Airborne exposure to gaseous and particle-associated organic substances in resin-based dental materials during restorative procedures

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Dental composite dust has been shown to act as a vehicle for methacrylates in vivo/in vitro. The objective of this study was to assess airborne exposure of dental personnel to gaseous and particle-associated organic constituents from resin-based dental materials in a simulated clinic. Sampling of total aerosol fractions and gaseous substances was performed by dental students carrying particle filters and gas sorbents attached to a personal pump during preclinical restorative procedures in phantom models ($n = 13$). Water from the phantoms was sampled. Organic substances were extracted from the sampled water, particle filters, and gas sorbents. Qualitative and quantitative analyses were performed by gas chromatography-mass spectrometry (GC-MS) and ultra-high-performance liquid chromatography-mass spectrometry (UHPLC-MS). The methacrylates 2-hydroxyethyl methacrylate (HEMA) and triethylene glycol dimethacrylate (TEGDMA) and the additives camphorquinone (CQ), butylated hydroxytoluene (BHT), and ethyl 4-(dimethylamino) benzoate (DMABEE), were quantified in the gas and particle fractions sampled. A positive-control experiment was conducted. No methacrylates were detected in the gas or particle fractions sampled, whereas strong signals for methacrylates were detected in the positive controls, matching the analysis of the uncured material. In addition, TEGDMA and DMABEE were quantified in the sampled water. Airborne exposure to constituents in resin-based dental materials was below the detection limit. However, the extent of exposure is probably dependent on the procedure, preventive measures, and type of materials used.

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Dental professionals (e.g. dental hygienists, dentists, and dental assistants) work in an environment in which a range of sensitizing and reactive substances are handled. Among the most common substances relevant for exposure and adverse effects for both patients and dental personnel are methacrylates, the main matrix constituents of resin-based dental materials (1, 2). For patients, relatively few adverse effects have been reported for these materials. This is probably related to the low dose and infrequent exposure to substances from cured resin-based materials. However, for dental personnel, the handling of resin-based dental materials has been associated with more severe and frequent cases of allergic contact dermatitis and airway-related diseases, that is, respiratory hypersensitivity, than is observed in patients (3–7). Furthermore, a recently published report indicated that dentists might be at higher risk than the general population of developing the life-threatening condition idiopathic pulmonary fibrosis (IPF) (8). While the etiology of IPF has not been verified, airborne exposure to chemicals and particulate matter in an occupational setting is thought to have a key role.

In the dental office, airborne exposure to methacrylates may occur through the inhalation of volatile elements (9–11). For example, semi-volatile and volatile methacrylates, such as 2-hydroxyethyl methacrylate (HEMA), methyl methacrylate (MMA), and triethylene glycol dimethacrylate (TEGDMA), have been measured to reach maximum concentrations of 79, 15, and 54 $\mu g\ m^{-3}$, respectively, depending on the clinical procedure monitored (9, 11). Additionally, airborne exposure to substances present in resin-based dental materials may occur through the inhalation of unreacted, particle-associated methacrylates and additives; that is, respirable dust from dental composite acts as a

vehicle for transferring unreacted constituents of resin-based composites into the lungs (12). Inhalable dust (aerodynamic diameter of ≤ 100 μm) is generated during dental procedures. It has also been shown that part of the dust generated is in the respirable size fraction (aerodynamic diameter of $<$ ~10 μm), thus able to penetrate into the deep inner gas-exchange areas of the lung. Inhaled dust may thus be an important source of methacrylate exposure (13–17). Yet, this exposure modality has only been investigated and documented to occur in laboratory studies (12).

In previous clinical investigations on exposure to gaseous, airborne methacrylate, the material-specific contribution to the methacrylates sampled was only partly addressed; that is, the content or the materials used by the clinicians were not disclosed (9–11). Furthermore, in laboratory studies on particle-associated exposure, water cooling and/or high-vacuum suction were not used during sampling. This implies that more clinically relevant data on this matter are needed. In the present study, gaseous and particle-associated exposure to organic substances in chemically well-characterized resin-based materials was investigated during restorative procedures in which water cooling and high-vacuum suction were available.

The primary objective of this study was to provide data on the total occupational airborne exposure to gaseous and particle-associated content of resin-based dental materials during restorative procedures in a simulated clinical environment. Our null hypothesis was that airborne methacrylates are not detectable during restorative procedures in which water cooling and high-vacuum suction are available.

Material and methods

Chemicals and dental materials

Analytical grade solvents and standards were obtained from Sigma–Aldrich (Oslo, Norway) (Table 1). Dental materials used in the simulation clinic were bought from Plandent (Oslo, Norway).

