to impurities in the monomer used. It could be expected that BPA leakage from the oxygen-inhibited layer on each filling surface could be associatied to the number of filling points in the samples collected directly after treatment. However, the BPA concentrations detected 10 minutes and 1 hour after placement did not show such an association. One possible explanation could be that factors with no correlations to the filling points (e.g. surplus material after placement, grinding and finishing) have contributed more substantially to the salivary

BPA concentration up to 1 hour after treatment. It cannot be excluded that use of rubber dam during the filling placement procedure, as well as rinsing the mouth after treatment, would have reduced the BPA exposure from these sources and thus would have strengthened the association between the number of filling points and salivary BPA concentration detected 10 minutes and 1 hour after placement.

The analyses did not identify significant associations between the amounts (weight in grams) of dental material placed and salivary BPA levels. This confirms previous findings in the literature and might be due to the observation period in the present study, which was too short to observe degradation and subsequent release of unbound compounds trapped in the polymer network (5).

The salivary BPA concentrations detected 10 minutes after treatment were high, and it could be expected that these levels could have been reflected in the subsequent urinary BPA concentrations. However, the urinary BPA level did not appear to be associated with the dental treatment. Neither the first posttreatment urine samples, collected 1 hour after treatment, nor the samples collected 24 hours and 1 week after treatment showed significant increases in BPA concentrations compared with baseline levels.

The BPA concentration in urine reflects the absorbed dose of BPA (73). As for saliva, it could be speculated as to whether the elevated urinary concentrations at 1 hour (146, 148) and 24 hours (28, 146-148, 155) after treatment reported in other studies might have been influenced by food intake.

The BPA concentrations in urine vary over time with exposure from the diet and other sources and exposure routes (54, 65, 66). Given that most of BPA is eliminated in urine after 5 to 7 hours (77), it is not unreasonable that 10 hour's fasting might decrease the BPA exposure, and thus decrease the urinary BPA level. Thus, a relatively low BPA exposure from dental polymer-based materials might not have

been sufficient to compensate for a lack of exposure from food and drink consumption.

Moreover, saliva sampling immediately after treatment might have considerably reduced the amount of BPA, that otherwise would have been absorbed, metabolized in the liver, and excreted via the urine (146). Furthermore, studies using single controlled doses of isotopically labeled BPA have indicated that following oral administration in adult humans, approximately half of the administered dose was eliminated in urine 1–3 hours after ingestion, and by 24 hours the urinary elimination was almost complete (64, 73). Thus, it could be speculated that the times chosen for posttreatment urine sampling in the present study were not the ideal time points for detecting levels of BPA after dental treatment. It is possible that collection of urine at later time points (e.g. 2 and 3 hours after treatment) could have been more useful for detecting BPA in the samples.

Two participants showed higher BPA levels in urine compared to the other subjects (Figure 16). Both of them were among five participants who handled receipts at work. Looking at these five as a group, there was considerable between and within-subject variability. However, as also found by Thayer et al. (66), average BPA levels were elevated. Thus, thermal paper could have been one source of exposure.

5.3.3 Paper III

This study revealed no increased risk of any of the following adverse birth outcomes – stillbirth, preterm birth, malformations, and low or high birth weight – among women who had white fillings placed during pregnancy. Gender-specific analyses showed generally similar results as when girls and boys were analyzed together.

This study might represent the very first to investigate potential associations between dental polymer-based fillings placed during pregnancy and birth outcomes. In a study by Michalowicz et al., no significant associations between adverse pregnancy

outcomes and use of anesthetic during nonsurgical periodontal treatment, treatment including temporary and permanent restorations, endodontic therapy, or extractions were found (156). However, in their study a selected sample of pregnant women diagnosed with periodontitis was examined. Moreover, the type of restorative materials, whether temporary, amalgam, or tooth-colored, was not recorded. Thus, although their findings are in agreement with the results described in Paper III, the studies are not directly comparable.

5.4 General discussion – clinical considerations

The results presented in this thesis suggest that treatment with dental polymer-based restorative materials might contribute to BPA exposure in humans. However, the exposure appears to be relatively short and transient. Women participating in MoBa who had dental polymer-based restorations (white fillings) placed during pregnancy had no increased risk for adverse birth outcomes compared with women who did not consult a dentist during pregnancy.

The daily dose of BPA from dental polymer-based restorative materials is probably relatively low compared with the total exposure from food and other sources and far below the current reference limit of Tolerable Daily Intake in Europe (i.e. 4 micrograms per kg bodyweight per day) (95). However, the biological effects of BPA have been reported to occur even within the range of the detection limits of most analytical procedures, and its influence on tissues might show a nonmonotonic dose response curve (85). Moreover, dental polymer-based materials cannot be considered on their own, but rather they must be added to other sources of BPA to which patients are exposed to on a daily basis. Exposure to BPA during developmental stages of life, such as prenatal life, infancy, and early childhood might be of particular concern (106-108). Fetuses and neonates might have lower capability to metabolize and excrete BPA from the body compared with adults and thus have greater potential for

negative health effects (109). In this context, it should be recognized that another synthetic estrogen, diethylstilbestrol (DES), which was commercialized during the 1940s, was found to be the causative factor to the higher incidence of adenocarcinomas of the vagina and cervix in daughters of women treated with the hormone during pregnancy (49).

Ideally, dental polymer-based restorative materials should be produced without substances having potential adverse effects. However, products presented as BPA-free have also shown estrogenic activity (157). As a guide to clinicians, safety data sheets should present essential information to identify potential harmful constituents in a material. However, studies have shown that data are often incomplete (29, 39, 158). It is hoped that new regulations and an updated ISO-4049 standard (2019) (https://www.iso.org/standard/67596.html) will force manufacturers to provide more complete information about the substances in their products.

Nevertheless, the benefit of dental polymer-based restorative material for oral health is well established, and these materials have become the first choice for restorative dental treatment (1). Given an extensive use of dental polymer-based materials, clinical procedures, limiting the release of BPA and other substances into the oral cavity after placement of such materials should be provided. Obviously, it is important to follow the manufacturers' instructions with regard to indications and handling procedures, including optimal polymerization. Use of rubber dam during insertion and grinding/finishing of the material might reduce the potential exposure (28). Moreover, rinsing with water for 30 seconds directly after treatment might reduce the exposure even more and should be highly recommended as a clinical routine (130).

6. Conclusions

In line with the specific aims of this thesis, the following main conclusions were drawn:

- There was some evidence that presence of dental polymer-based restorative fillings may be associated with slightly increased concentration of unconjugated BPA in saliva.

- The contribution from pre-existing dental polymer-based fillings to the total BPA exposure seemed to be low.

- Placement of dental polymer-based restorative materials might cause a substantial, short, and transient increase in salivary BPA concentration after treatment.

- There was no evidence that treatment with dental polymer-based restorative material was associated with increased BPA level in urine.

- The placement of dental polymer-based fillings (white fillings) during pregnancy was not associated with adverse birth outcomes including stillbirth, preterm birth, malformations and low or high birth weight.

7. Future perspectives

Due to their beneficial uses, dental polymer-based materials will continue to be an important contributor to restorative dental treatment. The results from the present work confirmed that these materials have the potential to release BPA immediately after placement. It is not likely that the manufacturers in the near future will be able to produce dental polymer-based restorative materials without any potential side effects. Thus, until further notice care must be taken to reduce exposure to potentially hazardous substances during and after dental treatment. Even though the risk for adverse effects might be very low, the precautionary principle (https://eur-lex.europa.eu/legal-content) could justify the implementation of preventive clinical procedures in clinical guidelines, especially regarding young children, and pregnant woman.

Future experiments should include the use of rubber dam and should allow participants to rinse their mouth after placement in order to reduce BPA in saliva directly after treatment. To obtain a more representative measure of systemic BPA exposure, saliva sampling should be avoided before urine collection. Furthermore, to monitor BPA concentration as a function of time, cumulative sampling of urine 1–6 hours after treatment should be considered.

With regard to potential adverse effects after placement of dental polymer-based materials during pregnancy, it might be interesting to design a case-control study with access to the dental records of the pregnant women. In this way, birth outcomes could be related to the exact dental history, including detailed information on type of material, teeth treated, and dates of treatment. However, such a study would pose ethical challenges.

8. References

1. Eklund SA. Trends in dental treatment, 1992 to 2007. J Am Dent Assoc. 2010;141(4):391-9.

2. United Nations Environment Programme (UNEP). The Minamata Convention on Mercury. 2013.

3. International Organization for Standardization. ISO 4049:2009. Dentistry − Polymer-based restorative materials. Geneva, Switzerland: ISO; 2009.

4. Mitra SB, Sakaguchi RL. Restorative materials − Composites and polymers. In: Sakaguchi RL, Powers JM, editors. Craig's restorative dental materials. 13th ed. Philadelphia: Elsevier Mosby; 2012. p. 161-98.

5. Van Landuyt KL, Nawrot T, Geebelen B, De Munck J, Snauwaert J, Yoshihara K, et al. How much do resin-based dental materials release? A meta-analytical approach. Dent Mater. 2011;27(8):723-47.

6. Rueggeberg FA. From vulcanite to vinyl, a history of resins in restorative dentistry. J Prosthet Dent. 2002;87(4):364-79.

7. Buonocore MG. A simple method of increasing the adhesion of acrylic filling materials to enamel surfaces. J Dent Res. 1955;34(6):849-53.

8. Bowen RL. Use of epoxy resins in restorative materials. J Dent Res. 1956;35(3):360-9.

9. Bowen RL. Method of preparing a monomer having phenoxy and methacrylate groups linked by hydroxyl glycerol groups. US Patent. 1965;3(179):623.

10. Rawls HR, Whang K. Resin-based composites. In: Anusavice KJ, Shen C, Rawls HR, editors. Phillips' Science of Dental Materials. 12 ed: Elsevier/Saunders; 2013. p. 275-306.

11. European Parliament and the Council of the European Union. Medical device regulation - Regulation (EU) 2017/745 of the European Parliament and of the Council of 5 April 2017 on medical devices, amending Directive 2001/83/EC, Regulation (EC) No 178/2002 and Regulation (EC) No 1223/2009 and repealing Council Direct. Off J Eur Union. 2017:L 117; 1-75.

12. European Commission. CE marking [cited 2019 June 19]. Available from: https://ec.europa.eu/growth/single-market/ce-marking_en].

13. European Parliament and the Council of the European Union. Regulation (EC) no 1272/2008 of the European Parliament and of the Council of 16 December

2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006. Off J Eur Union. 2008:L 353; 1-1355.

14. Dursun E, Fron-Chabouis H, Attal JP, Raskin A. Bisphenol A Release: Survey of the Composition of Dental Composite Resins. Open Dent J. 2016;10:446-53.

15. Peutzfeldt A. Resin composites in dentistry: the monomer systems. Eur J Oral Sci. 1997;105(2):97-116.

16. Fugolin APP, Pfeifer CS. New Resins for Dental Composites. J Dent Res. 2017;96(10):1085-91.

17. Zimmerli B, Strub M, Jeger F, Stadler O, Lussi A. Composite materials: composition, properties and clinical applications. A literature review. Schweiz Monatsschr Zahnmed. 2010;120(11):972-86.

18. Ferracane JL. Resin composite--state of the art. Dent Mater. 2011;27(1):29-38.

19. Söderholm KJ, Schmidseder J. Composites-Background. In: Rateitschak KH, Wolf HF, editors. Color Atlas of Dental Medicine - Aesthetic Dentistry. Stuttgart, New York: Thieme; 2000. p. 85-9.

20. Sofan E, Sofan A, Palaia G, Tenore G, Romeo U, Migliau G. Classification review of dental adhesive systems: from the IV generation to the universal type. Ann Stomatol (Roma). 2017;8(1):1-17.

21. European Chemicals Agency (ECHA). Community rolling action plan [cited 2019 June 19]. Available from: https://echa.europa.eu/regulations/reach/evaluation/substance-evaluation/community-rolling-action-plan].

22. Galvao MR, Caldas SG, Bagnato VS, de Souza Rastelli AN, de Andrade MF. Evaluation of degree of conversion and hardness of dental composites photo-activated with different light guide tips. Eur J Dent. 2013;7(1):86-93.

23. Schneider LF, Consani S, Ogliari F, Correr AB, Sobrinho LC, Sinhoreti MA. Effect of time and polymerization cycle on the degree of conversion of a resin composite. Oper Dent. 2006;31(4):489-95.

24. Sideridou I, Tserki V, Papanastasiou G. Effect of chemical structure on degree of conversion in light-cured dimethacrylate-based dental resins. Biomaterials. 2002;23(8):1819-29.

25. AlShaafi MM. Factors affecting polymerization of resin-based composites: A literature review. Saudi Dent J. 2017;29(2):48-58.

26. Ruyter IE. Unpolymerized surface layers on sealants. Acta Odontol Scand. 1981;39(1):27-32.

27. Gupta SK, Saxena P, Pant VA, Pant AB. Release and toxicity of dental resin composite. Toxicol Int. 2012;19(3):225-34.

28. Kingman A, Hyman J, Masten SA, Jayaram B, Smith C, Eichmiller F, et al. Bisphenol A and other compounds in human saliva and urine associated with the placement of composite restorations. J Am Dent Assoc. 2012;143(12):1292-302.

29. Michelsen VB, Kopperud HB, Lygre GB, Bjorkman L, Jensen E, Kleven IS, et al. Detection and quantification of monomers in unstimulated whole saliva after treatment with resin-based composite fillings in vivo. Eur J Oral Sci. 2012;120(1):89-95.

30. Putzeys E, De Nys S, Cokic SM, Duca RC, Vanoirbeek J, Godderis L, et al. Long-term elution of monomers from resin-based dental composites. Dent Mater. 2019;35(3):477-85.

31. Sevkusic M, Schuster L, Rothmund L, Dettinger K, Maier M, Hickel R, et al. The elution and breakdown behavior of constituents from various light-cured composites. Dent Mater. 2014;30(6):619-31.

32. Finer Y, Jaffer F, Santerre JP. Mutual influence of cholesterol esterase and pseudocholinesterase on the biodegradation of dental composites. Biomaterials. 2004;25(10):1787-93.

33. American Dental Association Council on Scientific A. Determination of bisphenol a released from resin-based composite dental restoratives. J Am Dent Assoc. 2014;145(7):763-5.

34. Cai K, Delaviz Y, Banh M, Guo Y, Santerre JP. Biodegradation of composite resin with ester linkages: identifying human salivary enzyme activity with a potential role in the esterolytic process. Dent Mater. 2014;30(8):848-60.

35. Drummond JL. Degradation, fatigue, and failure of resin dental composite materials. J Dent Res. 2008;87(8):710-9.

36. Finer Y, Santerre JP. The influence of resin chemistry on a dental composite's biodegradation. Journal of Biomedical Materials Research Part A. 2004;69a(2):233-46.

37. Jaffer F, Finer Y, Santerre JP. Interactions between resin monomers and commercial composite resins with human saliva derived esterases. Biomaterials. 2002;23(7):1707-19.

38. Tillberg A, Stenberg B, Berglund A. Reactions to resin-based dental materials in patients--type, time to onset, duration, and consequence of the reaction. Contact Dermatitis. 2009;61(6):313-9.

39. Örtengren U, Andreasson H, Karlsson S, Meding B, Barregard L. Prevalence of self-reported hand eczema and skin symptoms associated with dental materials among Swedish dentists. Eur J Oral Sci. 1999;107(6):496-505.

40. Becher R, Kopperud HM, Al RH, Samuelsen JT, Morisbak E, Dahlman HJ, et al. Pattern of cell death after in vitro exposure to GDMA, TEGDMA, HEMA and two compomer extracts. Dent Mater. 2006;22(7):630-40.

41. Geurtsen W. Substances released from dental resin composites and glass ionomer cements. Eur J Oral Sci. 1998;106(2 Pt 2):687-95.

42. Schweikl H, Spagnuolo G, Schmalz G. Genetic and cellular toxicology of dental resin monomers. J Dent Res. 2006;85(10):870-7.

43. Geurtsen W. Biocompatibility of resin-modified filling materials. Crit Rev Oral Biol Med. 2000;11(3):333-55.

44. Olea N, Pulgar R, Perez P, Olea-Serrano F, Rivas A, Novillo-Fertrell A, et al. Estrogenicity of resin-based composites and sealants used in dentistry. Environ Health Perspect. 1996;104(3):298-305.

45. Fleisch AF, Sheffield PE, Chinn C, Edelstein BL, Landrigan PJ. Bisphenol A and related compounds in dental materials. Pediatrics. 2010;126(4):760-8.

46. National Institutes of Health. PubChem Identifier: CID 6623 - Bisphenol A: National Institutes of Health (NIH) [cited 2019 June 19]. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/6623].

47. Jalal N, Surendranath AR, Pathak JL, Yu S, Chung CY. Bisphenol A (BPA) the mighty and the mutagenic. Toxicology Reports. 2018;5:76-84.

48. Dodds EC, Lawson W. Synthetic oestrogenic agents without the phenanthrene Nucleus. Nature. 1936;137:996.

49. Vogel SA. The politics of plastics: the making and unmaking of bisphenol a "safety". Am J Public Health. 2009;99 Suppl 3:S559-66.

50. The European Chemicals Agency (ECHA). Substance information: 4,4'-isopropylidenediphenol: ECHA; 2019 [Available from: https://echa.europa.eu/substance-information/-/substanceinfo/100.001.133].

51. Geens T, Goeyens L, Covaci A. Are potential sources for human exposure to bisphenol-A overlooked? Int J Hyg Environ Health. 2011;214(5):339-47.

52. Vandenberg LN, Chahoud I, Heindel JJ, Padmanabhan V, Paumgartten FJ, Schoenfelder G. Urinary, circulating, and tissue biomonitoring studies indicate widespread exposure to bisphenol A. Environ Health Perspect. 2010;118(8):1055-70.

53. Lorber M, Schecter A, Paepke O, Shropshire W, Christensen K, Birnbaum L. Exposure assessment of adult intake of bisphenol A (BPA) with emphasis on canned food dietary exposures. Environ Int. 2015;77:55-62.

54. Wilson NK, Chuang JC, Morgan MK, Lordo RA, Sheldon LS. An observational study of the potential exposures of preschool children to pentachlorophenol, bisphenol-A, and nonylphenol at home and daycare. Environ Res. 2007;103(1):9-20.

55. Stahlhut RW, Welshons WV, Swan SH. Bisphenol A data in NHANES suggest longer than expected half-life, substantial nonfood exposure, or both. Environ Health Perspect. 2009;117(5):784-9.

56. EFSA CEF Panel (EFSA Panel on Food Contact Materials E, Flavourings and Processing Aids). Scientific Opinion on the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs: Part I - Exposure assessment. EFSA Journal. 2015;13(1):1-396.

57. SCENIHR (Scientific Committee on Emerging and Newly-Identified Health Risks). Opinion on the safety of dental amalgam and alternative dental restoration materials for patients and users (update), 29 April, 2015: European Commission, DG Health and Food Safety; 2015. Available from: http://ec.europa.eu/health/sites/health/files/scientific_committees/emerging/docs/scenihr_o_046.pdf.

58. Söderholm KJ, Mariotti A. BIS-GMA--based resins in dentistry: are they safe? J Am Dent Assoc. 1999;130(2):201-9.

59. Atkinson JC, Diamond F, Eichmiller F, Selwitz R, Jones G. Stability of bisphenol A, triethylene-glycol dimethacrylate, and bisphenol A dimethacrylate in whole saliva. Dent Mater. 2002;18(2):128-35.

60. Kadoma Y, Tanaka M. Acid and base-catalyzed hydrolysis of bisphenol A-related compounds. Dent Mater J. 2000;19(2):139-52.

61. Schmalz G, Preiss A, Arenholt-Bindslev D. Bisphenol-A content of resin monomers and related degradation products. Clin Oral Investig. 1999;3(3):114-9.

62. Chen L, Suh BI. Bisphenol A in Dental Materials: A Review. JSM Dent. 2013;1(1004).

63. Gayrard V, Lacroix MZ, Collet SH, Viguie C, Bousquet-Melou A, Toutain PL, et al. High bioavailability of bisphenol A from sublingual exposure. Environ Health Perspect. 2013;121(8):951-6.

64. Teeguarden JG, Twaddle NC, Churchwell MI, Yang X, Fisher JW, Seryak LM, et al. 24-hour human urine and serum profiles of bisphenol A: Evidence against sublingual absorption following ingestion in soup. Toxicol Appl Pharmacol. 2015;288(2):131-42.

65. Biedermann S, Tschudin P, Grob K. Transfer of bisphenol A from thermal printer paper to the skin. Anal Bioanal Chem. 2010;398(1):571-6.

66. Thayer KA, Taylor KW, Garantziotis S, Schurman SH, Kissling GE, Hunt D, et al. Bisphenol A, Bisphenol S, and 4-Hydroxyphenyl 4-Isoprooxyphenylsulfone (BPSIP) in Urine and Blood of Cashiers. Environ Health Perspect. 2016;124(4):437-44.