Personal air samplers

Volunteers for the study were fifth-semester dental students, each of whom carried personal sampling pumps (SKC Sidekick, Pittsburgh, PA, USA) coupled to air samplers placed in their breathing zone during routine, restorative procedures that were part of their preclinical training (Fig. 1). In total, 13 restorative procedures were monitored. The samplers used were polytetrafluoroethylene (PTFE) filters, pore size 1.0 μm (Millipore Billerica, Burlington, MA, USA), placed in filter cassettes, and sorbent tubes containing XAD-7 resins (Cat. No. 226-95; SKC, Pittsburgh, PA, USA). The producer of gas sorbents, SKC, was asked to find the most suitable sorbents for our analytes. Filter cassettes made of conductive black polypropylene (Cat. No. 225-309; SKC) were used to minimize sample loss from electrostatic effects. The filter cassettes were taped across the seals/joins (Leukoflex; BNSmedical, Hamburg, Germany), to prevent contamination of the filter. A pump flow-rate of 2.2 l min^{-1} was used to sample total aerosol particles. XAD-7 sorbents with a pump flow-rate of 0.3 or 0.7 l min^{-1} were used for sampling gaseous organic substances. The airflows of the pumps were adjusted prior to the experiment and assessed at the end. A plastic cyclone (with a 37 mm cassette) for sampling respirable particles (Cat. No. 225-69-37; SKC) was used during initial pilot studies and for the positive-control experiments. The

Table 1

Chemicals and solvents used in the study

Abbreviation	Name (vendor, catalogue number)	Purity	CAS	Function	Purpose
HEMA	2-Hydroxyethyl methacrylate (Sigma-Aldrich, 477028)	$\geq 99\%$	868-77-9	Base monomers	Quantification/Identification (GC-MS/UPHLC-MS)
DMABEE	Ethyl 4-(dimethylamino)benzoate (Sigma-Aldrich, E24905)	$\geq 99\%$	10287-53-3	Co-initiator	Quantification/Identification (GC-MS)
CQ	Camphorquinone (Sigma-Aldrich, 124893)	97%	10373-78-1	Photoinitiator	Quantification/Identification (GC-MS)
BHT	Butylated hydroxytoluene (Sigma-Aldrich, W218405)	$\geq 99\%$	128-37-0	Inhibitor	Quantification/Identification (GC-MS/UPHLC-MS)
TEGDMA	Triethylene glycol dimethacrylate (Sigma-Aldrich, 759406)	99%	109-16-0	Base monomers	Quantification/Identification (GC-MS)
Bis-EMA	Bisphenol A ethoxylate dimethacrylate (Sigma-Aldrich, 455059) — number-average molecular weight (Mn) ~1700	Not reported	41637-38-1	Base monomers	Identification (UPHLC-MS)
UDMA	Diurethane dimethacrylate (Sigma-Aldrich, 436909)	$\geq 97\%$	72869-86-4	Base monomers	Identification (UPHLC-MS)
Bis-GMA	Bisphenol A glycidylmethacrylate (Sigma-Aldrich, 494356)	Not reported	1565-94-2	Base monomers	Identification (UPHLC-MS)
Methanol	Methanol (Sigma-Aldrich, 494291)	$\geq 99.9\%$	67-56-1	–	Solvent
IS	Diethyl phthalate (Sigma-Aldrich, 524972)	99.5%	84-66-2	–	Internal standard
–	Hexane (Sigma-Aldrich, 52750)	$\geq 99.7\%$	110-54-3	–	Solvent

CAS, Chemistry Abstracts Service registry number; GC-MS, gas chromatography-mass spectrometry; UPHLC-MS, ultra-high-performance liquid chromatography-mass spectrometry.

Fig. 1. Sampling equipment used by the students during restorative procedures. 1: Filter cassettes in conductive polypropylene for sampling of total aerosol particles. 2: XAD-7 sorbent for sampling of gaseous substances. 3: A cyclone for sampling respirable particles. *The cyclone was only used during initial pilot tests and in the positive control test.

cyclone was not used during the final monitoring of the students because of the results from the pilot study (in which no methacrylates were detected). The negative finding in the pilot studies also led us to limit the number of restorative procedures monitored to 13.

Study setting

Sampling was performed in the dental simulation clinic at the Department of Clinical Dentistry, The Arctic University of Norway. The preclinical training facility is equipped with 40 modified dental units (Planmeca, Helsinki, Finland) with phantom models (Frasaco, Tettnang, Germany). Ventilation of the room is performed using an 'intelligent' airflow management ventilation controlled by infrared sensors. All units had equivalent equipment and functionalities (e.g., water cooling, high-vacuum suction), as found in regular clinics.

On the day of the sampling, the students were instructed to perform restorative treatment with dental composite (ceram.x universal; Sirona Dentsply, Konstanz, Germany) and dental adhesive (Clearfil SE Bond; Kuraray, Tokyo, Japan). The safety data sheet information of the materials is listed in Table 2. The light-curing devices used by the students were Bluephase Style (IvoClar/Vivadent, Schaan, Lichtenstein). The students had been trained from the beginning of their clinical training to use water cooling and high-vacuum suction during cavity preparation and finishing/polishing of restorations. The sampling period was from the start of the bonding procedure until the clinical instructors had accepted the polished restoration(s) (range: 37–133 min). The polishing equipment available for the students was Identoflex Composite Polishers (Kerr, Orange, CA, USA); polishing diamonds with grit size 40 μm (red) and 20 μm (yellow) (Komet, Brasseler, Germany); and coarse, medium, fine, and superfine grits (Sof-Lex Contouring and Polishing Discs; 3M, Maplewood, MN, USA). The high-vacuum suction units were assessed on the day of sampling, and the suction rates measured ranged from 255 to 280 l min^{-1}. Rubber dam was used during the restoration procedure, but not during finishing/polishing, according to established restoration procedures at the university. After the restorations had been placed, but before the polishing procedure, the phantom masks were thoroughly washed with hot water and liquid detergent. After approval of the polished restoration by the clinical instructors, the total water content in the phantom was collected with a disposable polypropylene syringe (Terumo, Tokyo, Japan). The teeth were weighed (before and after the restorations were polished) using a microbalance (Sartorius, Goettingen, Germany) to assess the maximum amount of dust generated from each restorative procedure.