67. Dekant W, Völkel W. Human exposure to bisphenol A by biomonitoring: methods, results and assessment of environmental exposures. Toxicol Appl Pharmacol. 2008;228(1):114-34.

68. Matthews JB, Twomey K, Zacharewski TR. In vitro and in vivo interactions of bisphenol A and its metabolite, bisphenol A glucuronide, with estrogen receptors alpha and beta. Chem Res Toxicol. 2001;14(2):149-57.

69. Völkel W, Bittner N, Dekant W. Quantitation of bisphenol A and bisphenol A glucuronide in biological samples by high performance liquid chromatography-tandem mass spectrometry. Drug Metab Dispos. 2005;33(11):1748-57.

70. Snyder RW, Maness SC, Gaido KW, Welsch F, Sumner SC, Fennell TR. Metabolism and disposition of bisphenol A in female rats. Toxicol Appl Pharmacol. 2000;168(3):225-34.

71. Li M, Yang Y, Yang Y, Yin J, Zhang J, Feng Y, et al. Biotransformation of bisphenol AF to its major glucuronide metabolite reduces estrogenic activity. PLoS One. 2013;8(12):e83170.

72. Taylor JA, Vom Saal FS, Welshons WV, Drury B, Rottinghaus G, Hunt PA, et al. Similarity of bisphenol A pharmacokinetics in rhesus monkeys and mice: relevance for human exposure. Environ Health Perspect. 2011;119(4):422-30.

73. Thayer KA, Doerge DR, Hunt D, Schurman SH, Twaddle NC, Churchwell MI, et al. Pharmacokinetics of bisphenol A in humans following a single oral administration. Environ Int. 2015;83:107-15.

74. Negishi T, Tominaga T, Ishii Y, Kyuwa S, Hayasaka I, Kuroda Y, et al. Comparative study on toxicokinetics of bisphenol a in F344 rats, monkeys (Macaca fasciculans), and chimpanzees (Pan troglodytes). Exp Anim. 2004;53(4):391-4.

75. Pottenger LH, Domoradzki JY, Markham DA, Hansen SC, Cagen SZ, Waechter JM. The relative bioavailability and metabolism of bisphenol A in rats is dependent upon the route of administration. Toxicol Sci. 2000;54(1):3-18.

76. Tominaga T, Negishi T, Hirooka H, Miyachi A, Inoue A, Hayasaka I, et al. Toxicokinetics of bisphenol A in rats, monkeys and chimpanzees by the LC-MS/MS method. Toxicology. 2006;226(2-3):208-17.

77. Völkel W, Colnot T, Csanady GA, Filser JG, Dekant W. Metabolism and kinetics of bisphenol A in humans at low doses following oral administration. Chem Res Toxicol. 2002;15:1281-7.

78. Crain DA, Eriksen M, Iguchi T, Jobling S, Laufer H, LeBlanc GA, et al. An ecological assessment of bisphenol-A: evidence from comparative biology. Reprod Toxicol. 2007;24(2):225-39.

79. Rubin BS. Bisphenol A: an endocrine disruptor with widespread exposure and multiple effects. J Steroid Biochem Mol Biol. 2011;127(1-2):27-34.

80. European Chemicals Agency (ECHA). Agreement of the member state committee on the identification of 4,4'-isopropylidenediphenol (Bisphenol A) A substance of very high concern. ECHA; 2017.

81. International Programme on Chemical Safety. Global assessment of the state-of-the-science of endocrine disruptors. Geneva: World Health Organization; 2002.

82. Wetherill YB, Akingbemi BT, Kanno J, McLachlan JA, Nadal A, Sonnenschein C, et al. In vitro molecular mechanisms of bisphenol A action. Reprod Toxicol. 2007;24(2):178-98.

83. Vinas R, Jeng YJ, Watson CS. Non-genomic effects of xenoestrogen mixtures. Int J Environ Res Public Health. 2012;9(8):2694-714.

84. Andersson A-M. Hormonforstyrrende stoffer, in: Den Store Danske: Gyldendal; [cited 2019 June 19]. Available from: http://denstoredanske.dk/index.php?sideId=93064].

85. Vandenberg LN, Colborn T, Hayes TB, Heindel JJ, Jacobs DR, Jr., Lee DH, et al. Hormones and endocrine-disrupting chemicals: low-dose effects and nonmonotonic dose responses. Endocr Rev. 2012;33(3):378-455.

86. Richter CA, Birnbaum LS, Farabollini F, Newbold RR, Rubin BS, Talsness CE, et al. In vivo effects of bisphenol A in laboratory rodent studies. Reprod Toxicol. 2007;24(2):199-224.

87. Vandenberg LN, Ehrlich S, Belcher SM, Ben-Jonathan N, Dolinoy DC, Hugo ER, et al. Low dose effects of bisphenol A. Endocrine Disruptors. 2013;1(1):e26490.

88. Welshons WV, Nagel SC, vom Saal FS. Large effects from small exposures. III. Endocrine mechanisms mediating effects of bisphenol A at levels of human exposure. Endocrinology. 2006;147(6 Suppl):S56-S69.

89. Mikolajewska K, Stragierowicz J, Gromadzinska J. Bisphenol A - Application, sources of exposure and potential risks in infants, children and pregnant women. Int J Occup Med Environ Health. 2015;28(2):209-41.

90. Mustieles V, Perez-Lobato R, Olea N, Fernandez MF. Bisphenol A: Human exposure and neurobehavior. Neurotoxicology. 2015;49:174-84.

91. Rochester JR. Bisphenol A and human health: a review of the literature. Reprod Toxicol. 2013;42:132-55.

92. Beronius A, Ruden C, Hakansson H, Hanberg A. Risk to all or none? A comparative analysis of controversies in the health risk assessment of Bisphenol A. Reprod Toxicol. 2010;29(2):132-46.

93. Beronius A, Hanberg A, Zilliacus J, Ruden C. Bridging the gap between academic research and regulatory health risk assessment of Endocrine Disrupting Chemicals. Curr Opin Pharmacol. 2014;19:99-104.

94. Vandenberg LN, Chahoud I, Padmanabhan V, Paumgartten FJ, Schoenfelder G. Biomonitoring studies should be used by regulatory agencies to assess human exposure levels and safety of bisphenol A. Environ Health Perspect. 2010;118(8):1051-4.

95. EFSA CEF Panel (EFSA Panel on Food Contact Materials E, Flavourings and Processing Aids). Scientific Opinion on the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs: Executive summary. EFSA Journal. 2015;13(1):1-23.

96. Teeguarden JG, Calafat AM, Ye X, Doerge DR, Churchwell MI, Gunawan R, et al. Twenty-Four Hour Human Urine and Serum Profiles of Bisphenol a during High-Dietary Exposure. Toxicol Sci. 2011;123(1):48-57.

97. European Food Safety Authority. BPA update: working group to start reviewing new studies: EFSA; 2018 [Available from: https://www.efsa.europa.eu/en/press/news/180904].

98. Calafat AM, Ye X, Wong LY, Reidy JA, Needham LL. Exposure of the U.S. population to bisphenol A and 4-tertiary-octylphenol: 2003-2004. Environ Health Perspect. 2008;116(1):39-44.

99. Christensen KL, Lorber M, Ye X, Calafat AM. Reconstruction of bisphenol A intake using a simple pharmacokinetic model. J Expo Sci Environ Epidemiol. 2015;25(3):240-8.

100. LaKind JS, Naiman DQ. Temporal trends in bisphenol A exposure in the United States from 2003-2012 and factors associated with BPA exposure: Spot samples and urine dilution complicate data interpretation. Environ Res. 2015;142:84-95.

101. Koch HM, Kolossa-Gehring M, Schroter-Kermani C, Angerer J, Bruning T. Bisphenol A in 24 h urine and plasma samples of the German Environmental Specimen Bank from 1995 to 2009: A retrospective exposure evaluation. Journal of Exposure Science and Environmental Epidemiology. 2012;22(6):610-6.

102. Wolff MS, Teitelbaum SL, Windham G, Pinney SM, Britton JA, Chelimo C, et al. Pilot study of urinary biomarkers of phytoestrogens, phthalates, and phenols in girls. Environ Health Perspect. 2007;115(1):116-21.

103. von Goetz N, Wormuth M, Scheringer M, Hungerbuhler K. Bisphenol A: How the Most Relevant Exposure Sources Contribute to Total Consumer Exposure. Risk Analysis. 2010;30(3):473-87.

104. EU. European Union Risk Assessment Report: 4,4'-ISOPROPYLIDENEDIPHENOL (BISPHENOL-A), CAS No: 80-05-7, EINECS No: 201-245-8. Luxembourg: European Communities; 2003.

105. Teeguarden J, Hanson-Drury S, Fisher JW, Doerge DR. Are typical human serum BPA concentrations measurable and sufficient to be estrogenic in the general population? Food and Chemical Toxicology. 2013;62:949-63.

106. Liu J, Yu P, Qian W, Li Y, Zhao J, Huan F, et al. Perinatal bisphenol A exposure and adult glucose homeostasis: identifying critical windows of exposure. PLoS One. 2013;8(5):e64143.

107. Markey CM, Wadia PR, Rubin BS, Sonnenschein C, Soto AM. Long-term effects of fetal exposure to low doses of the Xenoestrogen bisphenol-A in the female mouse genital tract. Biology of Reproduction. 2005;72(6):1344-51.

108. Vandenberg LN, Maffini MV, Sonnenschein C, Rubin BS, Soto AM. Bisphenol-A and the great divide: a review of controversies in the field of endocrine disruption. Endocr Rev. 2009;30(1):75-95.

109. Divakaran K, Hines RN, McCarver DG. Human hepatic UGT2B15 developmental expression. Toxicol Sci. 2014;141(1):292-9.

110. Balakrishnan B, Henare K, Thorstensen EB, Ponnampalam AP, Mitchell MD. Transfer of bisphenol A across the human placenta. Am J Obstet Gynecol. 2010;202(4):393 e1-7.

111. Takahashi O, Oishi S. Disposition of orally administered 2,2-Bis(4-hydroxyphenyl)propane (Bisphenol A) in pregnant rats and the placental transfer to fetuses. Environ Health Perspect. 2000;108(10):931-5.

112. Sosial- og helsedirektoratet. Retningslinjer for bruk av tannrestaureringsmaterialer (IS-1086). Oslo: Sosial- og helsedirektoratet; 2003.

113. Magnus P, Birke C, Vejrup K, Haugan A, Alsaker E, Daltveit AK, et al. Cohort Profile Update: The Norwegian Mother and Child Cohort Study (MoBa). Int J Epidemiol. 2016;45(2):382-8.

114. Magnus P, Irgens LM, Haug K, Nystad W, Skjaerven R, Stoltenberg C, et al. Cohort profile: the Norwegian Mother and Child Cohort Study (MoBa). Int J Epidemiol. 2006;35(5):1146-50.

115. Tvinnereim HM, Lygre GB, Haug K, Schreuder P, Klock K. A biobank of primary teeth within the Norwegian Mother and Child Cohort Study (MoBa): a resource for the future. Paediatr Perinat Epidemiol. 2012;26(3):264-71.

116. Irgens LM. The Medical Birth Registry of Norway. Epidemiological research and surveillance throughout 30 years. Acta Obstet Gynecol Scand. 2000;79(6):435-9.

117. Olstad ML, Holland RI, Wandel N, Pettersen AH. Correlation between amalgam restorations and mercury concentrations in urine. J Dent Res. 1987;66(6):1179-82.

118. Cone EJ, Caplan YH, Moser F, Robert T, Shelby MK, Black DL. Normalization of urinary drug concentrations with specific gravity and creatinine. J Anal Toxicol. 2009;33(1):1-7.

119. Blencowe H, Cousens S, Oestergaard MZ, Chou D, Moller AB, Narwal R, et al. National, regional, and worldwide estimates of preterm birth rates in the year 2010 with time trends since 1990 for selected countries: a systematic analysis and implications. Lancet. 2012;379(9832):2162-72.

120. Fleischman AR, Oinuma M, Clark SL. Rethinking the definition of "term pregnancy". Obstet Gynecol. 2010;116(1):136-9.

121. World Health Organization. ICD-10 online versions 2010 [Available from: https://www.who.int/classifications/icd/icdonlineversions/en/].

122. World Health Organization. WHO: recommended definitions, terminology and format for statistical tables related to the perinatal period and use of a new certificate for cause of perinatal deaths. Modifications recommended by FIGO as amended October 14, 1976. Acta Obstet Gynecol Scand. 1977;56(3):247-53.

123. World Health Organization. Global Database on Body Mass Index. BMI classification 2007 2007 [Available from: http://www.who.int/bmi/index.jsp].

124. Hornung R, Reed L. Estimation of average concentration in the presence of nondetectable values. Appl Occup Hyg. 1990;5(1):46-51.

125. Zhang D, Fan C, Zhang J, Zhang CH. Nonparametric methods for measurements below detection limit. Stat Med. 2009;28(4):700-15.

126. Nilsen RM, Vollset SE, Gjessing HK, Skjaerven R, Melve KK, Schreuder P, et al. Self-selection and bias in a large prospective pregnancy cohort in Norway. Paediatr Perinat Epidemiol. 2009;23(6):597-608.

127. Longnecker MP, Harbak K, Kissling GE, Hoppin JA, Eggesbo M, Jusko TA, et al. The concentration of bisphenol A in urine is affected by specimen collection, a preservative, and handling. Environ Res. 2013;126:211-4.

128. Wu LH, Zhang XM, Wang F, Gao CJ, Chen D, Palumbo JR, et al. Occurrence of bisphenol S in the environment and implications for human exposure: A short review. Sci Total Environ. 2018;615:87-98.

129. Mullangi R, Agrawal S, Srinivas NR. Measurement of xenobiotics in saliva: is saliva an attractive alternative matrix? Case studies and analytical perspectives. Biomed Chromatogr. 2009;23(1):3-25.

130. Sasaki N, Okuda K, Kato T, Kakishima H, Okuma H, Abe K, et al. Salivary bisphenol-A levels detected by ELISA after restoration with composite resin. J Mater Sci Mater Med. 2005;16(4):297-300.

131. Genuis SJ. Elimination of persistent toxicants from the human body. Human & Experimental Toxicology. 2011;30(1):3-18.

132. Völkel W. Lesson of 15-year exposure to Bisphenol A: a critical discussion of biomonitoring studies. Arch Toxicol. 2017;91(11):3693-6.

133. Teeguarden JG, Calafat AM, Doerge DR. Adhering to Fundamental Principles of Biomonitoring, BPA Pharmacokinetics, and Mass Balance Is No "Flaw". Toxicol Sci. 2012;125(1):321-5.

134. Vandenberg LN, Gerona RR, Kannan K, Taylor JA, van Breemen RB, Dickenson CA, et al. A round robin approach to the analysis of bisphenol A (BPA) in human blood samples. Environ Health. 2014;13(1):25.

135. Vandenberg LN, Hunt PA, Myers JP, Vom Saal FS. Human exposures to bisphenol A: mismatches between data and assumptions. Rev Environ Health. 2013;28(1):37-58.

136. Teeguarden JG, Twaddle NC, Churchwell MI, Doerge DR. Urine and serum biomonitoring of exposure to environmental estrogens I: Bisphenol A in pregnant women. Food Chem Toxicol. 2016;92:129-42.

137. Fung EYK, Ewoldsen NO, St. Germain HA, Marx DB, Miaw C-L, Siew C, et al. Pharmacokinetics of Bisphenol a Released from a Dental Sealant. J Am Dent Assoc. 2000;131(1):51-8.

138. Zimmerman-Downs JM, Shuman D, Stull SC, Ratzlaff RE. Bisphenol A blood and saliva levels prior to and after dental sealant placement in adults. J Dent Hyg. 2010;84(3):145-50.

139. Putzeys E, Cokic SM, Chong H, Smet M, Vanoirbeek J, Godderis L, et al. Simultaneous analysis of bisphenol A based compounds and other monomers leaching from resin-based dental materials by UHPLC-MS/MS. J Sep Sci. 2017;40(5):1063-75.

140. Kopperud SE, Tveit AB, Opdam NJ, Espelid I. Occlusal caries management: Preferences among dentists in Norway. Caries Res. 2016;50(1):40-7.

141. Vidnes-Kopperud S, Tveit AB, Espelid I. Changes in the treatment concept for approximal caries from 1983 to 2009 in Norway. Caries Res. 2011;45(2):113-20.

142. Wang NJ. Tannhelseutvikling og bruk av tannrestaureringsmaterialer. In: Bruk av tannrestaureringsmaterialer i Norge IK-2652. 98-8. Oslo: Statens helsetilsyn; 1998. p. 69-74.

143. Selevan SG, Kimmel CA, Mendola P. Identifying critical windows of exposure for children's health. Environ Health Perspect. 2000;108 Suppl 3:451-5.

144. Vargesson N. Thalidomide-induced teratogenesis: history and mechanisms. Birth Defects Res C Embryo Today. 2015;105(2):140-56.

145. Greenland S, Senn SJ, Rothman KJ, Carlin JB, Poole C, Goodman SN, et al. Statistical tests, P values, confidence intervals, and power: a guide to misinterpretations. Eur J Epidemiol. 2016;31(4):337-50.

146. Joskow R, Barr DB, Barr JR, Calafat AM, Needham LL, Rubin C. Exposure to bisphenol A from bis-glycidyl dimethacrylate–based dental sealants. J Am Dent Assoc. 2006;137(3):353-62.

147. Kang YG, Kim JY, Kim J, Won PJ, Nam JH. Release of bisphenol A from resin composite used to bond orthodontic lingual retainers. Am J Orthod Dentofacial Orthop. 2011;140(6):779-89.

148. Moreira MR, Matos LG, de Souza ID, Brigante TA, Queiroz ME, Romano FL, et al. Bisphenol A release from orthodontic adhesives measured in vitro and in vivo with gas chromatography. Am J Orthod Dentofacial Orthop. 2017;151(3):477-83.

149. Arenholt-Bindslev D, Breinholt V, Preiss A, Schmalz G. Time-related bisphenol-A content and estrogenic activity in saliva samples collected in relation to placement of fissure sealants. Clin Oral Investig. 1999;3(3):120-5.

150. Kloukos D, Pandis N, Eliades T. In vivo bisphenol-a release from dental pit and fissure sealants: a systematic review. J Dent. 2013;41(8):659-67.

151. Gilbert GH, Litaker MS, Pihlstrom DJ, Amundson CW, Gordan VV, Group DC. Rubber dam use during routine operative dentistry procedures: findings from the Dental PBRN. Oper Dent. 2010;35(5):491-9.

152. Lynch CD, McConnell RJ. Attitudes and use of rubber dam by Irish general dental practitioners. Int Endod J. 2007;40(6):427-32.

153. Hamid A, Hume WR. A study of component release from resin pit and fissure sealants in vitro. Dent Mater. 1997;13(2):98-102.

154. Komurcuoglu E, Olmez S, Vural N. Evaluation of residual monomer elimination methods in three different fissure sealants in vitro. J Oral Rehabil. 2005;32(2):116-21.

155. Maserejian NN, Trachtenberg FL, Wheaton OB, Calafat AM, Ranganathan G, Kim HY, et al. Changes in urinary bisphenol A concentrations associated with placement of dental composite restorations in children and adolescents. J Am Dent Assoc. 2016.

156. Michalowicz BS, DiAngelis AJ, Novak MJ, Buchanan W, Papapanou PN, Mitchell DA, et al. Examining the safety of dental treatment in pregnant women. J Am Dent Assoc. 2008;139(6):685-95.

157. Bittner GD, Yang CZ, Stoner MA. Estrogenic chemicals often leach from BPA-free plastic products that are replacements for BPA-containing polycarbonate products. Environ Health. 2014;13(1):41.

158. Kanerva L, HenriksEckerman ML, Jolanki R, Estlander T. Plastics/acrylics: Material safety data sheets need to be improved. Clinics in Dermatology. 1997;15(4):533-46.

Paper I-III, Appendix I-V

Paper II

Bisphenol A in human saliva and urine before and after treatment with dental polymer-based restorative materials.

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Accepted for publication in European Journal of Oral Sciences, 29 May 2019.

II

Bisphenol A in human saliva and urine before and after treatment with dental polymer-based restorative materials

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Key words: Exposure to BPA; Dental resin-based material; Composite filling, Saliva; Urine;

Running title: BPA in saliva and urine after placement of composites

Abstract

The aim of this study was to quantify bisphenol A (BPA) concentrations in saliva and urine before and after treatment with dental polymer-based restorative materials to assess if placement of this material is associated with increased BPA levels in saliva and urine. Twenty individuals in need of at least one dental restoration with polymer-based restorative material were included in this study. The participants were instructed to abstain from eating, drinking, and brushing their teeth for at least 10 hours prior to sampling. Saliva and urine were collected before and 10 minutes (saliva only), 1 hour, 24 hours, and 1 week after treatment. Samples were stored at −80°C before analyses. BPA in saliva and urine was determined with liquid chromatography/mass spectrometry. Linear mixed effects regression models were used for statistical analyses. There was a statistically significant increase of salivary BPA concentration directly after placement of the dental polymer-based restorations. Following placement, the concentration of BPA decreased exponentially with time. One week after treatment the BPA level in saliva was only marginally higher than before treatment. In urine, no statistically significant change of the BPA concentration was detected after treatment.

Introduction

Bisphenol A (BPA, CAS no. 80-05-7), is a synthetic chemical substance, produced in large quantities and widely used in the production of polycarbonate plastics, epoxy resins, dental monomers, thermal paper, and numerous other products (1). BPA is known as an endocrine disruptor with the ability to interfere with and mimic estrogenic hormones (2-4). Concern has been raised about low level-exposure to BPA and the possible association with adverse health effects. In vitro and animal studies have linked BPA exposure to a variety of negative outcomes (5). In several epidemiological studies, BPA levels in human populations have been associated with reproductive abnormalities, adverse developmental effects, metabolic disease, and breast cancer among other health conditions (6-8). Although these findings are controversial (9), the current opinion of risk assessment agencies, such as the European Food Safety Authority (EFSA), is that negative health effects from BPA exposure could not be excluded (10). Of greatest concern is potential exposure during vulnerable periods like fetal and early postnatal development (4). In humans, the free (unconjugated) estrogenic form of BPA generally is conjugated to a nonestrogenic form via "the first pass metabolism" in the liver and eliminated in urine (11). However, the ability to metabolize and excrete BPA from the body may not be fully developed in the fetus and the neonate (12). In January 2015, the EFSA revised the recommended limit of "tolerable daily intake" of BPA from 50 to 4 µg/kg of bodyweight per day (10) and in December 2017 the European Chemicals Agency reclassified BPA as a chemical of very high concern (13). In November 2018, a working group from the EFSA started re-evaluating the potential hazards of BPA in food based on studies and data published after 2012. This new assessment is expected to be completed in 2020 (https://www.efsa.europa.eu/en/press/news/180904, downloaded 22.02.2019).

There is wide interest in the sources of BPA exposure. The primary source of human exposure is assumed to be through the diet because BPA can leach into the food and beverages from containers made of polycarbonate plastic or lined with epoxy resin coatings (1, 14, 15). However, results from studies have indicated human exposure also from numerous nondietary sources, including dust and indoor air, thermal paper, cosmetics, and dental materials (1, 10, 15, 16).

In dentistry, BPA is used as a raw material in the synthesis of several resin monomers and may be found as an impurity in dental materials (17). The most frequently used monomers synthesized from BPA include bisphenol A glycidyl methacrylate (Bis-GMA, CAS no. 1565-

94-2), bisphenol A ethoxylate dimethacrylate (Bis-EMA, CAS no. 41637-38-1), and bisphenol A dimethacrylate (Bis-DMA, CAS no. 3253-39-2) (18, 19). It has been shown that bis-DMA-based materials, such as Delton LC fissure sealant, may release BPA as a result of hydrolysis at the ester bond (20). Bis-GMA-based materials, which typically comprise restorative materials, do not undergo this form of biodegradation because its ether bond is resistant to hydrolysis (20-24). Several in vitro and some in vivo studies have focused on BPA leakage from dental polymer-based filling materials and have attempted to quantify the amounts detected in different solutions or biological media (17, 19, 25-30). However, probably because of differences in the materials examined and the methodological approaches, the amounts reported are diverging (19, 31). Moreover, exposure from other sources, e.g., participants' diet and its contribution to the BPA concentration in biological samples (e.g. urine), has not thoroughly been considered in previous clinical studies.

The aim of this study was to quantify BPA concentrations in saliva and urine before and after treatment with dental polymer-based restorative materials to assess if placement of these materials is associated with increased BPA levels in saliva and urine.

Material and methods

Study population

Patients in need of at least one dental restoration of two or more dental surfaces with a polymer-based restorative material were informed about the study by their dental hygienist or dentist at their regular dental examination at two public dental clinics in Bergen, Norway. Patients who chose to participate were given written information about the study for perusal at home. Individuals with removable dentures, dental splints, and those who currently were undergoing orthodontic treatment were excluded. Smokers, snuff users, and drug abusers were also excluded. We did not include individuals who had received polymer-based dental fillings during the previous 3 months, dental students, and dental health workers. Twenty volunteers, between 16 and 40 years of age, without any known diseases or medications at the time of the study, were included in the study from January 2016 to November 2017. All participants provided written informed consent.

Ethical approval

All procedures involving human participants were performed in accordance with the ethical standards of the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study protocol was reviewed and approved by The Regional Committee for Medical Research Ethics, South-East Norway (reference number 2014/1529). The study is registered at ClinicalTrials.gov, number NCT02575118.

Dental treatment procedures

One dentist (TLLB) recorded the number of tooth surfaces previously filled with tooth-colored restorative materials. The same dentist (TLLB) provided the dental treatment at one public dental clinic in Bergen, Norway. The treatment was performed according to standardized procedures and materials used at the clinic. Local anesthesia (Xylocain Dental adrenalin, Dentsply, Weybridge, England) was used in 19 of the participants; one participant preferred treatment without anesthesia. Cavity preparations were performed with diamond burs (Horico, Berlin, Germany) and round steel burs (Meisinger, Neuss, Germany). Cotton rolls and low-volume evacuator equipment (Hygoformic Saliva Ejector, Orsing, Helsingborg, Sweden) were used for moisture control. High-volume evacuator equipment (Hygovac aspirator tube, Orsing, Helsingborg, Sweden) was used during cavity preparations, etching, bonding and finishing procedures. Rubber dam isolation was not used. Contoured anatomical steel matrices (Polydentia SA, Mezzovico, Switzerland) were used to support and shape the restorations in premolars/molars and transparent curved strips (Hawe Neos, Kerr, Orange, California, USA) were used in front teeth. Dental approximal wooden wedges (Hawe Sycamore, Kerr, Orange, California, USA) were used in all cases. Etching with 37% phosphoric acid (ANA Etching Gel 37%, Directa, Upplands Väsby, Sweden) was performed according to the principles of the total etch technique (32). A two-part primer adhesive system (OptiBond FL, Kerr, Orange, California, USA) was used as the bonding agent. The cavities were restored with a widely used filling material (Tetric EvoCeram, 0.2 g compules, Color A2, LOT 14504, Ivoclar Vivadent AG, Schaan, Liechtenstein), tested to be assured it was bis-GMA-based. The bonding procedure and the application of the filling material were carried out according to the manufacturers' instructions. For each participant a new compule with filling material was used. The material was applied in incremental layers of <2.0 mm and each layer was cured for 20-30 seconds. Care was taken to avoid application of excessive amounts of material. Any surplus was removed and put back into the compule. Each compule was weighed before and after treatment, using an analytical balance (AG204 DeltaRange,

Mettler Toledo, Greifensee, Switzerland). The amount (weight in gram) of polymer-based material used in each participant was estimated by the difference between the two measurements. The curing lamp (Satelec Mini LED, Aceton, Meriganac, France) emitted a 600 to 700-mW/cm^2 light intensity at a range from 440 to 460-nm. The lamp was controlled prior to each treatment using a hand-held curing radiometer (Model 100, Dementron, Kerr, Danbury, Connecticut USA). After curing, the fillings were polished according to standard procedures using diamond polishing burs (FossViking, Fetsund, Norway), polishing disks (Sof-Lex XT Pop, 3M Espe, St. Paul, Minnesota USA) and silicone polishers (Identoflex Composite Polisher, Kerr, Bioggio, Switzerland).

The restorations differed in size depending on the tooth size and the extent of the prepared lesion. To adjust for differences, each filling surface was given scores from 1 to 3, depending on its area (33). Small restorations were given the lowest score of 1. Restorations of intermediate size, typically the approximal or occlusal surfaces of Class II restorations in premolars, were given a score of 2. The highest score, 3, was used for molars to denote restorations extending over the total occlusal fissure pattern or over the approximal surface of Class II restorations. The scores for all polymer-based filling surfaces treated in each patient were summed and yielded the variable "filling points". The tooth surfaces treated and the estimated "filling points" were recorded.

Sample collection

All treatment sessions were scheduled in the morning before 9 am. Prior to the treatment, the participants were asked about their dental hygiene habits and if they had work that involved handling of receipts (thermal paper). Each participant provided a total of five saliva samples and four urine samples. The first saliva and urine samples
were collected immediately before treatment, after a 10-h fast. Sampling of a second saliva sample was started 10 min after placement of the polymer-based fillings, and subsequent saliva and urine samples were collected 1 h, 24 h, and 1 wk after placement of the fillings (Fig. 1). On each day of sampling, the participants also answered questions regarding consumption of canned and microwaved food during the previous week and within the previous 24 hours. To reduce the exposure from other potential BPA sources, the participants were instructed to abstain from eating, drinking, and brushing their teeth for at least 10 hours prior to sampling. Only tap-water was allowed for drinking. The participants were asked not to use lip balm or lipstick during the same period. To identify possible contamination during sampling, transport and storage, field blanks were collected using ultra-pure water (Synergy

Water Purification Systems, Millipore, Billerica, Massachusetts, USA) instead of saliva and urine. The field blanks were treated like the biological samples in all aspects.

For saliva sampling, the participants were sitting in a relaxed position in the dental unit chair. They were instructed to spit in the dental unit sink, but rinsing was not allowed. Immediately after, they were instructed to do active tongue and cheek movements for 60 seconds and then spit the accumulated saliva into a polypropylene tube (15 ml, order number 62.554.001, Sarstedt AG & Co, Nümbrecht, Germany) until about 2 ml of saliva were sampled. To avoid contamination from the ambient air, they were asked to put the cap back on the tube between each spit. The sampling time was recorded.

Since the first morning urine void should not be collected, the participants were instructed to empty their bladder in the morning at home before entering the clinic. Urine specimens were collected in 100-ml polypropylene cups (order number 75.562.300, Sarstedt AG & Co, Nümbrecht, Germany) and aliquots were transferred into 15-ml polypropylene tubes. Immediately after collection, the samples were refrigerated (at 4°C) and within the same day stored frozen at −80°C until they were sent for analysis.

Determination of BPA in saliva and urine

Urine and saliva samples were analyzed at the laboratory of the Division of Occupational and Environmental Medicine at Lund University, Sweden, using liquid chromatography-triple quadrupole mass spectrometry (LC/MS/MS; QTRAP 5500; AB Sciex, Foster City, California, USA). Saliva samples were analyzed using a method described by BERGE et al (34) and urine samples were analyzed using a modified method described by GYLLENHAMMAR et al (35). Briefly, for the determination of total BPA in saliva, aliquots of 100 µl were digested with glucuronidase added with isotopically labeled internal standard for BPA (D_{16}-BPA) and proteins were precipitated using acetonitrile. Free (unconjugated) BPA in saliva was determined without using glucuronidase. For the analysis of total BPA in urine samples, aliquots of 200 µl were digested with glucuronidase added with D_{16}-BPA. The concentration of BPA in urine was adjusted for urinary density (36). Two different in-house prepared quality control (QC) samples and chemical blanks were analyzed in the analytical batches. The limit of detection (LOD) was determined to be 0.1 ng/ml. The method had acceptable between-day and within-run precision. The laboratory used is a European reference laboratory for BPA in urine (http://www.eu-bm.info/democophes) and a reference laboratory for BPA in

urine in the Erlangen Round Robin inter-laboratory control program. A detailed description of the analytical method is given in the Supplemental File.

Statistical method

Descriptive statistics were presented as mean, minimum, maximum values, and standard deviation (SD) for continuous variables, and as frequencies for categorical variables. For the analyses of repeated measures of the saliva and urine samples, linear mixed effects regression models were applied. In the mixed effects model, the repeated nature of the measurements of the data were accounted for using the patient ID, entered as a random factor, with an additional factor for time to account for the difference in variation (standard deviation) over time.

Time for the measurements was entered as a categorical variable in the models, comparing the succeeding measurements with the baseline (before treatment) measure. All available data, and hence data for participants with missing observations at some time points, were included in the model.

The decrease over time of the mean posttreatment BPA concentration in saliva was described using the equation: $Y = aX^b$, where Y is the BPA concentration and X is time. The equation was estimated by applying mixed effects regression on a log transform of the equation (which transforms the equation to a linear model).

For the descriptive statistics, SPSS (IBM SPSS, Version 25, NY, USA) was applied, while for the mixed effect analyses Stata (version 15, TX, USA) was applied.

Values below the limit of detection (LOD) were set to one half of the LOD in the statistical analysis (37). P-values less than 0.05 were considered statistically significant.

Results

Background and dental treatment characteristics

Background characteristics of the participants are shown in Table 1. Data regarding dental hygiene habits and consumption of canned and microwaved food are listed in Table 2. Details regarding dental treatment are shown in Table 3. Three of the participants attended the study without having any pre-existing polymer-based dental fillings and nine had polymer-based fillings removed during treatment.

Concentration of BPA in saliva

The saliva samples collected 10 minutes after treatment showed a statistically significant increase in BPA levels compared with the pretreatment samples. The concentrations remained significantly elevated 1 hour, 24 hours and 1 week after placement (Table 4; Fig. 2). After the immediate posttreatment increase, the concentration of BPA in saliva (Y; ng/ml) decreased exponentially. We estimated the relationship between the decrease and time (X; hours) to be $Y=54.2X^{-1.12}$ (Fig. 3). In saliva no conjugated BPA was detected. Pretreatment levels of BPA in saliva were low, and the mean value was estimated to be 0.11 ng/ml (Table 4). Before treatment, 11 of 20 (55%) participants had salivary BPA levels below the detection limit (0.1 ng/ml). In one saliva sample collected before treatment the BPA concentration was more than 100 times higher (11.6 ng/ml) than the mean value and more than 100 standard deviations from the mean of the remaining 19 samples. This saliva sample was excluded from the statistical analysis because of probable contamination. One participant had breakfast before the sampling 1 week after treatment and thus the samples collected this day from this participant were not included in the statistical analysis.

The levels of BPA concentrations in saliva were confirmed by analyzing nine samples using LC/MS/MS at an independent laboratory (Nordic Institute of Dental Materials, Oslo, Norway). These samples were selected to represent the full range of the values from the laboratory in Lund. The interclass correlation was high (0.91; 95% CI: 0.72–0.98), which indicates high agreement between the measurements.

Secondary explorative analysis showed that the number of filling points was associated with the BPA levels in saliva 24 hours ($p=0.011$) and 1 week ($p=0.029$) after treatment. However, neither number of filling surfaces nor the amount (weight) of dental polymer-based material placed was associated with the salivary BPA concentration at any time point (all $p>0.05$). Moreover, there were no statistically significant associations between the other covariates tested and the salivary BPA levels at the different time points (see Tables 1, 2, and 3 for tested variables).

Concentration of BPA in urine

Table 4 presents density adjusted concentrations of BPA in urine before and after treatment. Before treatment, 19 of 20 (95%) participants had detectable BPA levels in their urine. There were no statistically significant differences between urinary BPA levels before and after

placement of the dental polymer-based restorations (Table 4; Fig. 4). BPA levels in the urine samples collected 1 hour after treatment did not show a statistically significant association with the BPA level in the saliva samples collected within 10 minutes after treatment (Data not shown).

Fig. 4, presenting the urinary BPA levels over time, illustrates that two of the participants showed considerably higher BPA levels than the others. One of these participants had higher levels at all time points, while the other only had an elevated BPA concentration after one week. These participants were identified as two of five participants handling cash register receipts at work. The participant with highest levels in urine was also the one with the highest levels of BPA in saliva at all time points. Using receipts as a group variable (yes; n=5 / no; n=15) we found an overall elevated average level of urinary BPA in the group handling receipts ($p=0.031$, mean difference 0.83 ng/ml; 95% CI: 0.08-1.57).

Regression models testing additional potential factors that could contribute to the BPA concentration in urine (see Tables 1, 2, and 3 for tested variables) did not show a statistically significant influence at any time point (all $p>0.05$).

All field blanks had BPA concentrations below the detection limit.

Discussion

There was a considerable increase in the BPA concentration in saliva directly after placement of a dental polymer-based dental restorative material. The concentration of BPA then decreased exponentially with time. One week after treatment the concentration of BPA in saliva was only marginally higher than before treatment. This is in agreement with the results from other studies of BPA leakage from existing polymer-based fillings (29, 34). The time-course of the salivary BPA concentration after treatment is in accordance with other studies and supports a plausible pattern, which suggests that the main exposure to BPA from polymer-based dental filling materials is limited to a short period after placement (21, 26, 38, 39). In urine, no change of the BPA concentration was detected after treatment.

Over the last two decades, the amounts of BPA in saliva and urine after placement of dental polymer-based materials have been examined in several studies (26, 28, 30, 39). However, there are wide differences in the materials tested regarding composition, brands, and

application modes. Moreover, the size and number of tooth surfaces filled, sampling procedures, measurement time points and intervals, analytical methods, sensitivity of methods, and detection limits differ between studies. These differences may account for diverging results, which make the comparison between studies and calculations of the actual amount released difficult (19, 31). KLOUKOS et al. (31), searching the literature to assess the short- and long-term release of BPA in human tissues after treatment with dental sealants, concluded that only qualitative evaluation may be performed.

In the present study, the mean BPA concentration detected in saliva immediately after treatment was higher than expected. Our results are comparable with previous studies assessing salivary BPA concentrations directly after (21) and 1 to 3 hours after (38) placement of a bis-DMA-based dental fissure sealant (Delton LC). The range in BPA concentrations from these studies was 0.3–2.8 ppm and 5.8–105.6 ppb, respectively. OLEA et al. (25) reported that the cumulative salivary BPA concentration 1 hour after sealant placement was approximately 100 times higher than the BPA concentration detected directly after treatment in our study. However, the reliability of the analytical method used by OLEA et al. has been questioned (22) and the levels have not been confirmed by later studies. Other authors have evaluated the release of BPA in saliva after placement of bis-GMA-based dental materials used as restoratives (26, 29, 40, 41), and in orthodontic treatment, bonding lingual retainers (28), or brackets (30, 42). Compared with the salivary BPA levels reported in these studies, our levels are considerably higher.

The potential of dental polymer-based restorative materials to release BPA may differ depending on the BPA-derivatives included in the material. Because of the hydrolysis of bis-DMA, the BPA concentration in saliva collected directly after treatment with bis-DMA-based materials is expected to be higher than the amount of BPA found in salivary samples collected directly after placement of bis-GMA-based material (22, 24). However, the wide divergence in the salivary BPA concentration reported may in part have other explanations. The source of BPA in bis-GMA-based materials includes residual BPA present in the raw materials used in the manufacturing process (43). The amount of BPA left from the synthesis of bis-GMA may vary between different producers. A notable batch to batch variation could also be expected if different raw materials are used (43).

The release of components from dental polymer-based materials after curing could be a consequence of incomplete polymerization (44). During the polymerization process most of

the monomers should be converted into polymers to form a polymer network. However, the degree of conversion is reported never to be complete (45, 46). The extent to which monomers are converted may be influenced by several factors including the curing time, the light intensity, the composition of the polymer-based material and the thickness of the incremental layer (47). In the present study, the dental polymer-based material was placed in layers and cured according to the manufacturer's instructions. No association was found between the amount (weight) of material placed and salivary BPA concentration. This finding is in agreement with other studies and may be explained by the observation period used in the present study, which was too short to allow degradation and subsequent release of unbound compounds trapped in the polymer network (17). However, the exposure of materials to air inhibits polymerization and leads to a thin, liquid layer on the filling surface (48, 49). From this layer uncured components have the potential to leak immediately into the oral environment for a short time (17). Some studies have indicated that the BPA release from dental polymer-based fillings depends on the surface area that is exposed (50, 51). Our study found no association between the surface area of the polymer-based material placed and the salivary BPA levels immediately after and 1 hour after treatment. However, 24 hours and 1 week after treatment, the BPA concentration was associated with the number of filling points.

Moisture control during restorative dental treatment may include cotton rolls and a rubber dam combined with a saliva ejector. It has been reported that the increase in salivary BPA concentrations directly after placement of dental polymer-based material may be lower when a rubber dam is used (26); however, studies indicate that most dentists do not use rubber dam isolation during operative dentistry procedures (52, 53). We expected the leakage of BPA from newly placed polymer-based fillings to be low, so, in an effort to perform the most commonly used clinical procedures and detect the maximum BPA released after treatment, a rubber dam was not used during treatment in our study. Immediately after treatment, the participants were instructed to spit once in the unit sink. They then performed cheek and tongue movements and consecutively collected the accumulated saliva in tubes. Neither rinsing nor gargling was allowed before saliva sampling. Thus, the saliva samples collected immediately after treatment in our study may include BPA exposure from the uncured layer as well as surplus of material left over from the grinding and polishing procedure. Probably the association between surface area (filling points) and BPA concentration immediately after treatment would have been stronger if gargling had been permitted. Rinsing the mouth with water for 30 seconds after polymer-based filling placement has been shown to decrease the

BPA concentration in saliva to nearly baseline levels (41). In some studies, it was not reported whether the participants were instructed to rinse their mouth before sampling. Moreover, the time interval reflecting "immediately after treatment" may vary from 5 to 60 minutes across studies (26, 28, 29). Thus, the varied sampling procedures could influence the differences in the BPA concentrations detected and complicate estimation of the amount of BPA released immediately after treatment.