Questionnaire

A standardized questionnaire was given to all participants to document the procedures, namely, start and finishing time

Table 2

Safety data sheet (SDS) information of the materials used in the simulation clinic

Materials	Ingredients listed in the SDS	%	CAS
ceram.x universal*	Ethoxylated Bisphenol A Dimethacrylate[†]	2.5–10	41637-38-1
	Urethane modified Bis-GMA dimethacrylate resin	2.5–10	126646-17-1
	2,2′-Ethylendioxydiethyldimethacrylat[‡]	2.5–10	109-16-0
	ytterbium trifluoride		
	2,6-di-tert-butyl-p-cresol[§]	0.1–2.5	128-37-0
Clearfil SE Bond (primer)	2-hydroxyethyl methacrylate[¶]	20–40	868-77-9
Clearfil SE Bond (bond)*	2-hydroxyethyl methacrylate[¶]	20–40	868-77-9
	Bisphenol A diglycidylmethacrylate**	25–45	1565-94-2

*According to the scientific compendium of ceram.x universal, the composite is a nanohybrid with a filler load ranging from 77 to 79 wt% (59%–61% by volume). It contains a blend of spherical, pre-polymerized SphereTEC fillers, non-agglomerated barium glass, ytterbium fluoride, and methacrylic polysiloxane nanoparticles.
[†]Bisphenol A ethoxylate dimethacrylate (Bis-EMA).
[‡]Triethylene glycol dimethacrylate (TEGDMA).
[§]Butylated hydroxytoluene (BHT).
[¶]2-Hydroxyethyl methacrylate (HEMA).
**Bisphenol A glycidylmethacrylate (Bis-GMA).
CAS, Chemistry Abstracts Service registry number.

(filling and polishing), type of restoration, use of suction during the bonding procedures, type of polishing bur/tool used, and the use of water during the polishing procedures (contouring and finishing). The students were also asked to state the reason for omitting suction/water (if applicable).

Positive-control experiments

A positive-control experiment was performed in a rectangular chamber (25 cm × 14 cm × 14 cm), without ventilation, with the same personnel-borne samplers as used by the dental students. In addition, the cyclone sampler with the 37 mm cassette was used to collect respirable particles as per the MDHS 14/4 (18) and the ISO 7708 (13) criteria. The pump-rates for the cyclone and filter were 2.2 l min^{-1}, and 0.7 l min^{-1} for the XAD-7 sorbent. A round specimen of ceram.x universal was prepared in a Teflon mold (10 mm diameter, 6 mm height). A Bluephase Style in high-mode (irradiance ~1,200 mW cm^{-2}) for 30 s was used to cure the sample in a 2 mm layer. No means of preventing the inhibition layer were implemented. The sample was fixed in the chamber. The application of an airstream to a thin layer of primer and adhesive (Clearfil SE bond) was performed for approximately 7 s to assess release of gaseous substances. The composite specimen was polished with similar polishing burs as used by the students. The burs were operated at approximately 20,000 rounds per min. The total time of grinding and polishing was approximately 2.5 min (5 min in total). Approximately 2 mm of the cylinder height was polished. Total sampling time was 25 min.

Extraction of organic substances

Extraction of organic substances from the filters and sorbents was carried out immediately after sampling. The filters were inserted into petri dishes (37 mm diameter), and 1.5 ml of methanol with 5.33 μg ml^{-1} of internal standard (IS) (diethyl phthalate) was added. The dishes with the samples were kept in a sealed glass chamber at 20°C to prevent evaporation. The XAD-7 resin from the sorbent tubes were transferred to individual glass vials (Karl Hecht, Sondheim von der Rhön, Germany), and 1.5 ml of methanol with IS (5.33 μg ml^{-1}) was added. The sorbent samples were placed in an ultrasonic bath for 30 min to aid desorption. After 24 h, or 1 h for the positive-control samples, the methanol was transferred to labeled liquid chromatography-gas chromatography (LC-GC)-certified vials with screw-threaded caps and PTFE/silicon septum (Waters, Milford, MA, USA), using glass Pasteur pipettes (BRAND, Wertheim, Germany), for gas chromatography-mass spectrometry (GC-MS) analysis.

Organic substances in the water sampled from the phantoms were extracted with 1 ml of hexane in a three-step manner, prior to removal of hexane with a Centrivap SpeedVac (Labconco, Kansas City, MO, USA), at 40°C for 10 min. The samples were resuspended in methanol with IS (5.33 μg ml^{-1}) to make the samples compatible with the ultra-high-performance liquid chromatography-mass spectrometry (UHPLC-MS) and GC-MS analyses.

Preparation of uncured samples for GC-MS and UHPLC-MS analyses

In order to identify the composition of the materials used, approximately 2 mg each of Clearfil SE Bond and ceram.x universal were weighed in individual polypropylene microtubes (BRAND, Wertheim, Germany) using an analytic balance. The materials were dissolved in 1 ml of methanol. The tubes with Clearfil SE Bond and ceram.x universal were centrifuged at 20,000 **g** for 5 min to separate the filler and matrix phase. The supernatant of each sample was transferred to individual glass vials and diluted 30 times (based on the material weighted on the balance) before further analysis by GC-MS and UHPLC-MS.