Clinical studies examining saliva concentration of leachable chemicals from dental polymer-based materials show generally wide variations between participants and the relative standard deviation is usually high (25, 41, 54). Thus, the homogeneity and representativeness of the saliva samples could be questioned (55).

In the present study the participants were instructed to refrain from food and beverage intake 10 hours before sample collections. Urine samples were collected prior to treatment and 1 hour, 24 hours and 1 week after treatment. The salivary BPA concentrations detected immediately after treatment were high and could be expected to be reflected in the subsequent urinary BPA concentrations. However, neither the first posttreatment urine samples, collected 1 hour after treatment, nor any other posttreatment samples showed significant increases in BPA concentrations compared with pretreatment levels. This is in contrast to the findings by JOSKOW et al (39), which indicated that urinary BPA levels were highest 1 hour after sealant placement. Over time, there is a large variation of BPA in urine (56), and intake of food has a considerable influence on the concentration of BPA in urine (57). Thus, it could be speculated that the elevated urinary concentrations 1 hour after treatment reported in other studies may have been influenced by food intake before treatment. However, 10 hours of fasting may decrease the BPA exposure, and subsequently the urinary BPA level, and thus partly mask a potential increase in the urinary BPA concentration caused by the dental treatment. The BPA concentration in urine reflects the absorbed dose of BPA (58). Because saliva samples were collected immediately after treatment, we may have reduced a significant amount of BPA, which otherwise would have been absorbed, metabolized in the liver, and excreted via the urine. Thus, saliva sampling directly after treatment could have influenced the BPA concentrations in urine considerably (39).

In the present study we used LC/MS/MS with an isotopically labeled internal standard (D_{16}-BPA) for the analysis of BPA. This is the method of choice for the determination of BPA in biological samples (59). Quality control samples and field blanks were analyzed together with

the samples of saliva and urine. In addition, a limited number of saliva samples were analyzed at a second laboratory and showed similar results. Thus, we evaluated the analysis to be reliable and the accuracy and precision acceptable for the present purpose.

To standardize the procedures as much as possible, the dental treatment was provided by one dentist at one dental clinic using the same procedures for all patients. Moreover, the same batch of filling material was used for all fillings.

The participants in the present study acted as their own control as reflected by pretreatment sampling. Including an unexposed control group would have enabled us to better interpret the contribution of the polymer-based material to urinary BPA levels. Although data from the study by JOSKOW et al. (39) indicate an increase of BPA in urine 1 hour after treatment, it is possible that the peak concentration of BPA in urine after exposure is between 1 and 4 hours (58). Thus, the sample collection at 1 hour may have been too early for detection of the maximum concentration of BPA in urine.

Two participants showed significantly higher urinary BPA levels compared with the other participants (Fig. 4). One of them had elevated BPA levels in urine at all time points. Both participants handled receipts at work and consequently, thermal paper could be one source of exposure. Looking at the group of participants handling receipts, there were substantial between- and within-subject variability, but average levels were elevated, as was also found by THAYER et al (60).

There may be considerable differences regarding potential BPA exposure depending on materials used. Because only one batch of one material was tested in this study, the detected amounts of BPA must be interpreted with care. Materials with less BPA contamination may cause lower exposure. Hence, the daily dose of BPA from dental polymer-based restorative materials is probably relatively low compared with the total exposure from food and other sources (10). However, the biologic effects of BPA have been reported to occur even within the range of the detection threshold of most analytical procedures, and its influence on tissues may show a nonmonotonic dose response curve pattern (61). This is characterized by intense reactivity at low levels and no response at high levels (62).

Data on the chemical composition of dental polymer-based material from the material Safety Data Sheets are incomplete (19). Therefore, manufacturers should be required to provide more exact information about the composition of their products (19). Ideally, dental polymer-

based materials should be produced without components having estrogenic effects. However, materials introduced as BPA free have also shown estrogenic activity (63). Thus, methods to reduce the release of BPA after placement of dental polymer-based materials should be provided. Using a rubber dam to control the operative field would limit the potential exposure (26). Moreover, rinsing with water directly after treatment should be highly recommended (41).

In conclusion, the findings in this study confirm that placement of dental polymer-based restorative materials may cause a substantial increase in salivary BPA concentrations after treatment. The results indicate that the exposure to BPA is relatively short and transient. After 1 week, the concentration of BPA in saliva was only slightly elevated compared with the levels before treatment. This study did not show changes in the BPA concentration in urine after treatment with a dental polymer-based restorative material.

Acknowledgements

The authors want to give special thanks to the participants of this study. We also appreciate the support from The Public Dental Services in Hordaland County and skillful help from personnel at the dental clinics (Årstad and Sammen/Student Welfare Organization in Bergen). We acknowledge Carina S Nilsson and Margareta Maxe, Lund University, Sweden, for technical assistance with the analyses of BPA. Hilde M Kopperud and Lene A Grutle, Nordic Institute of Dental Materials, Norway, are acknowledge for skillful help with the additional analyses of BPA.

The Norwegian Dental Biomaterials Adverse Reaction Unit and the Oral Health Centre of Expertise in Western Norway are funded by the Norwegian Ministry of Health and Care Services. The study was supported by the Norwegian Directorate of Health.

Conflicts of interest

The authors declare no conflicts of interest.

References

1. GEENS T, GOEYENS L, COVACI A. Are potential sources for human exposure to bisphenol-A overlooked? *Int J Hyg Environ Health* 2011; **214**: 339-347.

2. CRAIN DA, ERIKSEN M, IGUCHI T, JOBLING S, LAUFER H, LEBLANC GA, GUILLETTE JR. LJ. An ecological assessment of bisphenol-A: evidence from comparative biology. *Reprod Toxicol* 2007; **24**: 225-239.

3. RUBIN BS. Bisphenol A: an endocrine disruptor with widespread exposure and multiple effects. *J Steroid Biochem Mol Biol* 2011; **127**: 27-34.

4. VANDENBERG LN, MAFFINI MV, SONNENSCHEIN C, RUBIN BS, SOTO AM. Bisphenol-A and the great divide: a review of controversies in the field of endocrine disruption. *Endocr Rev* 2009; **30**: 75-95.

5. Richter CA, Birnbaum LS, Farabollini F, Newbold RR, Rubin BS, Talsness CE, Vandenbergh JG, Walser-Kuntz DR, vom Saal FS. In vivo effects of bisphenol A in laboratory rodent studies. *Reprod Toxicol* 2007; **24**: 199-224.

6. MIKOLAJEWSKA K, STRAGIEROWICZ J, GROMADZINSKA J. Bisphenol A - Application, sources of exposure and potential risks in infants, children and pregnant women. *Int J Occup Med Environ Health* 2015; **28**: 209-241.

7. MUSTIELES V, PEREZ-LOBATO R, OLEA N, FERNANDEZ MF. Bisphenol A: Human exposure and neurobehavior. *Neurotoxicology* 2015; **49**: 174-184.

8. ROCHESTER JR. Bisphenol A and human health: a review of the literature. *Reprod Toxicol* 2013; **42**: 132-155.

9. BERONIUS A, RUDEN C, HAKANSSON H, HANBERG A. Risk to all or none? A comparative analysis of controversies in the health risk assessment of Bisphenol A. *Reprod Toxicol* 2010; **29**: 132-146.

10. EFSA CEF PANEL (EFSA PANEL ON FOOD CONTACT MATERIALS E, FLAVOURINGS AND PROCESSING AIDS). Scientific Opinion on the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs: Executive summary. *EFSA Journal* 2015; **13**: 1-23.

11. VÖLKEL W, COLNOT T, CSANADY GA, FILSER JG, DEKANT W. Metabolism and kinetics of bisphenol A in humans at low doses following oral administration. *Chem Res Toxicol* 2002; **15**: 1281-1287.

12. DIVAKARAN K, HINES RN, MCCARVER DG. Human hepatic UGT2B15 developmental expression. *Toxicol Sci* 2014; **141**: 292-299.

13. EUROPEAN CHEMICALS AGENCY. Agreement of the member state committee on the identification of 4,4'-isopropylidenediphenol (Bisphenol A) A substance of very high concern. 2017.

14. LORBER M, SCHECTER A, PAEPKE O, SHROPSHIRE W, CHRISTENSEN K, BIRNBAUM L. Exposure assessment of adult intake of bisphenol A (BPA) with emphasis on canned food dietary exposures. *Environ Int* 2015; **77**: 55-62.

15. WILSON NK, CHUANG JC, MORGAN MK, LORDO RA, SHELDON LS. An observational study of the potential exposures of preschool children to pentachlorophenol, bisphenol-A, and nonylphenol at home and daycare. *Environ Res* 2007; **103**: 9-20.

16. STAHLHUT RW, WELSHONS WV, SWAN SH. Bisphenol A data in NHANES suggest longer than expected half-life, substantial nonfood exposure, or both. *Environ Health Perspect* 2009; **117**: 784-789.

17. Van Landuyt KL, Nawrot T, Geebelen B, De Munck J, Snauwaert J, Yoshihara K, Scheers H, Godderis L, Hoet P, Van Meerbeek B. How much do resin-based dental materials release? A meta-analytical approach. *Dent Mater* 2011; **27**: 723-747.

18. BOWEN RL. Properties of a silica-reinforced polymer for dental restorations. *J Am Dent Assoc* 1963; **66**: 57-64.

19. FLEISCH AF, SHEFFIELD PE, CHINN C, EDELSTEIN BL, LANDRIGAN PJ. Bisphenol A and related compounds in dental materials. *Pediatrics* 2010; **126**: 760-768.

20. SCHMALZ G, PREISS A, ARENHOLT-BINDSLEV D. Bisphenol-A content of resin monomers and related degradation products. *Clin Oral Investig* 1999; **3**: 114-119.

21. ARENHOLT-BINDSLEV D, BREINHOLT V, PREISS A, SCHMALZ G. Time-related bisphenol-A content and estrogenic activity in saliva samples collected in relation to placement of fissure sealants. *Clin Oral Investig* 1999; **3**: 120-125.

22. ATKINSON JC, DIAMOND F, EICHMILLER F, SELWITZ R, JONES G. Stability of bisphenol A, triethylene-glycol dimethacrylate, and bisphenol A dimethacrylate in whole saliva. *Dent Mater* 2002; **18**: 128-135.

23. CHEN L, SUH BI. Bisphenol A in Dental Materials: A Review. *JSM Dent* 2013; **1**.

24. KADOMA Y, TANAKA M. Acid and base-catalyzed hydrolysis of bisphenol A-related compounds. *Dent Mater J* 2000; **19**: 139-152.

25. OLEA N, PULGAR R, PEREZ P, OLEA-SERRANO F, RIVAS A, NOVILLO-FERTRELL A, PEDRAZA V, SOTO AM, SONNENSCHEIN C. Estrogenicity of resin-based composites and sealants used in dentistry. *Environ Health Perspect* 1996; **104**: 298-305.

26. KINGMAN A, HYMAN J, MASTEN SA, JAYARAM B, SMITH C, EICHMILLER F, ARNOLD MC, WONG PA, SCHAEFFER JM, SOLANKI S, DUNN WJ. Bisphenol A and other compounds in human saliva and urine associated with the placement of composite restorations. *J Am Dent Assoc* 2012; **143**: 1292-1302.

27. MASEREJIAN NN, HAUSER R, TAVARES M, TRACHTENBERG FL, SHRADER P, MCKINLAY S. Dental composites and amalgam and physical development in children. *J Dent Res* 2012; **91**: 1019-1025.

28. KANG YG, KIM JY, KIM J, WON PJ, NAM JH. Release of bisphenol A from resin composite used to bond orthodontic lingual retainers. *Am J Orthod Dentofacial Orthop* 2011; **140**: 779-789.

29. LEE JH, YI SK, KIM SY, KIM JS, SON SA, JEONG SH, KIM JB. Salivary bisphenol A levels and their association with composite resin restoration. *Chemosphere* 2017; **172**: 46-51.

30. MOREIRA MR, MATOS LG, DE SOUZA ID, BRIGANTE TA, QUEIROZ ME, ROMANO FL, NELSON-FILHO P, MATSUMOTO MA. Bisphenol A release from orthodontic adhesives measured in vitro and in vivo with gas chromatography. *Am J Orthod Dentofacial Orthop* 2017; **151**: 477-483.

31. KLOUKOS D, PANDIS N, ELIADES T. In vivo bisphenol-a release from dental pit and fissure sealants: a systematic review. *J Dent* 2013; **41**: 659-667.

32. SOFAN E, SOFAN A, PALAIA G, TENORE G, ROMEO U, MIGLIAU G. Classification review of dental adhesive systems: from the IV generation to the universal type. *Ann Stomatol (Roma)* 2017; **8**: 1-17.

33. OLSTAD ML, HOLLAND RI, WANDEL N, PETTERSEN AH. Correlation between amalgam restorations and mercury concentrations in urine. *J Dent Res* 1987; **66**: 1179-1182.

34. BERGE TLL, LYGRE GB, JONSSON BAG, LINDH CH, BJORKMAN L. Bisphenol A concentration in human saliva related to dental polymer-based fillings. *Clin Oral Investig* 2017; **21**: 2561-2568.

35. GYLLENHAMMAR I, GLYNN A, JONSSON BA, LINDH CH, DARNERUD PO, SVENSSON K, LIGNELL S. Diverging temporal trends of human exposure to bisphenols and plastizisers, such as phthalates, caused by substitution of legacy EDCs? *Environ Res* 2017; **153**: 48-54.

36. CONE EJ, CAPLAN YH, MOSER F, ROBERT T, SHELBY MK, BLACK DL. Normalization of urinary drug concentrations with specific gravity and creatinine. *J Anal Toxicol* 2009; **33**: 1-7.

37. HORNUNG R, REED L. Estimation of average concentration in the presence of nondetectable values. *Appl Occup Hyg* 1990; **5**: 46-51.

38. FUNG EYK, EWOLDSEN NO, ST. GERMAIN HA, MARX DB, MIAW C-L, SIEW C, CHOU H-N, GRUNINGER SE, MEYER DM. Pharmacokinetics of Bisphenol a Released from a Dental Sealant. *J Am Dent Assoc* 2000; **131**: 51-58.

39. JOSKOW R, BARR DB, BARR JR, CALAFAT AM, NEEDHAM LL, RUBIN C. Exposure to bisphenol A from bis-glycidyl dimethacrylate–based dental sealants. *J Am Dent Assoc* 2006; **137**: 353-362.

40. POLYDOROU O, HUBERTY C, WOLKEWITZ M, BOLEK R, HELLWIG E, KUMMERER K. The effect of storage medium on the elution of monomers from composite materials. *J Biomed Mater Res B Appl Biomater* 2012; **100**: 68-74.

41. SASAKI N, OKUDA K, KATO T, KAKISHIMA H, OKUMA H, ABE K, TACHINO H, TUCHIDA K, KUBONO K. Salivary bisphenol-A levels detected by ELISA after restoration with composite resin. *J Mater Sci Mater Med* 2005; **16**: 297-300.

42. KLOUKOS D, SIFAKAKIS I, VOUTSA D, DOULIS I, ELIADES G, KATSAROS C, ELIADES T. BPA qualtitative and quantitative assessment associated with orthodontic bonding in vivo. *Dent Mater* 2015; **31**: 887-894.

43. AMERICAN DENTAL ASSOCIATION COUNCIL ON SCIENTIFIC A. Determination of bisphenol a released from resin-based composite dental restoratives. *J Am Dent Assoc* 2014; **145**: 763-765.

44. GUPTA SK, SAXENA P, PANT VA, PANT AB. Release and toxicity of dental resin composite. *Toxicol Int* 2012; **19**: 225-234.

45. SIDERIDOU I, TSERKI V, PAPANASTASIOU G. Effect of chemical structure on degree of conversion in light-cured dimethacrylate-based dental resins. *Biomaterials* 2002; **23**: 1819-1829.

46. PEUTZFELDT A. Resin composites in dentistry: the monomer systems. *Eur J Oral Sci* 1997; **105**: 97-116.

47. ALSHAAFI MM. Factors affecting polymerization of resin-based composites: A literature review. *Saudi Dent J* 2017; **29**: 48-58.

48. RUYTER IE. Unpolymerized surface layers on sealants. *Acta Odontol Scand* 1981; **39**: 27-32.

49. RAWLS HR, WHANG K. Resin-based composites. In: ANUSAVICE KJ, SHEN C, RAWLS HR, eds. *Phillips' Science of Dental Materials*. Elsevier/Saunders, 2013; 275-306.

50. HAMID A, HUME WR. A study of component release from resin pit and fissure sealants in vitro. *Dent Mater* 1997; **13**: 98-102.

51. KOMURCUOGLU E, OLMEZ S, VURAL N. Evaluation of residual monomer elimination methods in three different fissure sealants in vitro. *J Oral Rehabil* 2005; **32**: 116-121.

52. GILBERT GH, LITAKER MS, PIHLSTROM DJ, AMUNDSON CW, GORDAN VV, GROUP DC. Rubber dam use during routine operative dentistry procedures: findings from the Dental PBRN. *Oper Dent* 2010; **35**: 491-499.

53. LYNCH CD, MCCONNELL RJ. Attitudes and use of rubber dam by Irish general dental practitioners. *Int Endod J* 2007; **40**: 427-432.

54. MICHELSEN VB, KOPPERUD HB, LYGRE GB, BJORKMAN L, JENSEN E, KLEVEN IS, SVAHN J, LYGRE H. Detection and quantification of monomers in unstimulated whole saliva after treatment with resin-based composite fillings in vivo. *Eur J Oral Sci* 2012; **120**: 89-95.

55. MULLANGI R, AGRAWAL S, SRINIVAS NR. Measurement of xenobiotics in saliva: is saliva an attractive alternative matrix? Case studies and analytical perspectives. *Biomed Chromatogr* 2009; **23**: 3-25.

56. YE X, WONG LY, BISHOP AM, CALAFAT AM. Variability of urinary concentrations of bisphenol A in spot samples, first morning voids, and 24-hour collections. *Environ Health Perspect* 2011; **119**: 983-988.

57. VANDENBERG LN, HAUSER R, MARCUS M, OLEA N, WELSHONS WV. Human exposure to bisphenol A (BPA). *Reprod Toxicol* 2007; **24**: 139-177.

58. Thayer KA, Doerge DR, Hunt D, Schurman SH, Twaddle NC, Churchwell MI, Garantziotis S, Kissling GE, Easterling MR, Bucher JR, Birnbaum LS. Pharmacokinetics of bisphenol A in humans following a single oral administration. *Environ Int* 2015; **83**: 107-115.

59. PUTZEYS E, COKIC SM, CHONG H, SMET M, VANOIRBEEK J, GODDERIS L, VAN MEERBEEK B, VAN LANDUYT KL, DUCA RC. Simultaneous analysis of bisphenol A based compounds and other monomers leaching from resin-based dental materials by UHPLC-MS/MS. *J Sep Sci* 2017; **40**: 1063-1075.

60. Thayer KA, Taylor KW, Garantziotis S, Schurman SH, Kissling GE, Hunt D, Herbert B, Church R, Jankowich R, Churchwell MI, Scheri RC, Birnbaum LS, Bucher JR. Bisphenol A, Bisphenol S, and 4-Hydroxyphenyl 4-Isoprooxyphenylsulfone (BPSIP) in Urine and Blood of Cashiers. *Environ Health Perspect* 2016; **124**: 437-444.

61. Vandenberg LN, Colborn T, Hayes TB, Heindel JJ, Jacobs DR, Jr., Lee DH, Shioda T, Soto AM, vom Saal FS, Welshons WV, Zoeller RT, Myers JP. Hormones and endocrine-disrupting chemicals: low-dose effects and nonmonotonic dose responses. *Endocr Rev* 2012; **33**: 378-455.

62. WELSHONS WV, NAGEL SC, VOM SAAL FS. Large effects from small exposures. III. Endocrine mechanisms mediating effects of bisphenol A at levels of human exposure. *Endocrinology* 2006; **147**: S56-S69.

63. BITTNER GD, YANG CZ, STONER MA. Estrogenic chemicals often leach from BPA-free plastic products that are replacements for BPA-containing polycarbonate products. *Environ Health* 2014; **13**: 41.

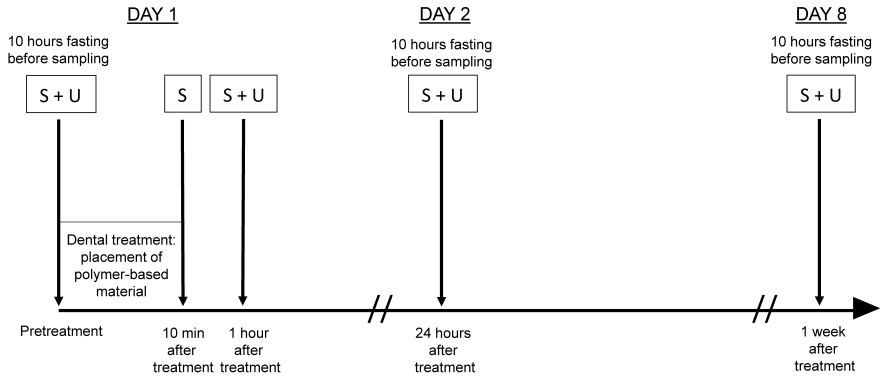

Fig. 1. Time schedule of saliva (S) and urine (U) sampling.

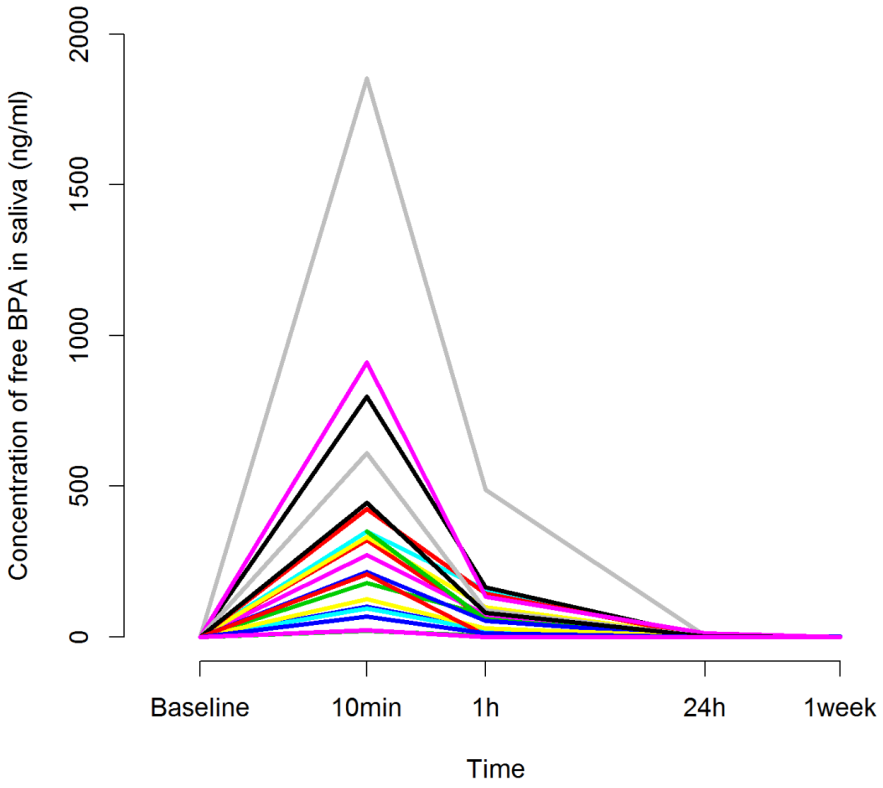

Fig. 2. Salivary concentrations (ng/ml) of bisphenol A (BPA) among participants (individual patterns) before treatment (baseline) and 10 minutes, 1 hour, 24 hours and 1 week after treatment with polymer-based filling material (n=20).

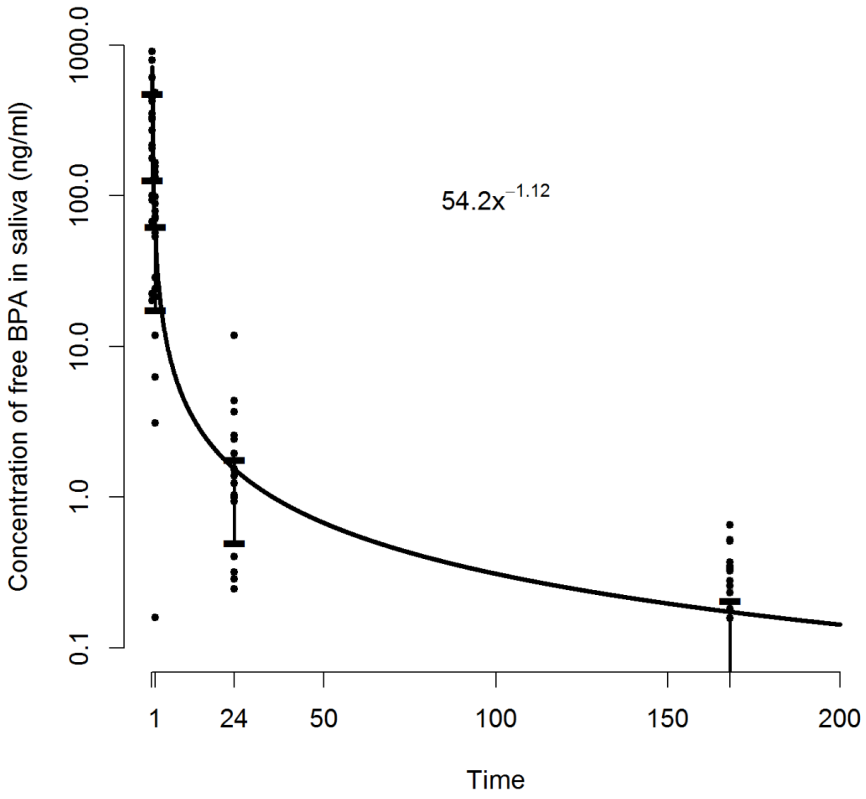

Fig. 3. Concentration (ng/ml) of bisphenol A (BPA) in saliva after treatment with polymer-based dental restorative materials as a function of time (hours).

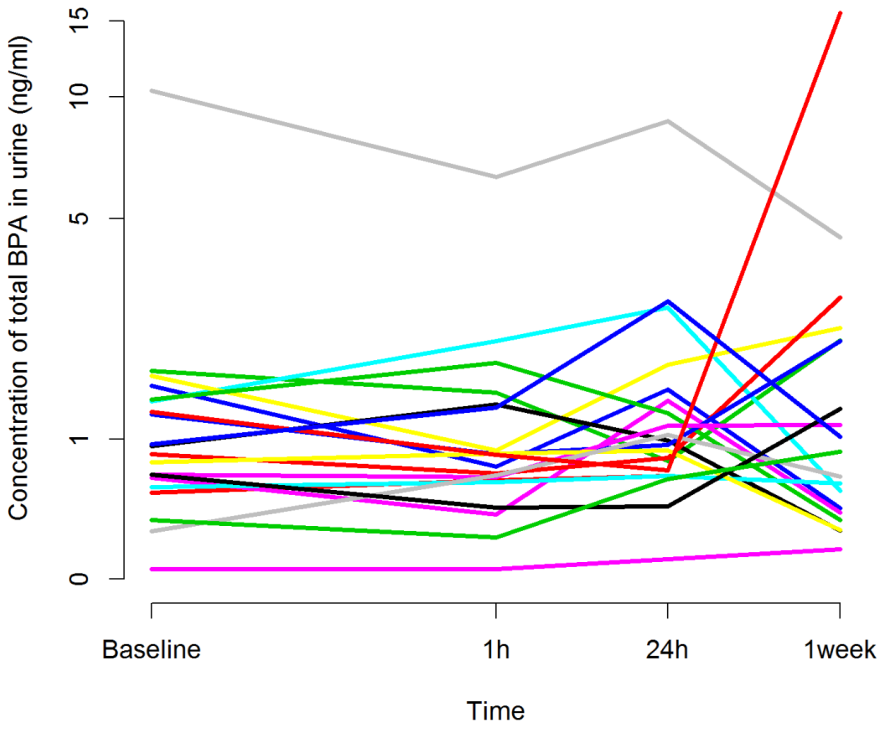

Fig. 4. Urinary concentrations (ng/ml) of bisphenol A (BPA) among participants (individual patterns) before treatment (baseline) and 1 hour, 24 hours and 1 week after treatment with polymer-based filling material (n=20).

Table 1
Background characteristics of the participants (n=20)

Variables	
Sex	
Women/Men	13/7
Age (years)	
Mean (SD)[1]	23.4 (5.7)
Min[2]-Max[3]	17 - 36
Education (years)	
Mean (SD)	13.8 (1.8)
Min-Max	10 - 17
Daily use of chewing gum	
Yes/No	4/16
Daily use of toothpaste	
Yes/No	20/0
Use of rinsing agents	
Yes/No	11/9
Job handling receipts	
Yes/No	5/15

[1] Standard deviation; [2] Minimum; [3] Maximum

Table 2

Data regarding participants' dental hygiene habits and intake of canned and microwaved food by sampling day (n=20)

Variables	Day 1 (Day of treatment)	Day 2 (24 hours after treatment)	Day 8 (1 week after treatment)
Refrained from food and drink at least 10 hours before sampling			
Yes/No	20/0	20/0	19/1
Brushed teeth during the morning before sampling			
Yes/No	5/15	2/18	2/18
Use of mouth rinse before sampling			
Yes/No	1/19	1/19	0/20
Intake of canned food last 24 hours			
Yes/No	4/16	2/18	4/16
Intake of microwaved food last 24 hours			
Yes/No	2/18	2/18	1/19
Intake of canned food last week			
Yes/No	13/7	NA	12/8
Intake of microwaved food last week			
Yes/No	7/13	NA	2/18

NA: Not applicable

Table 3

Data regarding previous and current dental restorative treatment of the participants (n=20)

Variables	Mean (SD)[1]	Min[2]	Max[3]
Number of preexisting tooth-colored filling surfaces	11.8 (9.6)	0	26
Number of preexisting tooth-colored filling points	25.7 (21.7)	0	68
Number of polymer-based filling surfaces removed during treatment	0.70 (0.92)	0	3
Number of polymer-based filling points removed during treatment	1.8 (2.7)	0	9
Number of treated filling surfaces	3.7 (1.9)	2	10
Number of treated filling points	7.7 (0.7)	4	15
Weight of polymer-based material used (g)	0.158 (0.067)	0.065	0.309

[1] Standard deviation; [2] Minimum; [3] Maximum

Table 4

Estimated BPA concentration (ng/ml) in saliva and urine samples before and after treatment with polymer-based dental restorative material

	Time point	N	Mean	(95% CI)	P value
Saliva	Before treatment	19	0.11	(<LOD–0.16)	Reference
	10 minutes after treatment	20	385	(205–565)	< 0.001
	1 hour after treatment	20	88.2	(42.4–134)	< 0.001
	24 hours after treatment	20	1.85	(0.72–2.98)	0.003
	1 week after treatment	19	0.25	(0.17–0.33)	0.002
Urine[1]	Before treatment	20	1.41	(0.42–2.41)	Reference
	1 hour after treatment	20	1.17	(0.18–2.16)	0.674
	24 hours after treatment	20	1.53	(0.54–2.53)	0.834
	1 week after treatment	19	1.99	(0.97–3.00)	0.321

Limit of detection (LOD) = 0.1 ng/ml, values < 0.1 ng/ml were set to half of the LOD

[1] Density adjusted urine concentrations (1.016 g/l)

Paper III

Polymer-based dental filling materials placed during pregnancy and risk to the foetus.

Berge TLL, Lygre GB, Lie SA, Björkman L.

BMC Oral Health. 2018;18(144).

III

Berge et al. BMC Oral Health (2018) 18:144
https://doi.org/10.1186/s12903-018-0608-1

BMC Oral Health

RESEARCH ARTICLE Open Access

Polymer-based dental filling materials placed during pregnancy and risk to the foetus

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Abstract

Background: Tooth-coloured polymer-based dental filling materials are currently the first choice for dental restorative treatment in many countries. However, there are some concerns about their safety. It has been shown that substances known as endocrine disrupters, which might pass through the placental barrier, are released from these materials within the first hours after curing. Thus, the placement of polymer-based dental fillings in pregnant women may put the vulnerable foetus at risk. Large epidemiological studies exploring the risk of having polymer-based dental materials placed during pregnancy are lacking. The aim of this study was to investigate the association between the placement of polymer-based dental fillings during pregnancy and adverse birth outcomes.

Methods: This study is based on data from the large Norwegian Mother and Child Cohort Study (MoBa). The information about dental treatment during pregnancy was obtained from questionnaires sent to the participating women during weeks 17 and 30 of pregnancy. Reported placement of "white fillings" was used as exposure marker for having received polymer-based dental filling materials. Only singleton births were included in the present study. Data were linked to the Medical Birth Registry of Norway. Logistic regression models that included the mother's age, level of education, body mass index, parity, and smoking and alcohol consumption during pregnancy were used to estimate the odds ratio (OR) and 95% confidence interval (CI). Different adverse birth outcomes were of interest in the present study.

Results: Valid data were available from 90,886 pregnancies. Dentist consultation during pregnancy was reported by 33,727 women, 10,972 of whom had white fillings placed. The adjusted logistic regression models showed no statistically significant association between having white dental fillings placed during pregnancy and stillbirth, malformations, preterm births, and low or high birth weight.

Conclusions: In this study, women who reported white fillings placed during pregnancy had no increased risk for adverse birth outcomes compared with women who did not consult a dentist during pregnancy. Thus, our findings do not support the hypothesis of an association between placement of polymer-based fillings during pregnancy and adverse birth outcomes.

Keywords: Polymer-based dental filling materials, Pregnancy, Adverse birth outcomes, Congenital malformation, Birth weight, Stillbirth, Premature birth, Bisphenol A, BPA, The Norwegian mother and child cohort study

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Background

Tooth-coloured polymer-based materials are the first choice for dental restorative treatment in many countries [1, 2]. However, there are concerns about the safety of these materials [3]. Results of in vitro and in vivo studies have shown that substances that potentially could lead to adverse effects in the patient are released from these materials within 24 h after curing [4–8]. Elution may initially be due to incomplete polymerization [9, 10] and contaminants [11, 12]. The local adverse effects [13] caused by the leachable components are rare [14]. However, the possibility of systemic adverse effects could not be ruled out [15].

The elution of bisphenol A (BPA) has been of particular concern [16]. BPA is a chemical known to be an endocrine disruptor, mimicking oestrogen [17, 18]. Polymer-based dental filling materials may contain BPA as an impurity from the production process of bisphenol-A glycidyl dimethacrylate (Bis-GMA) [8, 11, 19, 20] or, less probable, a degradation product of monomers [12, 21, 22]. Results from animal studies have indicated that BPA has reproductive, developmental and systemic toxic effects [23, 24]. It has been shown that newly placed composite restorations in humans may be associated with short-term elevated BPA levels in both saliva and urine [4, 7].

The impact of exposure to BPA on human health remains uncertain. However, data from the literature indicate that exposure to BPA, even at relatively low doses, could potentially result in adverse health effects [15]. Moreover, studies suggest that BPA might pass through the placental barrier [25], and thus, maternal exposure to BPA may offer a potential risk to the vulnerable foetus.

Even though substances with potential toxicity are released from dental polymer-based materials [4, 5], studies exploring the risk of having these materials placed during pregnancy are lacking.

The aim of the present study was to investigate whether the placement of polymer-based dental fillings during pregnancy is associated with adverse birth outcomes including stillbirth, preterm birth, malformations and low or high birth weight.

Methods

Data from the ongoing Norwegian Mother and Child Cohort Study (MoBa), a prospective population-based cohort study conducted by the Norwegian Institute of Public Health, were used. From 1999 to the end of 2008, pregnant women in Norway were invited to MoBa through a postal invitation in connection with their first routine ultrasound examination. The participation rate was approximately 41%, and the cohort currently comprises more than 108,000 pregnancies, 114,000 children, 95,000 mothers and 75,000 fathers. Written informed consent was obtained from each participant upon recruitment [26, 27].

In the present study, data were gathered from two questionnaires that were sent to the participating women in weeks 17 and 30 of pregnancy [28]. Each woman could participate with multiple pregnancies. Only singleton births were included in the present study.

Information about white fillings placed during pregnancy was obtained from the questionnaires sent to the participants in week 30. Reported placement of white fillings was used as exposure marker. The participants reported if they had consulted a dentist during pregnancy ("Have you been to the dentist during this pregnancy? Yes/No") and if so, whether they had received white fillings ("If, yes, did the dentist put in new white fillings? Yes/No").

Women without valid information about dental treatment during pregnancy and those with missing data on birth outcomes were excluded, leaving a study population that included 90,886 pregnancies (Fig. 1).

Information about gender, preterm delivery, stillbirth, malformations, birth weight and mother's age at delivery was obtained from the Medical Birth Registry of Norway (MBRN) [29]. The mother's 11-digit unique personal identification number assigned to every citizen in Norway was used to link data sources. Gestational age was based on ultrasound examination in the 17th week of pregnancy.

Infants were classified as late preterm if they were born between gestational week 33 and 37, and very preterm if they were born before or during the 32nd gestational week [30, 31]. Infants with a birth weight less than 2500 g at birth were classified as low-birth weight infants, and infants with a birth weight more than 4000 g were classified as high-birth weight infants [32].

Maternal body mass index (BMI; kg/m^2) was calculated from self-reported pre-pregnancy height and weight. The BMI was categorized according to the WHO classification [33].

Information about parity, defined as the number of former births with a gestational age of 12 weeks or more, was based on data reported by the mothers in the MoBa study and from the MBRN.

Information about education, smoking habits and alcohol consumption during pregnancy was obtained from the first questionnaire completed at approximately the 17th week.

The present study is based on version 8 of the quality-assured MoBa data files. We defined dental treatment during pregnancy as follows: participants who did not consult a dentist during pregnancy (reference category); participants who consulted a dentist but had no white fillings placed; and participants who consulted a dentist and had white fillings placed (Fig. 1).

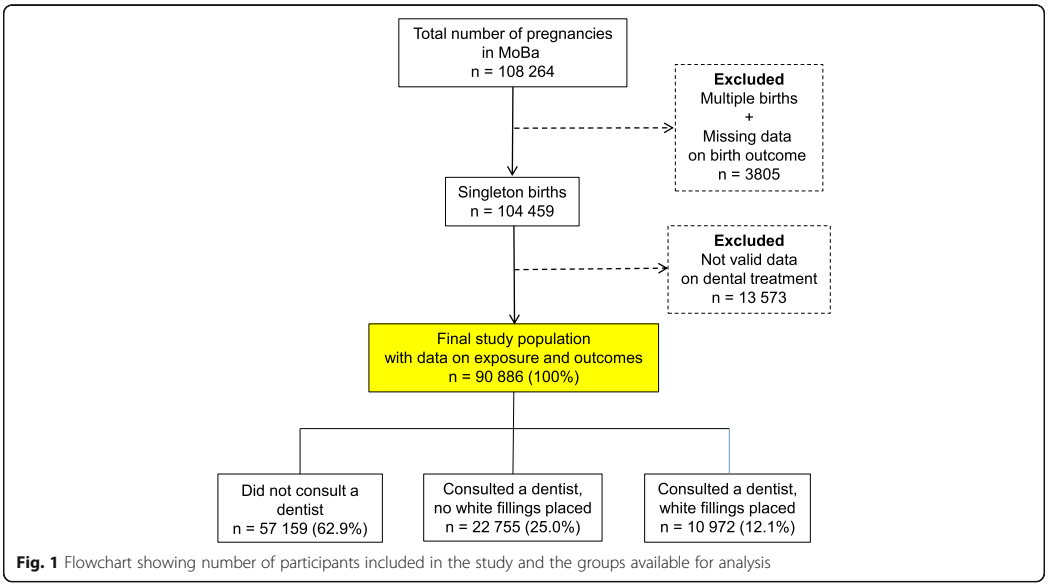

Fig. 1 Flowchart showing number of participants included in the study and the groups available for analysis

Infants were defined as small for gestational age (SGA) if the weight at birth was less than the 10th percentile for gestational age and large for gestational age (LGA) if they were larger than the 90th percentile. Very small for gestational age was defined as weight below the 2.5th percentile [34].

The odds ratio (OR) with a 95% confidence interval was calculated using logistic regression. The OR was adjusted for maternal age (≤19, 20–24, 25–29, 30–34, 35–39, 40+ years), length of education (≤12, 13–16, ≥17 years), pre-pregnancy BMI (< 18.5, 18.5–24.9, 25.0–29.9, 30.0–34.9, 35.0–39.9, ≥ 40 kg/m^2), parity (first, second and more), smoking during pregnancy (never, occasionally, daily) and alcohol consumption during pregnancy (never, less than once a week, once a week, more than once a week).

Analyses were performed using IBM-SPSS (IBM Corp. Released 2016. IBM SPSS Statistics for Windows, Version 24.0, Armonk, NY, USA: IBM Corp.). *p*-values less than 0.05 were considered statistically significant.

The MoBa cohort study obtained a license from the Norwegian Data Inspectorate, and this research project was approved by the Regional Ethics Committee for Medical Research (REC South-East D, 2011/727).

Results

Dentist consultation during pregnancy was reported by 33,727 women, and of these, 10,972 had white fillings placed (Fig. 1). Detailed descriptive information regarding the characteristics of the participants is included in Table 1. Of the included pregnancies, 204 (0.2%) resulted in a stillbirth. The overall proportion of malformation was 4.8%, and the proportion of very preterm births and late preterm births was 0.6 and 3.8%, respectively (Table 2).

Compared to the reference group, there was no statistically significant increased risk for any adverse birth outcomes for participants who had consulted a dentist during pregnancy without having white fillings placed or for those who had white fillings placed (Table 3).