Gas chromatography–mass spectrometry

The combination of GC and MS was used to analyze volatile and semi-volatile substances. The GC-MS instrumentation consisted of a 7891A autosampler and an HP6890 GC (Agilent, Santa Clara, CA, USA) connected to a QuattroMicro MS (Micromass, Cary, NC, USA). Instrument control, data sampling, and handling were controlled by MassLynx 4.1 (Waters, Milford, MA, USA). The GC was equipped with a capillary column (30 m; 0.25 mm internal diameter; 0.25 μm film thickness) (Rxi-1MS; Restek, Bellefonte, PA, USA). Helium (5.0 grade) was used as a carrier gas with a flowrate of 1 ml min^{-1}. Splitless injection was used. The injection volume was 1 μl, and the injector temperature was 250°C. The column start temperature was 50°C, which was then increased to 120°C at a rate of 10°C min^{-1}, held at 120°C for 3 min, increased from 120°C to 160°C at a rate of 20°C min^{-1}, held at 160°C for 4 min, increased from 160°C to 280°C at a rate of 20°C min^{-1}, then held for 1 min.

Identification of substances in the uncured and extracted samples was performed using the mass spectrometer in full-scan mode from 50 to 350 mass-to-charge ratio (m/z). Identification of substances in the samples was performed by comparing the retention times and mass spectra obtained with the corresponding retention times and spectra of reference substances. Substances not identified by reference substance were compared with data from the NIST library (National Institute of Science and Technology, Gaithersburg, MD, USA).

Calibration curves and quantification (GC-MS)

The reference substances HEMA, camphorquinone (CQ), butylated hydroxytoluene (BHT), ethyl 4-(dimethylamino) benzoate (DMABEE), and triethylene glycol dimethacrylate (TEGDMA) were weighed in separate glassware, using a scientific balance, and diluted in methanol. The solutions were mixed to make a stock solution containing all the reference substances. The stock was serially diluted in eight steps. Then, 1 ml of each dilution was transferred to a GC vial, to which 0.5 ml of methanol with diethyl phthalate was added, for a final IS concentration of 5.33 μg ml^{-1}. Calibration curves were created by plotting the area of the analytes/IS against the concentration of each analyte in the eight analyte mixtures (0.001–30 μg ml^{-1}).

Quantification of substances in the extracted methanol was performed by Selected Ion Recording (SIR) analysis of abundant ions characteristic for each analyte (Table 3). Comparison of area under the analyte peak with the area of the internal standard peak was performed for each analyte. The ratio thus obtained was used in conjunction with the calibration curve to determine the concentration of each substance. The amount of eluate was then calculated and expressed in μg m^{-3} air for the samples collected

Table 3

Molecular weight (MW) values, retention times, and molecular and characteristic ions of the reference substances used for quantification in the gas chromatography-mass spectrometry (GC-MS) analyses

Substance	MW	Retention time (GC-MS) (min)	Molecular ion	Characteristic ions
HEMA	130.14	5.63	130	69*, 87, 130
CQ	166.22	10.46	166	95*, 138, 166
BHT	220.35	14.14	220	205*, 220
DEPH (IS)	222.24	15.17	222	149*, 177
DMABEE	193.24	17.43	193	148*, 164, 193
TEGDMA	286.32	19.27	286	69*, 113

*Quantifying ions.

BHT, butylated hydroxytoluene; CQ, camphorquinone; DEPH (IS), diethyl phthalate (internal standard); DMABEE, ethyl 4-(dimethylamino)benzoate; HEMA, 2-hydroxyethyl methacrylate; TEGDMA, triethylen glycol dimethacrylate

with the personnel-borne samplers. Organic substances extracted from the water from the phantoms were expressed in μg.

UHPLC-quadrupole time-of-flight MS identification

The combination of LC and MS (LC-MS) is used to analyze substances with low vapor pressure even at elevated temperatures. All samples were analyzed using an Acquity I-class UHPLC system connected to a Xevo G2 quadrupole time-of-flight (Q-TOF) mass spectrometer (both from Waters, Milford, MA, USA). Full-scan spectra in electrospray ionization (ESI+) mode were obtained in the mass range 95–3,500 Da with a scan time of 300 ms and an interscan time of 14 ms. The column used was an ACQUITY UPLC BEH C18 1.7 μm (Waters) with an internal diameter of 2.1 mm and a length of 100 mm. The mobile phase was water (A) and acetonitrile (B) with 0.1% formic acid mobile phase, gradient 95/5 (A/B) at 0 min and 5/95 at 10 min (linear gradient). From 10.1 to 13.5 min the gradient was 95/5 (A/B). The equilibrium time represented approximately 10 column volumes. A flowrate of 0.6 ml min^{-1} and a column temperature 65.0°C was used. The injection volume was 5 μl. The injection needle was flushed between samples with methanol (weak and strong wash).

In addition to the standards used to prepare the calibration curve in the GC-MS analysis, bisphenol A ethoxylate dimethacrylate (Bis-EMA), bisphenol A glycidylmethacrylate (Bis-GMA), and urethane dimethacrylate (UDMA) standards were prepared (Table 1). Identification of substances in the samples was performed by comparing the obtained retention times and mass spectra with the corresponding retention times and spectra of all the reference substances.