Separate analyses by gender showed that girls born to mothers who had white fillings placed during pregnancy had an increased risk of being small for gestational age (below the 10th percentile) compared to the reference group. The unadjusted OR was 1.14 (95% CI 1.01–1.28; $p = 0.029$) while after adjustment for potential confounders, the OR was reduced and not statistically significant (OR = 1.10, 95% CI 0.97–1.24; Table 3).

Boys born to mothers who received white fillings during pregnancy had a slightly increased risk of being born late preterm compared to the boys born in the reference group. The unadjusted OR was 1.16 (95% CI 1.01–1.34; $p = 0.041$), and the adjusted OR was 1.13 (95% CI 0.98–1.31; $p = 0.082$; Table 3).

Discussion

The aim of the present study was to investigate whether the placement of polymer-based dental fillings during pregnancy was associated with outcomes including stillbirth, preterm delivery, malformations, and low or high

Table 1 Characteristics of the participants related to dental treatment during pregnancy ($n = 90,886$)

	Did not consult a dentist	Consulted a dentist, no white fillings placed	Consulted a dentist, white filling placed	Total
Number of participating pregnancies, *n* (%)	57,159 (62.9)	22,755 (25.0)	10,972 (12.1)	90,886 (100)
Maternal age (years), *n* (%)				
≤ 19	525 (0.9)	207 (0.9)	127 (1.2)	859 (0.9)
20–24	6056 (10.6)	1767 (7.8)	1195 (10.9)	9018 (9.9)
25–29	19,288 (33.7)	7159 (31.5)	3686 (33.6)	30,133 (33.2)
30–34	21,928 (38.4)	9273 (40.8)	3990 (36.4)	35,191 (38.7)
35–39	8315 (14.5)	3856 (16.9)	1711 (15.6)	13,882 (15.3)
≥ 40	1047 (1.8)	493 (2.2)	263 (2.4)	1803 (2.0)
Maternal pre-pregnant Body Mass Index, *n* (%)				
< 18.5	1663 (2.9)	660 (2.9)	319 (2.9)	2642 (2.9)
18.5–24.9	36,021 (63.0)	14,956 (65.7)	6655 (60.7)	57,632 (63.4)
25.0–29.9	12,003 (21.0)	4542 (20.0)	2443 (22.3)	18,988 (20.9)
30.0–34.9	3837 (6.7)	1402 (6.2)	842 (7.7)	6081 (6.7)
35.0–39.9	1091 (1.9)	354 (1.6)	232 (2.1)	1677 (1.8)
≥ 40	341 (0.6)	124 (0.5)	68 (0.6)	533 (0.6)
Missing	2203 (3.9)	717 (3.2)	413 (3.8)	3333 (3.7)
Parity, *n* (%)				
Para 0 (first pregnancy)	25,428 (44.5)	10,897 (47.9)	4836 (44.1)	41,161 (45.3)
Para 1+ (second pregnancy or more)	31,731 (55.5)	11,858 (52.1)	6136 (55.9)	49,725 (54.7)
Maternal education, *n* (%)				
≤ 12 years	18,849 (33.0)	6831 (30.0)	4177 (38.1)	29,857 (32.9)
13–16 years	22,042 (38.6)	9226 (40.5)	4018 (36.6)	35,286 (38.8)
≥17 years	12,725 (22.3)	5366 (23.6)	2097 (19.1)	20,188 (22.2)
Missing	3543 (6.2)	1332 (5.9)	680 (6.2)	5555 (6.1)
Smoking during pregnancy, *n* (%)				
Never	45,831 (80.2)	18,208 (80.0)	8420 (76.7)	72,459 (79.7)
Occasionally	1421 (2.5)	567 (2.5)	374 (3.4)	2362 (2.6)
Daily	2848 (5.0)	968 (4.3)	821 (7.5)	4637 (5.1)
Missing	7059 (12.3)	3012 (13.2)	1357 (12.4)	11,428 (12.6)
Alcohol during pregnancy, *n* (%)				
Never	42,203 (73.8)	16,731 (73.5)	7834 (71.4)	66,768 (73.5)
Less than once a week	5709 (10.0)	2512 (11.0)	1251 (11.4)	9472 (10.4)
Once a week	233 (0.4)	100 (0.4)	46 (0.4)	379 (0.4)
More than once a week	39 (0.1)	25 (0.1)	6 (0.1)	70 (0.1)
Missing	8975 (15.7)	3387 (14.9)	1835 (16.7)	14,197 (15.6)

Body Mass Index = weight (kg)/height^2 (m)^2

birth weight. No evidence of an increased risk of adverse birth outcomes after placement of white fillings during pregnancy was found. Gender-specific analyses showed generally similar results for girls and boys analysed together.

The main strengths of the present study are the overall large sample size and the large number of participants who had white fillings placed. These large numbers enabled us to study even rare birth outcomes. Furthermore, the prospective design of the study reduced the risk for recall bias. Additionally, the information on health-related and lifestyle data that was derived from both the MBRN and the MoBa questionnaires enabled us to control for some potential confounding factors.

To the best of our knowledge, the present study is the first to investigate potential associations between

Table 2 Birth outcomes by dental treatment during pregnancy ($n = 90,886$)

	Did not consult a dentist	Consulted a dentist, no white fillings placed	Consulted a dentist, white filling placed	Total
Number of boys, *n* (%)	29,387 (51.4)	11,607 (51.0)	5582 (50.9)	46,576 (51.2)
Number of preterm births, *n* (%)				
Very preterm births (≤ 32 weeks)	337 (0.6)	124 (0.5)	65 (0.6)	526 (0.6)
Late preterm births (33–36 weeks)	2150 (3.8)	884 (3.9)	454 (4.1)	3488 (3.8)
Mean birth weight (g)				
Mean birth weight (SD)	3611 (546)	3603 (538)	3607 (549)	3608 (544)
Number of children with low birth weight, *n* (%)				
Low birth weight (< 2500 g)	1465 (2.6)	576 (2.5)	290 (2.6)	2331 (2.6)
Small for gestational age (SGA) 10 percentile	3660 (6.4)	1475 (6.5)	726 (6.6)	5861 (6.5)
Small for gestational age (SGA) 2.5 percentile	793 (1.4)	293 (1.3)	145 (1,3)	1231 (1.4)
Number of children with high birth weight, *n* (%)				
High birth weight children (> 4000 g)	12,515 (21.9)	4905 (21.6)	2390 (21.8)	19,810 (21.8)
Large for gestational age (LGA) 10 percentile	6633 (11.7)	2557 (11.3)	1285 (11.8)	10,475 (11.6)
Large for gestational age (LGA) 2.5 percentile	2110 (3.7)	809 (3.6)	418 (3.8)	3337 (3.7)
Number of children with malformation, *n* (%)	2697(4.7)	1108 (4.9)	519 (4.7)	4324 (4.8)
Number of stillbirths, *n* (%)	125 (0.2)	49 (0.2)	30 (0.3)	204 (0.2)

polymer-based fillings placed during pregnancy and birth outcomes. Michalowicz et al. found no significant associations between adverse pregnancy outcomes and periodontal treatment, the use of anaesthetic during nonsurgical periodontal treatment, treatment including temporary and permanent restorations, endodontic therapy, and extractions [35]. These results are in agreement with our findings. However, in the study of Michalowicz et al., the type of restorative material was not specified. Thus, the results are not directly comparable.

A limitation of the MoBa study is the low response rate, with a possible self-selection of the healthiest women. The MoBa has an underrepresentation of young mothers (< 25 years). The participants have a higher level of education and are more likely to be non-smokers than the general population of pregnant women in Norway [36].

However, self-selection to the cohort is not a validity problem in studies of associations between exposure and outcomes [36].

The MoBa study is based on questionnaires filled in by the participating women. To achieve reliable answers from all participants in this large cohort, an effort was made to make the questions as easy and achievable as possible. Thus, information about dental treatment is sparse. Detailed information about the type and manufacturer of the polymer-based filling material and size and number of fillings placed, would be of interest. However, to obtain accurate information about this, access to dental records would be needed. In large epidemiological studies, like the MoBa study, access to updated dental records would be unfeasible. Accordingly, reliable knowledge about the number and size of possible pre-existing composite restorations is lacking. Since leakage of BPA from existing polymer-based restorative materials is very low compared with other sources [37], this information would most likely be of minor importance.

The participants were asked if they had received "white fillings" during pregnancy. In Norway, white fillings would practically be the same as polymer-based restorative fillings or so called polymer-based or resin-based composites. However, the term "white fillings" may include materials like resin-modified cements, compomers and water-based glass ionomer cements (GIC). In the period of this study, the vast majority of Norwegian dentists used polymer-based filling materials when restoring cavities in adults. Kopperud et al. described management of occlusal caries in adults by Norwegian dentists in 2009 and stated that polymer-based composite was the preferred restorative material (91.9%) [38]. In the same study the use of other filling materials was reported to be less than 4%. This is in accordance with another study examining treatment concept for approximal caries in Norway [39]. In 2009 polymer-based filling material was preferred by 94.9% of the responding dentists. Preference for other filling materials was: 1.1% compomer, 1.1% GIC, 0.5% resin-modified GIC and 1.8% a combination of resin composite and GIC [39]. In 1997, 2 years before recruitment started in MoBa, Norwegian data showed that approximately 70% of the tooth-coloured fillings placed in adults were polymer-based [40].

Table 3 Crude and adjusted odds ratio (OR) and confidence interval (CI) for adverse birth outcomes related to dental treatment during pregnancy. (Reference category: Women who did not consult a dentist, OR = 1)

		Consulted a dentist, no white fillings placed OR (95% CI)	Consulted a dentist, white filling placed OR (95% CI)
Very preterm birth (≤ 32 weeks)			
Girls	Crude	0.96 (0.71–1.30)	0.91 (0.60–1.37)
	Adjusted	0.94 (0.69–1.27)	0.88 (0.58–1.33)
Boys	Crude	0.91 (0.69–1.21)	1.08 (0.76–1.53)
	Adjusted	0.88 (0.67–1.17)	1.02 (0.72–1.45)
All	Crude	0.92 (0.75–1.14)	1.01 (0.77–1.31)
	Adjusted	0.90 (0.73–1.11)	0.97 (0.74–1.26)
Late preterm birth (33–36 weeks)			
Girls	Crude	1.00 (0.89–1.13)	1.05 (0.90–1.22)
	Adjusted	1.00 (0.89–1.12)	1.03 (0.88–1.19)
Boys	Crude	1.06 (0.95–1.18)	1.16*(1.01–1.34)
	Adjusted	1.05 (0.94–1.18)	1.14 (0.99–1.31)
All	Crude	1.03 (0.96–1.12)	1.10 (1.00–1.23)
	Adjusted	1.03 (0.95–1.11)	1.08 (0.97–1.20)
Low birth weight (< 2500 g)			
Girls	Crude	1.01 (0.88–1.51)	1.03 (0.86–1.23)
	Adjusted	0.98 (0.86–1.12)	0.99 (0.83–1.18)
Boys	Crude	0.96 (0.84–1.11)	1.03 (0.86–1.24)
	Adjusted	0.94 (0.82–1.09)	0.99 (0.83–1.19)
All	Crude	0.99 (0.90–1.09)	1.03 (0.91–1.17)
	Adjusted	0.96 (0.87–1.06)	0.99 (0.87–1.13)
Small for gestational age (SGA) 10 percentile			
Girls	Crude	1.07 (0.97–1.17)	1.14*(1.01–1.28)
	Adjusted	1.03 (0.94–1.13)	1.10 (0.97–1.24)
Boys	Crude	0.97 (0.89–1.05)	0.95 (0.85–1.07)
	Adjusted	0.92 (0.84–1.00)	0.93 (0.83–1.04)
All	Crude	1.01 (0.95–1.08)	1.04 (0.95–1.13)
	Adjusted	0.97 (0.91–1.03)	1.00 (0.92–1.09)
Very small for gestational age (SGA) 2.5 percentile			
Girls	Crude	0.86 (0.71–1.06)	1.04 (0.81–1.34)
	Adjusted	0.84 (0.68–1.03)	0.97 (0.75–1.25)
Boys	Crude	0.98 (0.82–1.17)	0.88 (0.68–1.13)
	Adjusted	0.93 (0.77–1.11)	0.84 (0.65–1.08)
All	Crude	0.93 (0.81–1.06)	0.95 (0.80–1.14)
	Adjusted	0.89 (0.77–1.02)	0.90 (0.75–1.08)
High birth weight (> 4000 g)			
Girls	Crude	0.99 (0.94–1.05)	0.98 (0.91–1.06)
	Adjusted	1.03 (0.97–1.09)	0.98 (0.91–1.06)
Boys	Crude	0.98 (0.93–1.03)	1.01 (0.94–1.07)
	Adjusted	1.01 (0.96–1.06)	1.00 (0.93–1.07)
All	Crude	0.98 (0.94–1.02)	0.99 (0.95–1.04)
	Adjusted	1.01 (0.98–1.05)	0.99 (0.94–1.04)

Table 3 Crude and adjusted odds ratio (OR) and confidence interval (CI) for adverse birth outcomes related to dental treatment during pregnancy. (Reference category: Women who did not consult a dentist, OR = 1) *(Continued)*

		Consulted a dentist, no white fillings placed OR (95% CI)	Consulted a dentist, white filling placed OR (95% CI)
Large for gestational age (LGA) 10 percentile			
Girls	Crude	0.96 (0.90–1.02)	0.99 (0.91–1.08)
	Adjusted	1.00 (0.93–1.07)	0.98 (0.90–1.08)
Boys	Crude	0.97 (0.90–1.04)	1.03 (0.94–1.13)
	Adjusted	1.01 (0.94–1.08)	1.01 (0.93–1.11)
All	Crude	0.96 (0.92–1.01)	1.01 (0.95–1.08)
	Adjusted	1.01 (0.96–1.06)	1.00 (0.94–1.06)
Large for gestational age (LGA) 2.5 percentile			
Girls	Crude	0.97 (0.87–1.09)	1.01 (0.87–1.17)
	Adjusted	1.02 (0.91–1.15)	0.99 (0.86–1.15)
Boys	Crude	0.95 (0.84–1.07)	1.06 (0.90–1.24)
	Adjusted	0.98 (0.87–1.11)	1.03 (0.88–1.20)
All	Crude	0.96 (0.88–1.04)	1.03 (0.93–1.15)
	Adjusted	1.00 (0.92–1.09)	1.01 (0.91–1.12)
Malformation			
Girls	Crude	1.02 (0.92–1.14)	0.99 (0.86–1.15)
	Adjusted	1.00 (0.90–1.12)	1.00 (0.86–1.15)
Boys	Crude	1.05 (0.95–1.15)	1.01 (0.89–1.15)
	Adjusted	1.03 (0.94–1.14)	1.00 (0.88–1.14)
All	Crude	1.03 (0.96–1.11)	1.00 (0.91–1.10)
	Adjusted	1.02 (0.95–1.09)	1.00 (0.91–1.10)
Stillbirth			
Girls	Crude	0.96 (0.59–1.55)	1.20 (0.67–2.15)
	Adjusted	0.92 (0.57–1.50)	1.16 (0.64-2.07)
Boys	Crude	0.97 (0.61–1.54)	1.30 (0.75–2.24)
	Adjusted	0.95 (0.60–1.51)	1.22 (0.70–2.11)
All	Crude	0.98 (0.71–1.37)	1.25 (0.84–1.86)
	Adjusted	0.96 (0.69–1.33)	1.18 (0.79–1.76)

The OR was adjusted for mothers age (≤19, 20–24, 25–29, 30–34, 35–39, 40+), parity (0, 1 or more previous viable pregnancies), education (≤ 12 years, 13–16 years, ≥ 17 years), pre-pregnancy body mass index (< 18.5, 18.5–24.9, 25.0–29.9, 30.0–34.9, 35.0–39.9, ≥ 40), smoking (never, occasionally, daily) and alcohol consumption during pregnancy (never, less than once a week, once a week, more than once a week). *$p < 0.05$

The participants answered questions regarding dental treatment during the first 30 weeks of pregnancy but were not asked to specify in which week of pregnancy they visited the dentist. Hence, a limitation is that we could not study if treatment with polymer-based filling materials could be a factor of importance at specific time windows during pregnancy. The severity of the effects of prenatal exposure to toxic agents appears to be influenced by the degree and timing of the exposure during gestation [41]. Some teratogens cause damage only during specific days or weeks early in pregnancy, when a particular part of the body is formed [41]. A well-known example is the thalidomide-tragedy in the late 1950s and the early 1960s, where the medication taken during days 20–36 after fertilization resulted in serious malformations of the foetus [42, 43].

Some women with the need for dental treatment do not seek or do not receive dental care during pregnancy [44]. This may, in part, be due to their concerns about the potential risk to the foetus, as well as dentists and other health care providers' attitudes and beliefs about the safety of dental treatment during pregnancy [44].

The findings from the present study, including more than 90,000 pregnancies, are reassuring. However, taken the limitations of a prospective cohort study into account, these findings could be corroborated in case

control studies. Thus, access to dental records and thereby accurate and detailed information regarding dental treatment could be possible to obtain.

Conclusion

In this study, women who had white fillings placed during pregnancy had no increased risk for adverse birth outcomes compared with women who did not consult a dentist during pregnancy. Thus, our findings do not support the hypothesis of an association between placement of polymer-based fillings during pregnancy and adverse birth outcomes.

Abbreviations

Bis-GMA: Bisphenol-A glycidyl dimethacrylate; BMI: Body mass index; BPA: Bisphenol A; LGA: Large for gestational age; MBRN: Medical Birth Registry of Norway; MoBa: the Norwegian Mother and Child Cohort Study; SGA: Small for gestational age; WHO: World Health Organization

Acknowledgements

The Norwegian Mother and Child Cohort Study is supported by the Norwegian Ministry of Health and Care Services and the Ministry of Education and Research, NIH/NINDS (grant no.1 UO1 NS 047537-01 and grant no.2 UO1 NS 047537-06A1). We are grateful to all the participating families in Norway who take part in this on-going cohort study.

Funding

The Norwegian Dental Biomaterials Adverse Reaction Unit and the Oral Health Centre of Expertise in Western Norway are funded by the Norwegian Ministry of Health and Care Services.

Availability of data and materials

Due to data protection regulations no data can be shared. Information about data access from the Norwegian Mother and Child Cohort Study is given by the Norwegian Institute of Public Health [45].

Authors' contributions

GBL and LB conceptualized the study, critically reviewed the results of the analyses, reviewed and revised the manuscript. TLLB carried out the analyses, drafted the initial manuscript and coordinated the editing. SAL critically reviewed the results of analyses, reviewed and revised the manuscript. All authors approved the final manuscript as submitted.

Ethics approval and consent to participate

The MoBa study was conducted according to the guidelines in the Declaration of Helsinki. The Regional Committee for Medical and Health Research Ethics in South-East Norway and the Norwegian Data Inspectorate approved the study. All MoBa participants provided written informed consent before enrolment into the study, permitting repeated assessment, the use and publication of data, and linkage to other health registries. The MoBa study protocol (revised October 2012) including patient consent form is available from Norwegian Institute of Public Health [46]. The present study was approved by the Regional Ethics Committee for medical research (REC South-East D, 2011/727).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Publisher's Note

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Received: 19 January 2018 Accepted: 13 August 2018
Published online: 22 August 2018

References

1. Alexander G, Hopcraft MS, Tyas MJ, Wong RH. Dentists' restorative decision-making and implications for an 'amalgamless' profession. Part 1: a review. Aust Dent J. 2014;59:408–19. https://doi.org/10.1111/adj.12209.
2. Eklund SA. Trends in dental treatment, 1992 to 2007. J Am Dent Assoc. 2010;141:391–9. https://www.ncbi.nlm.nih.gov/pubmed/20354088
3. SCENIHR (Scientific Committee on Emerging and Newly-Identified Health Risks). Opinion on the safety of dental amalgam and alternative dental restoration materials for patients and users (update), 29 April. European commission. DG Health and Food Safety. 2015:2015. http://ec.europa.eu/health/sites/health/files/scientific_committees/emerging/docs/scenihr_o_046.pdf. Accessed 14 Dec 2017
4. Kingman A, Hyman J, Masten SA, Jayaram B, Smith C, Eichmiller F, et al. Bisphenol A and other compounds in human saliva and urine associated with the placement of composite restorations. J Am Dent Assoc. 2012;143:1292–302. http://www.ncbi.nlm.nih.gov/pubmed/23204083
5. Michelsen VB, Kopperud HB, Lygre GB, Bjorkman L, Jensen E, Kleven IS, et al. Detection and quantification of monomers in unstimulated whole saliva after treatment with resin-based composite fillings in vivo. Eur J Oral Sci. 2012;120:89–95. https://doi.org/10.1111/j.1600-0722.2011.00897.x.
6. Sevkusic M, Schuster L, Rothmund L, Dettinger K, Maier M, Hickel R, et al. The elution and breakdown behavior of constituents from various light-cured composites. Dent Mater. 2014;30:619–31. https://doi.org/10.1016/j.dental.2014.02.022.
7. Maserejian NN, Trachtenberg FL, Wheaton OB, Calafat AM, Ranganathan G, Kim HY, et al. Changes in urinary bisphenol a concentrations associated with placement of dental composite restorations in children and adolescents. J Am Dent Assoc. 2016; https://doi.org/10.1016/j.adaj.2016.02.020.
8. Van Landuyt KL, Nawrot T, Geebelen B, De Munck J, Snauwaert J, Yoshihara K, et al. How much do resin-based dental materials release? A meta-analytical approach. Dent Mater. 2011;27:723–47. https://doi.org/10.1016/j.dental.2011.05.001.
9. Durner J, Obermaier J, Draenert M, Ilie N. Correlation of the degree of conversion with the amount of elutable substances in nano-hybrid dental composites. Dent Mater. 2012;28:1146–53. https://doi.org/10.1016/j.dental.2012.08.006.
10. Ferracane JL. Elution of leachable components from composites. J Oral Rehabil. 1994;21:441–52. https://www.ncbi.nlm.nih.gov/pubmed/7965355
11. American Dental Association Council on Scientific Affairs. Determination of bisphenol a released from resin-based composite dental restoratives. American Dental Association Professional Product Review. 2014;9(3). http://www.ada.org/en/publications/ada-professional-product-review-ppr. Accessed 5 Sept 2014.
12. Schmalz G, Preiss A, Arenholt-Bindslev D. Bisphenol-A content of resin monomers and related degradation products. Clin Oral Investig. 1999;3:114–9. https://www.ncbi.nlm.nih.gov/pubmed/10803121
13. Schedle A, Ortengren U, Eidler N, Gabauer M, Hensten A. Do adverse effects of dental materials exist? What are the consequences, and how can they be diagnosed and treated? Clin Oral Implants Res. 2007;18(Suppl 3):232–56. https://doi.org/10.1111/j.1600-0501.2007.01481.x.
14. Bakopoulou A, Papadopoulos T, Garefis P. Molecular toxicology of substances released from resin-based dental restorative materials. Int J Mol Sci. 2009;10:3861–99. https://doi.org/10.3390/ijms10093861.
15. Rochester JR. Bisphenol a and human health: a review of the literature. Reprod Toxicol. 2013;42:132–55. https://doi.org/10.1016/j.reprotox.2013.08.008.
16. Fleisch AF, Sheffield PE, Chinn C, Edelstein BL, Landrigan PJ. Bisphenol a and related compounds in dental materials. Pediatrics. 2010;126:760–8. https://doi.org/10.1542/peds.2009-2693.