Validation

Blank samples of chemicals (water, ethanol, methanol) and eluates from equipment [glassware, plastic teeth (Frasaco), polyester films, pipettes, polypropylene tubes, and rubber bulb] used during sample preparation were collected and analyzed to identify contaminants that might interfere with the analysis. Carryover was assessed by analyzing blanks between samples.

Limit of detection (LD) and lowest limit of quantification (LLOQ) were set as $\geq$3 and $\geq$10 signal-to-noise ratio, respectively, and were determined by analyzing reference substances in concentrations ranging from 0.001 to 30 μg ml^{-1}. The signal-to-noise was determined visually by inspecting the chromatograms. Precision was assessed by analyzing the 2- and 5-μg ml^{-1} concentrations of reference substance samples between repeated measurements within and between days.

Ethical approval

Monitoring of routines performed daily was performed and did not involve an intervention on human subjects. No personal data on the participants were recorded. Thus, in accordance with the guidelines given by the Norwegian Regional Committee for Medical and Health Research Ethics (REK), this study did not require an ethical approval.

Statistics

The outcome variables examined were the concentration of methacrylates and additives detected in particle-associated, gaseous, and water samples. Descriptive statistics, that is, mean, median, and SD, were generated using Microsoft Excel 2013 (Microsoft, Redmond, WA, USA).

Results

Screening of the dental materials used during the procedure

The content of the materials used during the restorative procedure was investigated using GC-MS and UHPLC-MS. Ceram.x universal contained DMABEE, CQ, BHT, and TEGDMA, as shown by the GC-MS and UHPLC-MS analyses (Fig. 2). The TEGDMA signal was much stronger than the signals for the other analytes. Clearfil SE Bond encompasses a primer and an adhesive (called 'bond' by the manufacturer). The primer of Clearfil SE Bond contained HEMA, while the adhesive contained Bis-GMA and HEMA, as identified by the GC-MS and LC-MS analyses (in line with the safety data sheet of the material; Table 2). It is worth noting that in the safety data sheet of ceram.x universal, Bis-EMA is listed as an ingredient; however, despite having a similar CAS number, no match for our reference substance (Bis-EMA) was seen in the UHPLC-MS analysis.

Questionnaire and sampling parameters

The reported use of high-vacuum suction and water cooling varied depending on the procedure (Table 4). Suction and water were used during the whole procedure in, respectively, 10 and 11 of 13 contouring procedures. For polishing, six students reported using suction, while nine reported using water. The use of polishing disks was the most common reason for omitting water or suction during parts of the procedure.
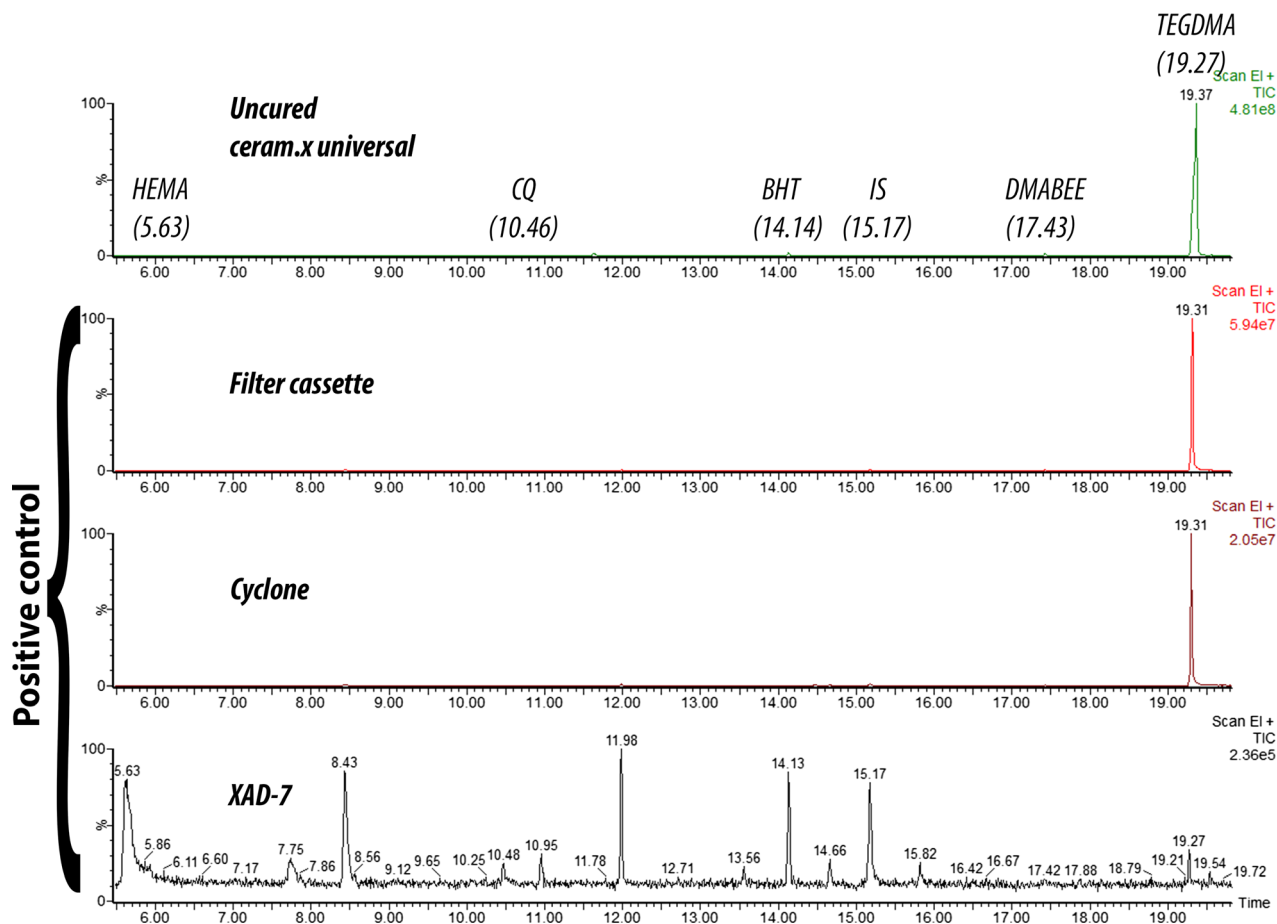
Fig. 2. Gas chromatography-mass spectrometry (GC-MS) analyses of uncured ceram.x universal and the positive control. *y*-axis: relative intensity of signal; *x*-axis, retention time (min). The extracts from the filter cassette and cyclone show a strong signal for triethylene glycol dimethacrylate (TEGDMA) (approximately 80 times higher than the other peaks), in addition to a weak signal for the other analytes and internal standard (with exception of 2-hydroxyethyl methacrylate (HEMA)). The chromatogram of the uncured ceram.x universal shows similarities to the chromatograms of the particles collected in the positive controls. In the gas samples (XAD-7), the signal of the analytes was much weaker than in the particle fractions sampled. Chromatograms for uncured ceram.x universal and the positive controls of the particles sampled, omitting the TEGDMA signal, is available in the supporting information (Figure S1). BHT, butylated hydroxytoluene; CQ, camphorquinone; DMABEE, ethyl 4-(dimethylamino)benzoate; IS, diethyl phthalate.

The suction device was used during the application of an airstream to the adhesive/primer in nine out of 13 restoration procedures.

There was a large variation in the amount of restoration polished and accordingly the potential amount of dust generated (Table 4). In five of the 13 procedures monitored, two restorations were placed (same quadrant). In all other procedures, one restoration was placed. The amount of dust generated was 14.3 ± 17.5 mg and 6.2 (1.2–55.2) mg [(mean$\pm$SD and median (range)]; the sampling time was 69 ± 26 min and 68 (37–133) min; and the volume of water collected from the phantoms was 149 ± 123 ml and 108 (7–444 ml).

Exposure to gaseous substances (personal air samplers)

No gaseous substances were detected in the GC-MS or UHPLC-MS analyses performed on the samples sampled with the XAD-7 sorbent.

Exposure to particle-associated substances (personal air samplers)

Butylated hydroxytoluene was the only substance detected and quantified from the samples collected in the filter cassette (range: 0.5–2.5 μg m^{-3}).

Positive-control experiments

In the extracts from the XAD-7 sorbent, a weak signal was found for all analytes in SIR analysis. In the full scan of the same sample, the analytes HEMA, BHT, and TEGDMA were observed (Fig. 2).

In the positive control of the particles sampled, signals were found for all analytes. In particular, TEGDMA had a strong signal (Figs 2 and 3). In the UHPLC-MS analysis, TEGDMA was found. In addition, the high-molecular-weight substances found in uncured material matched the substances found in the positive control (Fig. 3).

Table 4

Results for the quantitative gas chromatography-mass spectrometry (GC-MS) analysis and for the questionnaire on each restorative procedure that was performed by the students

Procedure (cavity)	Substances detected/quantified*		Dust produced (mg)	Sampling duration (min)	Reported use of suction†			Reported use of water†	
	From particles (μg m^{-3})	In water‡			Bonding	Contouring	Polishing	Contouring	Polishing
45d, 45o	BHT: 2.25	None	1.2	45	Yes	No	No	No	No
26m,o	BHT: 0.72	TEGDMA: 1.5 μg	6.8	61	Yes	Yes	Yes	Yes	Yes
26m,o	BHT: 0.62	TEGDMA: 1.6 μg	52.4	75	Yes	Yes	No	Yes	No
45d,o	BHT: 0.50	None	8.5	77	Yes	Yes	Yes	Yes	Yes
26m,o, 22d	BHT: 0.83	TEGDMA: 0.7 μg	55.2	70	Yes	Yes	Yes	Yes	Yes
26m,o, 22d	BHT: 0.97	TEGDMA: 1.5 μg	29.3	89	Yes	Yes	No	Yes	Yes
24m,o,d, 26m,o	BHT: 0.73	TEGDMA: 11.4 μg	4.8	133	Yes	Yes	Yes	Yes	Yes
26m,o, 22d	None	TEGDMA: 4.2 μg	12.2	62	Yes	No	No	Yes	No
16o	BHT: 1.46	TEGDMA: 1.7 μg, DMABEE: 0.4 μg	26.1	37	No	Yes	No	Yes	Yes
35b	BHT: 2.55	TEGDMA: 2.0 μg	3.8	40	No	Yes	Yes	Yes	Yes
26m,o	None	None	–	68	No	Yes	Yes	Yes	Yes
14m,o	None	None	–	97	Yes	No	No	No	No
36o,d	BHT: 2.18	TEGDMA: 8.3 μg, DMABEE: 1.3 μg	–	55	No	Yes	No	Yes	Yes

Only quantifiable results are shown. No detectable results were found for the gaseous samples.

b, buccal surface; BHT, butylated hydroxytoluene; CQ, camphorquinone; d, distal surface; DMABEE, ethyl 4-(dimethylamino)benzoate; HEMA, 2-hydroxyethyl methacrylate; m, mesial surface; o, occlusal surface; TEGDMA, triethylene glycol dimethacrylate.