17. Schug TT, Janesick A, Blumberg B, Heindel JJ. Endocrine disrupting chemicals and disease susceptibility. J Steroid Biochem Mol Biol. 2011;127:204–15. https://doi.org/10.1016/j.jsbmb.2011.08.007.
18. Crain DA, Eriksen M, Iguchi T, Jobling S, Laufer H, LeBlanc GA, et al. An ecological assessment of bisphenol-a: evidence from comparative biology. Reprod Toxicol. 2007;24:225–39. https://doi.org/10.1016/j.reprotox.2007.05.008.
19. Soderholm KJ, Mariotti A. BIS-GMA--based resins in dentistry: are they safe? J Am Dent Assoc. 1999;130:201–9. https://www.ncbi.nlm.nih.gov/pubmed/10036843
20. Olea N, Pulgar R, Perez P, Olea-Serrano F, Rivas A, Novillo-Fertrell A, et al. Estrogenicity of resin-based composites and sealants used in dentistry. Environ Health Perspect. 1996;104:298–305. http://www.ncbi.nlm.nih.gov/pubmed/8919768
21. Imai Y, Watanabe M, Ohsaki A. Analysis of major components and bisphenol a in commercial Bis-GMA and Bis-GMA-based resins using high performance liquid chromatography. Dent Mater J. 2000;19:263–9. https://www.ncbi.nlm.nih.gov/pubmed/11218846
22. Atkinson JC, Diamond F, Eichmiller F, Selwitz R, Jones G. Stability of bisphenol a, triethylene-glycol dimethacrylate, and bisphenol a dimethacrylate in whole saliva. Dent Mater. 2002;18:128–35. http://www.ncbi.nlm.nih.gov/pubmed/11755591
23. Vandenberg LN, Colborn T, Hayes TB, Heindel JJ, Jacobs DR Jr, Lee DH, et al. Hormones and endocrine-disrupting chemicals: low-dose effects and nonmonotonic dose responses. Endocr Rev. 2012;33:378–455. https://doi.org/10.1210/er.2011-1050.
24. Richter CA, Birnbaum LS, Farabollini F, Newbold RR, Rubin BS, Talsness CE, et al. In vivo effects of bisphenol a in laboratory rodent studies. Reprod Toxicol. 2007;24:199–224. https://doi.org/10.1016/j.reprotox.2007.06.004.
25. Miyakoda HT, Masako T, Onodera SO, Takeda K. Passage of bisphenol a into the fetus of pregnant rat. J Health Sci. 1999;45:318–23.
26. Magnus P, Irgens LM, Haug K, Nystad W, Skjaerven R, Stoltenberg C, et al. Cohort profile: the Norwegian mother and child cohort study (MoBa). Int J Epidemiol. 2006;35:1146–50. https://doi.org/10.1093/ije/dyl170.
27. Magnus P, Birke C, Vejrup K, Haugan A, Alsaker E, Daltveit AK, et al. Cohort profile update: the Norwegian mother and child cohort study (MoBa). Int J Epidemiol. 2016; https://doi.org/10.1093/ije/dyw029.
28. Norwegian Institute of Public Health. Questionnaires from MoBa. 2005 [updated 15 September 2016]. https://www.fhi.no/en/studies/moba/for-forskere-artikler/questionnaires-from-moba/. Accessed 15 Dec 2017.
29. Irgens LM. The medical birth registry of Norway. Epidemiological research and surveillance throughout 30 years. Acta Obstet Gynecol Scand. 2000;79:435–9. https://www.ncbi.nlm.nih.gov/pubmed/10857866
30. Fleischman AR, Oinuma M, Clark SL. Rethinking the definition of "term pregnancy". Obstet Gynecol. 2010;116:136–9. https://doi.org/10.1097/AOG.0b013e3181e24f28.
31. Blencowe H, Cousens S, Oestergaard MZ, Chou D, Moller AB, Narwal R, et al. National, regional, and worldwide estimates of preterm birth rates in the year 2010 with time trends since 1990 for selected countries: a systematic analysis and implications. Lancet. 2012;379:2162–72. https://doi.org/10.1016/S0140-6736(12)60820-4.
32. World Health Organization. WHO: recommended definitions, terminology and format for statistical tables related to the perinatal period and use of a new certificate for cause of perinatal deaths. Modifications recommended by FIGO as amended October 14, 1976. Acta Obstet Gynecol Scand. 1977;56:247–53. https://www.ncbi.nlm.nih.gov/pubmed/560099
33. World Health Organization. Global Database on Body Mass Index. BMI classification 2007. 2007. http://www.who.int/. Accessed 22.11.2017.
34. Skjaerven R, Gjessing HK, Bakketeig LS. Birthweight by gestational age in Norway. Acta Obstet Gynecol Scand. 2000;79:440–9. https://www.ncbi.nlm.nih.gov/pubmed/10857867
35. Michalowicz BS, DiAngelis AJ, Novak MJ, Buchanan W, Papapanou PN, Mitchell DA, et al. Examining the safety of dental treatment in pregnant women. J Am Dent Assoc. 2008;139:685–95. https://www.ncbi.nlm.nih.gov/pubmed/18519992
36. Nilsen RM, Vollset SE, Gjessing HK, Skjaerven R, Melve KK, Schreuder P, et al. Self-selection and bias in a large prospective pregnancy cohort in Norway. Paediatr Perinat Epidemiol. 2009;23:597–608. https://doi.org/10.1111/j.1365-3016.2009.01062.x.
37. Berge TL, Lygre GB, Jonsson BA, Lindh CH, Bjorkman L. Bisphenol a concentration in human saliva related to dental polymer-based fillings. Clin Oral Investig. 2017; https://doi.org/10.1007/s00784-017-2055-9.
38. Kopperud SE, Tveit AB, Opdam NJ, Espelid I. Occlusal caries management: preferences among dentists in Norway. Caries Res. 2016;50:40–7. https://doi.org/10.1159/000442796.
39. Vidnes-Kopperud S, Tveit AB, Espelid I. Changes in the treatment concept for approximal caries from 1983 to 2009 in Norway. Caries Res. 2011;45:113–20. https://doi.org/10.1159/000324810.
40. Wang NJ. Tannhelseutvikling og bruk av tannrestaureringsmaterialer. In: Bruk av tannrestaureringsmaterialer i Norge IK-2652. 98–8. Oslo: Statens helsetilsyn; 1998. p. 69–74.
41. Selevan SG, Kimmel CA, Mendola P. Identifying critical windows of exposure for children's health. Environ Health Perspect. 2000;108(Suppl 3):451–5. https://www.ncbi.nlm.nih.gov/pubmed/10852844
42. Miller MT, Ventura L, Stromland K. Thalidomide and misoprostol: ophthalmologic manifestations and associations both expected and unexpected. Birth Defects Res A Clin Mol Teratol. 2009;85:667–76. https://doi.org/10.1002/bdra.20609.
43. Rogers JM, Kavlok RJ. Developmental toxicology. In: Klaassen CD, editor. Casarett and Doull's toxicology: the basic science of poisons. 7th ed. New York: McGraw-Hill; 2008. p. 417–8.
44. Gaffield ML, Gilbert BJ, Malvitz DM, Romaguera R. Oral health during pregnancy: An analysis of information collected by the pregnancy risk assessment monitoring system. J Am Dent Assoc. 2001;132:1009–16. https://www.ncbi.nlm.nih.gov/pubmed/11480627
45. Norwegian Institute of Public Health. Research and data access from the Norwegian Mother and Child Cohort Study. 2010 [updated 11 January 2018]. https://www.fhi.no/div/datatilgang/. Accessed 19 Jan 2018.
46. Norwegian Institute of Public Health. MoBa study protocol. 2010 [updated October 2012]. https://www.fhi.no/globalassets/dokumenterfiler/moba/dokumenter/moba-protokoll-norsk-versjon-oktober-2012-pdf.pdf. Accessed 19 Jan 2018.

Appendix I

Paper I - Questionnaire

Spørreskjema (inkluderte pasienter)
(rev 11.07.13)

Dato for utfylling:.....

Initialer/journalnr. i prosjekt:.....

Kjønn: Kvinne ❑ Mann ❑ Fødselsår: .....

Sivilstand:

Utdannelse:

Yrke/arbeidssituasjon: .....

Kassajobb: Ja ❑ Nei ❑

Sykdommer:

Medikamenter/Naturpreparater: Ja ❑ Nei ❑

Navn på preparat(er):

Allergier:

Nytelsesmidler/alkoholvaner:
Tobakk Ja ❑ Nei ❑ Mengde..... Snus ❑
Alkohol ❑
Andre ❑

Tyggegummi daglig: Ja ❑ Nei ❑

Tannkrem daglig (navn): .....

Munnskyllemiddel (hyppighet/navn): daglig ❑ ukentlig ❑ månedlig ❑

Inntak av hermetiske fødeemner (siste uken): Ja ❑ Nei ❑

Hvilke (angi navn):

Har du spist frokost i dag? Ja ❑ Nei ❑

Gi en mest mulig detaljert liste over hva du inntok:

II

Appendix II

Paper I - Supplemental material

Analysis of bisphenol A in saliva

Bisphenol A (BPA) and D_{16}-BPA, were obtained from Sigma-Aldrich Inc. (St. Louis, MO, USA). Acetonitrile, ammonium acetate and methanol were from Merck (Darmstadt, Germany). Water was from a Milli-Q Integral 5 system (Millipore, Billerica, MA, USA). β-Glucuronidase (Escherichia coli K12) was obtained from Roche Diagnostics with a specific activity of ~80 units/mg protein (Glucuronidase at 25°C, or 140 U/mg at 37 °C, at pH 7 with 4-nitrophenyl-β-D-glucuronide as substrate. (Mannheim, Germany).

Stock solutions were prepared by dissolving accurately weighed amounts of BPA in methanol. Standard solutions were prepared by further dilution of the stock solutions in methanol. Serum was used for the calibration standards and for quality control (QC) samples and was obtained from healthy volunteers at our laboratory. The levels were quantified and samples with low amount of BPA were selected for the calibration standards. Calibration standards were prepared by adding 25 µl standard solution to serum. Two quality control serum samples were prepared by additions of small amounts of BPA into pooled samples.

The samples were prepared in 96-well plates with 2 ml flat bottom glass vials (Biotech solutions, Vineland, NJ, USA). For the analysis of total BPA in the samples, aliquots of 100 µl saliva were added with 10 µl glucuronidase, 10 µl 1M ammonium acetate buffer at pH 6.5. The samples were digested at 37°C for 90 min. Then 25 µl of methanol containing D_{16}-BPA as internal standard and an additional 25 µl methanol was added, thereafter the proteins were precipitated with 200 µl acetonitrile followed by vigorous shaking for 30 min. For the calibration standards, aliquots of 100 µl serum were used and treated as above, but standards were added 25 µl methanol. The samples were thereafter centrifuged at 2600g for 10 min. The supernatant (0.2 ml) was transferred to a new 96-well plate with 0.5 ml conical glass vials (MicroLiter Analytical Supplies, Inc., Suwanee, GA, USA) for analysis. Samples analyzed for free

BPA in saliva was determined as above but digestion using glucuronidase was omitted. Concentration of conjugated BPA was estimated by the difference between total and free BPA.

Quantitative analysis was conducted using a triple quadrupole linear ion trap mass spectrometry (QTRAP 5500; AB Sciex, Foster City, CA, USA) coupled to a liquid chromatography system (UFLCXR, Shimadzu Corporation, Kyoto, Japan; LC/MS/MS). Air was used as nebulizer and auxiliary gas. Pure nitrogen was used as curtain gas and collision gas. The temperature of the auxiliary gas was set at 630 °C and the ion spray voltage was -4500 V. The MS analyzes were carried out using selected reaction monitoring (SRM) in negative ion mode. BPA was analyzed using the transitions m/z 227-212 as quantifier ion, m/z 227-133 as qualifier ion and m/z 241-142 was used for the internal standard.

For the analysis of BPA a C18 column (2.1 mm i.d. x 50 mm, Genesis Lightn; Grace, Deerfield, IL, USA) was used prior to the injector to filter the mobile phases from contaminating BPA. Aliquots of 3 µl of the samples were injected on a C18 column (1.5 µm, 2.0 mm i.d. x 100 mm VisionHT; Grace, Deerfield, IL, USA). The mobile phases were A: water and B: methanol. The mobile phase was kept at 15% B for 1 min after injection. A gradient was then applied in 4 min to 95% B where it was kept for 2.6 min. The column was then conditioned at 15% B for 2 min. A diverter valve was used and the column effluent was diverted to the MS between 6.0 and 7.5 min. The flow rate was 0.25 ml/min and the column was maintained at 60 °C.

Concentrations were determined by peak area ratios between the analytes and the IS. The levels of BPA in the pooled serum used for preparation of standards were quantified in each batch and the calibration standards were corrected for the concentration found in this pooled sample. Also, all values were corrected for the mean of chemical blanks, run within each batch.

The LOD was calculated as three times the standard deviation of the ratio between the peak area at the analyte retention time and the peak area of IS, divided by the slope of the calibration line. The LOD was determined to 0.1 ng/ml.

In all analytical batches there were two different in-house prepared quality control (QC) samples and chemical blanks analyzed. The samples were prepared and analyzed in duplicates and the mean of the two concentrations were used.
Between-day precision was estimated from the in house prepared QC samples analyzed several times within an analysis batch data from a different project where 76 batches were analyzed are included. The CV of the two quality control sample was at 2.6 ng/ml 14% and at LOD at 0.1ng/ml 42%.
The within run precision were determined in spiked serum samples (n=10) at three different levels. The CVs were at 13 ng/ml 2.8%, at 7 ng/ml 3.4% and at 2 ng/ml 4%.
The laboratory in Lund performing the analyzes is a European reference laboratory for BPA in urine (www.eu-hbm.info/democophes) and a reference laboratory for BPA in urine in the Erlangen Round Robin inter-laboratory control program.

Appendix III

Paper II - Questionnaires 1-3

III

Spørreskjema 1

«Konsentrasjon av bisfenol A i saliva og urin etter fyllingsterapi med plastbaserte tannmaterialer»

Dato for utfylling:…… Initialer/journalnr. i prosjekt:……

Fødselsår: …… Alder:……..

Kjønn: Kvinne ❑ Mann ❑

Utdannelse: Yrke/arbeidssituasjon: ❑…….

Sykdommer: Ingen ❑ Hvilke: ……..

Allergier: Ingen ❑ Hvilke:……...

Medikamenter/Naturpreparater: Ingen: ❑ Hvilke:……

Kassajobb: Nei ❑ Ja ❑ Daglig ❑ Ukentlig ❑ Månedlig ❑

Nytelsesmidler/alkoholvaner:

Røyk	Nei ❑	Daglig ❑	Ukentlig ❑	Månedlig❑
Snus	Nei ❑	Daglig ❑	Ukentlig ❑	Månedlig❑
Alkohol	Nei ❑	Daglig ❑	Ukentlig ❑	Månedlig❑
Andre	Nei ❑	Daglig ❑	Ukentlig ❑	Månedlig❑

Bruk av tyggegummi daglig: Nei ❑ Ja❑

Bruk av tannkrem daglig: Nei ❑ Ja ❑ Hvilken:……
Benyttet i dag: Nei ❑ Ja❑

Munnskyllemiddel: Nei ❑ Ja ❑ Hvilket:…….....
Daglig ❑ Ukentlig ❑ Månedlig ❑
Benyttet i dag: Nei ❑ Ja❑

Inntak av hermetiske fødeemner:

Siste 24 timer: Nei ❑ Ja ❑ Hvilke: ……..

Siste uken: Nei ❑ Ja ❑ Hvilke: ……

Inntak mikrobølgevarmet mat, varmet i plastemballasje:

Siste 24 timer: Nei ❑ Ja ❑ Spesifiser……..

Siste uken: Nei ❑ Ja ❑ Spesifiser…….

Har du spist frokost i dag? Nei ❑ Ja ❑ Hvilke fødeemner: ……

Spørreskjema 2

«Konsentrasjon av bisfenol A i saliva og urin etter fyllingsterapi med plastbaserte tannmaterialer»

Dato for utfylling:…… Initialer/journalnr. i prosjekt:……

Inntak av hermetiske fødeemner:

Siste 24 timer: Nei ❑ Ja ❑ Hvilke:…….. ……

Inntak mikrobølgevarmet mat, varmet i plastemballasje:

Siste 24 timer: Nei ❑ Ja ❑ Spesifiser: ……...

Har du pusset tenner i dag? Nei ❑ Ja ❑ Hvilken tannkrem……

Har du brukt munnskyllemiddel i dag? Nei ❑ Ja ❑ Hvilket middel ……

Har du spist frokost i dag? Nei ❑ Ja ❑ Hvilke fødeemner: ……

Spørreskjema 3

«Konsentrasjon av bisfenol A i saliva og urin etter fyllingsterapi med plastbaserte tannmaterialer»

Dato for utfylling:…… Initialer/journalnr. i prosjekt:……

Inntak av hermetiske fødeemner:

Siste 24 timer: Nei ❑ Ja ❑ Hvilke:……..

Siste uken: Nei ❑ Ja ❑ Hvilke: ……

Inntak mikrobølgevarmet mat, varmet i plastemballasje:

Siste 24 timer: Nei ❑ Ja ❑ Spesifiser:……

Siste uken: Nei ❑ Ja ❑ Spesifiser:…… ……...

Har du pusset tenner i dag? Nei ❑ Ja ❑ Hvilken tannkrem……

Har du brukt munnskyllemiddel i dag? Nei ❑ Ja ❑ Hvilket middel ……

Har du spist frokost i dag? Nei ❑ Ja ❑ Hvilke fødeemner: ……

Appendix IV

Paper II - Supplemental material

IV

Analysis of bisphenol A

Bisphenol A (BPA) and D_{16}-BPA were obtained from Sigma-Aldrich Inc. (St. Louis, Missouri, USA). Acetonitrile (AcN) and ammonium acetate were from Merck (Darmstadt, Germany). Water was from a Milli-Q Integral 5 system (Millipore, Billerica, Massachusetts, USA). Serum (Fetal Bovine Serum) was from Gibco Thermo Fisher Scientific (Waltham, Massachusetts, USA). β-Glucuronidase (Escherichia coli K12) was obtained from Roche Diagnostics (Mannheim, Germany).

Preparation of standard samples and control samples (quality control)

Stock solutions were prepared by dissolving accurately weighed amounts of BPA in 50% AcN. Standard solutions were prepared by further dilution of the stock solutions in 50% AcN. Serum was used for the calibration standards for the saliva analysis and urine (obtained from healthy volunteers at our laboratory) for the urine analysis Calibration standards were prepared by adding 25 µl standard solution each to serum and urine. Two control samples were used for quality control (QC). Samples were prepared by additions of small amounts of BPA into pooled urine samples.