*No substances were detected in any of the gas sorbents.

†During the total duration of the procedure.

‡From Frasaco phantoms.

Exposure to organic substances in resin-based dental materials via water

The quantifiable substances in the samples collected from the phantoms were TEGDMA and DMABEE: TEGDMA was detected in 11 samples and quantified in 9 (range: 0.7 – 11.4 μg); and DMABEE was quantified in two samples (0.4 and 1.3 μg) (Table 4).

Validation of GC-MS quantification

The coefficient of determination (r^2) was calculated to be >0.99 for all calibration curves for the analytes in the range 0.001–10 μg ml^{-1}. The LLOQ was determined to be 0.1 μg ml^{-1} (corresponding to 100 pg injected on the column) for all substances, with the exception of TEGDMA detected at 0.01 μg ml^{-1} (corresponding to 10 pg injected on the column). A summary of precision calculations is presented in Table 5. The between-day relative standard deviation (RSD) observed for TEGDMA was most likely to be the result of a random instrument error.

Discussion

In the present study, no detectable exposure to gaseous or particle-associated methacrylates was found in the samples collected by the personal air samplers carried by students performing restorative procedures. However, substantial amounts of the constituents of ceram.x universal, including non-volatile substances, were found in the positive control (Figs 2 and 3). Our data confirm previous data demonstrating that airborne exposure to methacrylates could occur through inhalation of dust from dental composite (12).

Quantifiable amounts of ingredients from ceram.x universal were found in the water collected in the phantoms. This indicates that patients may be exposed to microgram amounts of constituents from resin-based material during certain dental procedures, for example, polishing. The antioxidant BHT was quantifiable in the majority of the dust samples collected from students. However, because the quantified BHT did not show a relationship with the amount of restoration polished, and were the only substance detected from the dust samples (in contrast to the positive control (Fig. 2)), this result may not be attributed to dust particles from the polished restoration. As the blanks used between samples showed no signs of BHT carry-over, it was speculated that the BHT may have come from cosmetic products used by the participants, as cosmetic products may contain up to 0.5% BHT (19); however, this could not be verified.

Our findings concerning particle-associated exposure to methacrylates may partly be explained by factors influencing the amounts of particle reaching the filters. It has previously been demonstrated that dental
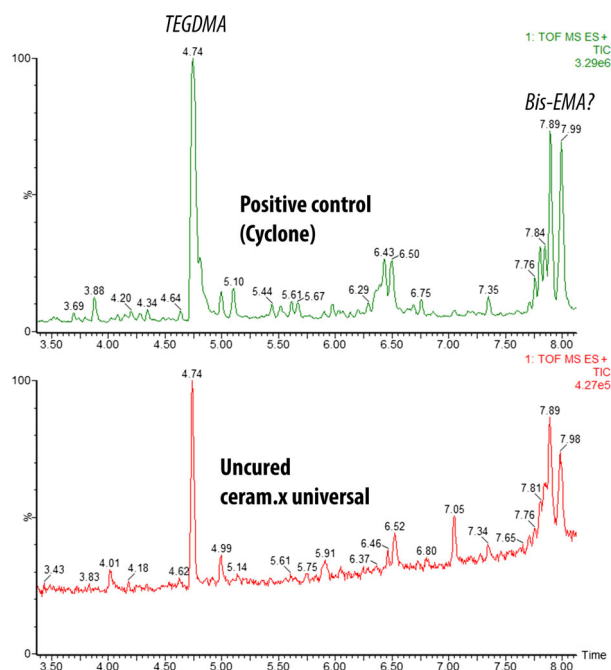
Fig. *3*. Ultra-high-performance liquid chromatography-mass spectrometry chromatograms of positive-control (respirable particles) and uncured ceram.x universal. *y*-axis, relative intensity of signal. *x*-axis, retention time (min). A peak corresponding to triethylene glycol dimethacrylate (TEGDMA) is shown at 4.74 min in both samples. The mass spectra at 7.89 and 7.98–7.99 min indicate the presence of the same substance in the uncured sample and the positive control. Bis-EMA, bisphenol A ethoxylate dimethacrylate.

personnel can be exposed to inhalable particles during restorative procedures performed in both laboratory and clinical settings (14–16). However, in these studies, the use of water cooling and high-flow suction was only occasionally reported in clinical measurements and not at all during the laboratory assessment of particle

Table 5

Within- and between-day precision values for the substances quantified

Substance	$\mu g\ ml^{-1}$	Within-day[†] RSD (%)*	Between-day[†] RSD (%)*
HEMA	5	10.3	11.2
	2	3.7	12.7
CQ	5	3.6	0.3
	2	1.6	3
BHT	5	1.0	5.2
	2	0.7	2.9
DMABEE	5	2.9	7.9
	2	3.8	6.5
TEGDMA	5	2.1	22.4
	2	5.8	84.3

*Relative standard deviation: $(=100 * s/|\bar{X}|)$, s = the sample standard deviation, $\bar{X}$ = analyte/internal standard mean).
[†]$n = 3$.
BHT, butylated hydroxytoluene; CQ, camphorquinone; DMABEE, ethyl 4-(dimethylamino)benzoate; HEMA, 2-hydroxyethyl methacrylate; TEGDMA, triethylene glycol dimethacrylate.

concentration (14–16). In our study, all of the students, with the exception of one, reported using either water cooling or high-flow suction for the total duration of the contouring procedure – the process by which the unpolymerized monomer layer (i.e., the oxygen inhibition layer) of the composite is removed (20). The use of both suction and water during particle-producing procedures is recognized to reduce dust exposure (21). The use of either preventive measure initially may partly explain the lack of signal observed in the procedures monitored. Our negative findings may also relate to the amount of dust produced during the procedures monitored, that is, the average amount of dust produced during the polishing was 14.3 mg; however, during esthetic build-ups, the amount of particles generated is likely to be considerably higher (14). Thus, the risk of airborne exposure to methacrylates from dust should be further explored in more comprehensive clinical procedures.

Location of the sampling equipment will probably also influence the results obtained. HENRIKS-ECKERMAN *et al*. (9) performed exposure assessment of gaseous methacrylates during procedure-specific tasks. They found quantifiable amounts of TEGDMA when the sampling was performed 20–30 cm from the mouth of the patients (9). By contrast, in the present study, the sampling equipment was located near the breathing zone of the dental student (which may imply a longer distance from the exposure source). Another difference is that the former study used a combination of up to three samples to achieve a detectable signal of methacrylates and had a short sampling time. In the present study, the long sampling times were probably a consequence of the inexperience of the operators. Yet, the inability to detect methacrylates in our samples suggests that ambient exposure to particle-associated and gaseous substances was very low for student participants and instructors in the simulation clinic.

The materials used during the restorative procedure have been shown to affect the severity and type of substances/particles dental personnel are exposed to during restorative procedures (12). There may be several reasons for this. First, the brand/type of dental composite affects the size and type of particles produced during grinding (14, 16). This may influence the number of inhalable particles and their leachability, owing to their varying surface area (22, 23). Second, the amount of unreacted substances in the material will also influence the potential exposure. It has been shown that the composition of composites influences the degree of cure and/or monomer conversion (24, 25). Analysis of extracts from standardized, pre-cured composite samples shows that eluates vary considerably among brands of composite (26). In relation to our findings, a previous study has shown that ceram.x, the predecessor to ceram.x universal, had no detectable eluates, even after 28 d in acetone. By contrast, Filtek Supreme XT eluted quantifiable levels of TEGDMA, Bis-GMA, and UDMA (27). Our negative results should therefore be extrapolated with caution to other composite resin-based materials as the amount of unreacted and freely available monomers may vary considerably.

Still, organic substances from ceram.x universal were found in the water collected after the polishing procedure. This indicates that patients might be exposed to the constituents from the resin-based materials if they swallow the water used during polishing. If no water is used during the polishing procedure, it may be speculated that these substances would be a source of airborne exposure for patient and dental personnel. If it is not possible to use a rubber dam during the polishing procedure, removing the oxygen inhibition layer (20) and maximizing the efficacy of the high-vacuum suction by correctly angling and positioning the tip, will probably minimize exposure to substances from resin-based materials.

Based on the chemicals listed in the safety data sheet of ceram.x universal, we planned to quantify particle-associated exposure to Bis-EMA. However, despite having a similar CAS number, the substances in the reference substance did not match the substances present in ceram.x universal. A similar problem concerning reference substances and CAS numbers has been previously reported (28). A CAS registry number is a unique numeric identifier that designates only one substance (29). However, a search with the CAS number of Bis-EMA on Sigma-Aldrich's webpage yields two different reference substances for Bis-EMA (with dissimilar number average molecular weight, M_n), one being discontinued. Thus, Bis-EMA oligomers seem to have the same CAS number, as also reported by VERVILET *et al.* (30). From a health, safety, and environmental perspective, molecular weight (or M_n) should accompany CAS numbers in the safety data sheet, so that dentists can find the content of the material they are using and for which they are responsible. Indeed, it would be preferable for researchers to provide full information of the catalogue number and/or M_n of polymers when publishing, as this may aid other researchers to obtain the correct reference standard.

In conclusion, dust particles may contribute to exposure to organic substances in resin-based materials, as quantifiable amounts of methacrylates were detected in the positive control and in the water collected in the phantoms. Yet, neither particle-associated nor gaseous exposure to airborne methacrylates was detected when sampling was performed in a simulated clinical set-up where water and high-vacuum suction were available. Future studies should look into clinical procedures that involve other resin-based dental materials and extensive (dry) polishing (e.g., multiple-unit temporary restorations, orthodontic bracket removal, and/or esthetic build-ups) as there may be serious, albeit rare, conditions associated with the work environment of dental personnel (8). In addition, the roles of water cooling and high-vacuum suction in preventing exposure to methacrylates should be examined further.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Chromatograms for uncured ceram.x universal and the positive controls of the particles sampled, omitting the TEGDMA signal.