Preparation of saliva samples

The samples were prepared in 96-well plates with 2-ml flat bottom glass vials (Biotech solutions, Vineland, New Jersey, USA). For the analysis of total BPA in the samples, aliquots of 100 µl of saliva were added with 10 µl glucuronidase and 10 µl of 1M ammonium acetate buffer at pH 6.5. The samples were digested at 37°C for 90 min. Then, 25 µl of 50% AcN containing D_{16}-BPA as the internal standard and an additional 25 µl of 50% AcN was added. Thereafter, the proteins were precipitated with 200 µl acetonitrile followed by vigorous shaking for 30 min. For the calibration standards, aliquots of 100 µl of serum were used and treated as above, but standards were added in 25 µl of AcN. The samples were thereafter centrifuged at 2600 ×g for 10 min. The supernatant (0.2 ml) was transferred to a new 96-well plate with 0.5-ml

conical glass vials (MicroLiter Analytical Supplies, Inc., Suwanee, Georgia, USA) for analysis and centrifuged again at 3000 ×g for 10 min before analysis.

Preparation of urine samples

The samples were prepared in 96-well plates with 1-ml glass inserts (1-ml SQW Micro-Inserts, La-Pha-Pack, Langerwehe, Germany). For the analysis of total BPA in the samples, aliquots of 200 µl of urine were added with 10 µl of glucuronidase and 100 µl of 1M ammonium acetate buffer at pH 6.5. The samples were digested at 37°C for 30 min. Then, 25 µl of 50% AcN containing D_{16}-BPA as the internal standard and an additional 25 µl of 50% AcN was added. For the calibration standards, aliquots of 200 µl of urine were used and treated as above, but standards were added in 25 µl of 50% AcN. The samples were thereafter centrifuged at 3000 ×g for 10 min.

Samples analyzed for free BPA in saliva were determined as above but digestion using glucuronidase was omitted. The concentration of conjugated BPA was estimated by the difference between total and free BPA.

Quantitative analysis

Quantitative analysis was conducted using triple quadrupole linear ion trap mass spectrometry (QTRAP 5500; AB Sciex, Foster City, California, USA) coupled to a liquid chromatography system (UFLCXR, Shimadzu Corporation, Kyoto, Japan; LC/MS/MS). Pure Nitrogen was used as the nebulizer, auxiliary gas, curtain gas, and collision gas. The temperature of the auxiliary gas was set at 630°C and the ion spray voltage was −4500 V. The MS analyses were carried out using selected reaction monitoring (SRM) in negative ion mode. BPA was analyzed using the transitions m/z 227-212 as the quantifier ion, m/z 227-133 as the qualifier ion, and m/z 241-142 was used for the internal standard.

In the LC system, a C18 column (4 µm, 2.1-mm i.d. x 50-mm, Genesis Lightning; Grace, Hichrom, Reading, United Kingdom) was used prior to the injector to filter the mobile phases from contaminating BPA. Aliquots of 5 µl of the samples were injected on a C18 column (same as above). The mobile phases were A: water and B: methanol, both containing 0.08% NH3. The mobile phase was maintained at 5% B for 0.2 min

after injection. A gradient was then applied in 7 min to 70% B and another 0.5-min increase of B to 95%. The column was then conditioned at 5% B for 1.5 min. A diverter valve was used and the column effluent was diverted to the MS between 4 and 7 min. The flow rate was 0.6 ml/min and the column was maintained at 45°C. Concentrations were determined by peak area ratios between the analytes and the internal standard (IS). In addition, all values were corrected for the mean of the chemical blanks, which were run within each batch.

The limit of detection (LOD) was calculated as three times the standard deviation of the ratio between the peak area in the chemical blank samples at the analyte retention time and the peak area of IS, divided by the slope of the calibration line. The LOD was determined to be 0.1 ng/ml for both saliva/serum and urine analysis.

In all analytical batches there were two different in-house prepared QC samples and chemical blanks analyzed. The samples were prepared and analyzed in duplicates and the mean of the two concentrations was used.

Between-run precision was estimated from the above QC samples analyzed in 76 batches of urine samples. The CV of the two QC samples was at 2.6 ng/ml 14% and at LOD at 0.1 ng/ml 42%.

The within-run precision was determined in spiked serum samples (n=10) at three levels. The CVs were at 13 ng/ml 2.8%, at 7 ng/ml 3.4% and at 2 ng/ml 4%. The laboratory in Lund performing the analyses is a European reference laboratory for BPA in urine (www.eu-hbm.info/democophes) and a reference laboratory for BPA in urine in the Erlangen Round Robin inter-laboratory control program.

Qualitative analysis

Because, in the HPLC-MSMS run of the saliva samples, there was a small shift in the retention time between the BPA peak in the standard samples and the BPA peak in the saliva samples, a more thorough investigation was made to be able to confirm that the peaks in the saliva samples originate from BPA. The samples were also analyzed on a quadropol time of flight mass spectrometer (QTOF; Triple TOF 5600; AB Sciex,

Foster City, CA, USA) coupled to a liquid chromatography system (UFLCXR, Shimadzu Corporation, Kyoto, Japan).

The columns and mobile phases including the settings were the same as for quantitative analysis. The mobile phase was kept at 5% B for 0.5 min after injection. A gradient was then applied in 3.5 min to 95% B and was maintained for 1 min. The column was then conditioned at 5% B for 1.5 min. The column effluent was diverted to the MS between 3.0 and 4.7 min.

The temperature of the auxiliary gas on the MS was 600°C and the ion spray voltage was −4500 V. The MS analysis was carried out using product ion scan in negative ion mode. For accurate mass determination, BPA was analyzed using the precursor ions m/z 227.1 for BPA, and m/z 241.1 was used for the BPA-D_{16} internal standard.

The QTOF spectra of the chromatographic BPA peak from the standard were compared with the QTOF spectra of the chromatographic BPA peak from the saliva samples. The spectra found in the standard sample were comparable to the peak found in saliva, thus suggesting that the peak in the saliva samples is an isomer of BPA and this causes a small shift in the retention time.

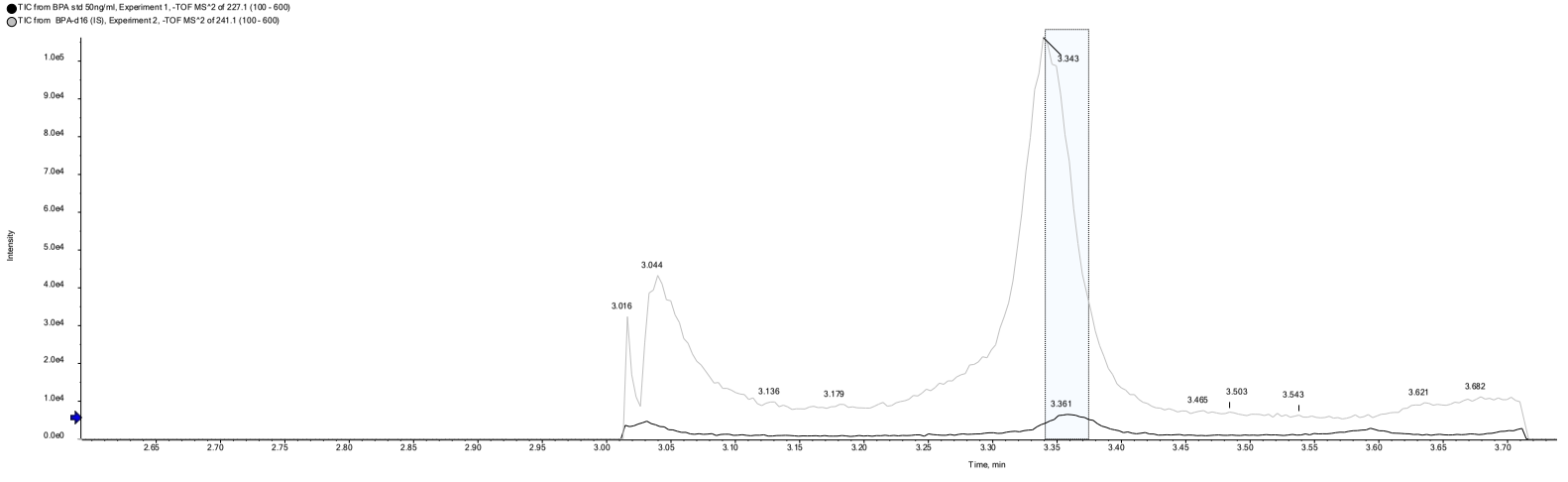


Fig. S1. Total ion chromatogram for the BPA peak from the standard sample of 50 ng/ml (retention times: BPA, 3.36 min (black); BPA-D16, 3.34 min (gray)).

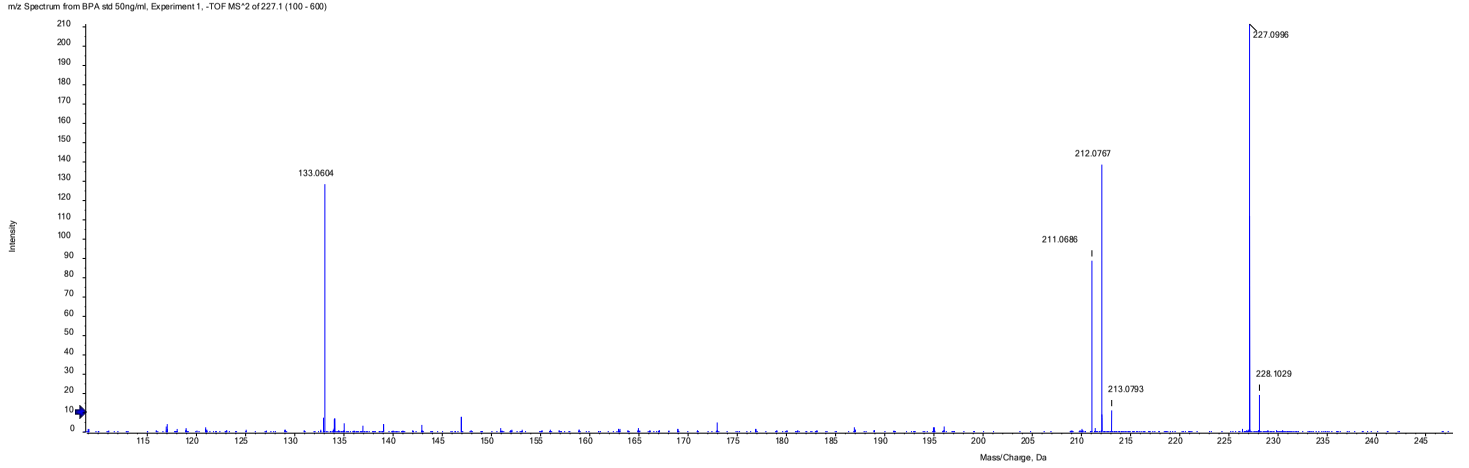


Fig. S2. Quadropol time of flight mass spectra for the BPA peak from the standard sample of 50 ng/ml (retention time: 3.36 min).

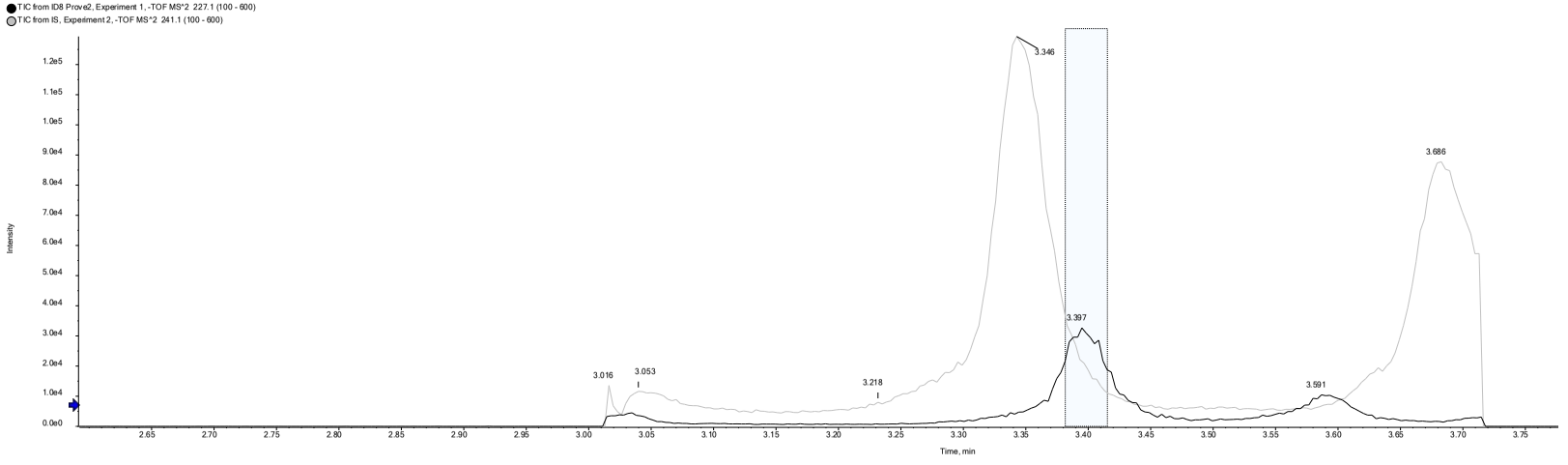


Fig. S3. Total ion chromatogram for the BPA peak from a saliva sample with a concentration of 800 ng BPA/ml (retention time: BPA, 3.40 min (black); BPA-D16, 3.34 min (gray)).

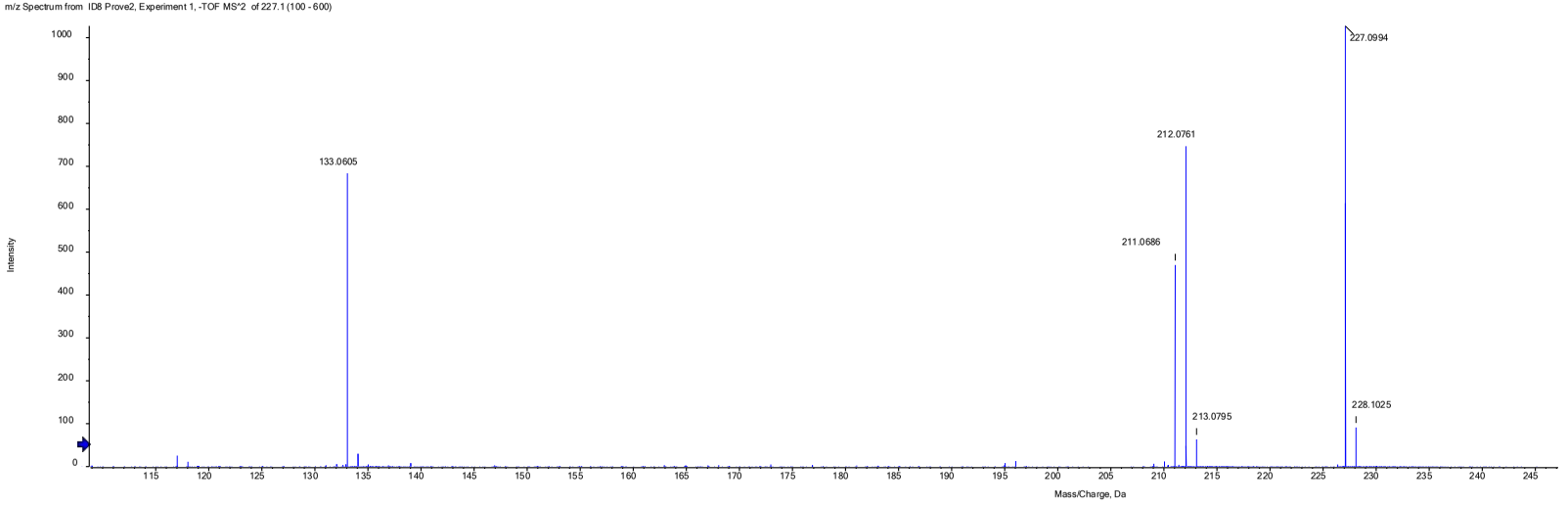
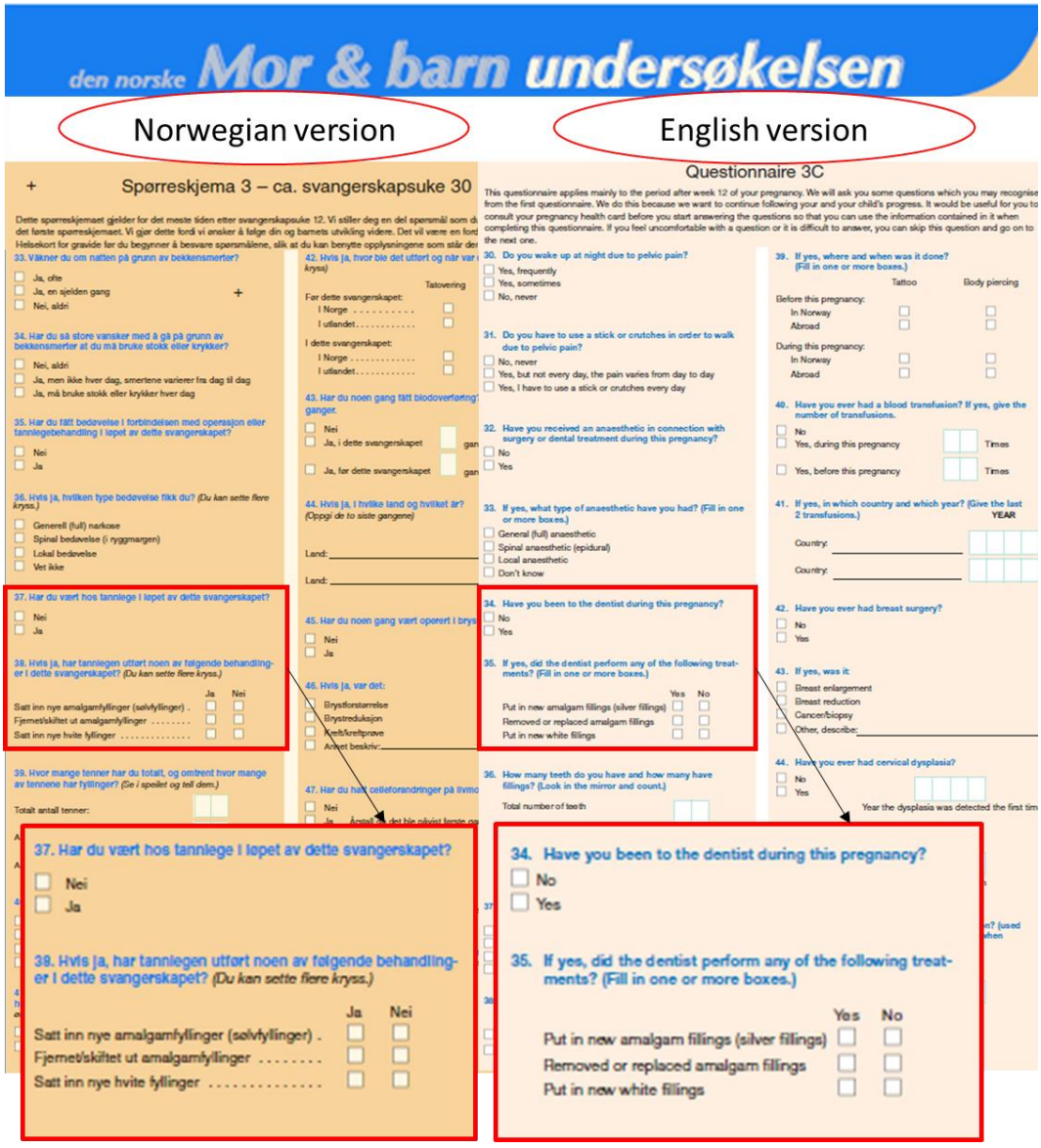


Fig. S4. Quadropol time of flight mass spectra for the BPA peak from the saliva sample shown in Figure 3 (retention time: 3.40 min).

Appendix V

Paper III - MoBa Questionnaire 3 (Q3)

V


den norske Mor & barn undersøkelsen
Norwegian version
English version
Spørreskjema 3 – ca. svangerskapsuke 30
Questionnaire 3C
37. Har du vært hos tannlege i løpet av dette svangerskapet?
Nei
Ja
38. Hvis ja, har tannlegen utført noen av følgende behandlinger i dette svangerskapet? (Du kan sette flere kryss.)
Ja
Nei
Satt inn nye amalgamfyllinger (sølvfyllinger) .
Fjernet/skiftet ut amalgamfyllinger .....
Satt inn nye hvite fyllinger .....
34. Have you been to the dentist during this pregnancy?
No
Yes
35. If yes, did the dentist perform any of the following treatments? (Fill in one or more boxes.)
Yes
No
Put in new amalgam fillings (silver fillings)
Removed or replaced amalgam fillings
Put in new white fillings

Graphic design: Communication Division, UiB / Print: Skipnes Kommunikasjon AS

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ISBN: 9788230851593 (print)
9788230861974 (PDF)