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VISEU

TOXICITY OF RESIN-MATRIX COMPOSITES IN DENTISTRY - A SYSTEMATIC REVIEW

Dissertation presented to the Universidade Católica Portuguesa to obtain the degree of Master in Dental Medicine

Maria Ana Cordeiro

Viseu, 2024

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Dedicatória

Aos meus avós,

Por me guiarem sempre pelo caminho certo, me protegerem, apoiarem e ajudarem durante todo o meu percurso.

Aos meus pais, irmã e tio pelo apoio incondicional e esforço, sem eles nada disto seria possível. Uma Conquista que também é deles.

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Abstract

Purpose: This systematic review aims to comprehensively explore the factors contributing to the toxicity associated with resin-based dental materials and to establish clinical criteria that reduce the release of residual monomers.

Methods: A systematic review was performed according to the PRISMA criteria. A PICO question was established: Which factors(C) influence the toxicity (O) of dental resin composites (I) in humans (P), and what are their local or systemic adverse effects (O)? and a bibliographic review was performed in three databases, PubMed, Cochrane Central and Web of Science. Inclusion and exclusion criteria were established to retrieve articles in the last 20 years, in English language.

Results: A total of 1261 articles were retrieved from the three electronic databases. Following the elimination of duplicates, a total of 1227 articles remained for further selection by title and abstract. After which 20 articles were subjected to comprehensive reading, 12 articles were included and 8 were excluded. The analysis of the selected articles indicated that low levels of monomers were detected in the participants´ saliva and urine, suggesting relatively low local and systemic toxicity.

Conclusions: In all the selected studies, the changes observed were not considered significant enough to substantially affect a patient's health, however, the adherence to manufacturer’s instructions is important. If preventive measures are followed, including adequate photopolymerization for the appropriate exposure time, a higher degree of monomer conversion can be guaranteed. This, in turn, results in a lower release of free monomers and thus a lower probability of any type of toxicity occurring.

Keywords: toxicity; resin monomers; BPA; resin-matrix composite; dentistry

Resumo

Objetivo: A presente revisão sistemática tem como objetivo explorar de forma abrangente os factores que contribuem para a toxicidade associada aos materiais de matriz de resina e estabelecer critérios clínicos que reduzam a libertação de monómeros residuais.

Métodos: Foi efectuada uma revisão sistemática de acordo com os critérios PRISMA. Foi estabelecida uma questão PICO: Quais os factores (C) que influenciam a toxicidade (O) dos compósitos de resina dentária (I) em humanos (P), e quais os seus efeitos adversos locais ou sistémicos (O)? e foi realizada uma revisão bibliográfica em três bases de dados, PubMed, Cochrane Central e Web of Science. Os critérios de inclusão e exclusão foram estabelecidos para abranger artigos dos últimos 20 anos, em língua inglesa.

Resultados: Um total de 1261 artigos foram slecionadosdas três bases de dados electrónicas. Após a eliminação dos duplicados, restaram 1227 artigos para uma nova seleção por título e resumo. Depois disso, 20 artigos foram submetidos a uma leitura exaustiva, 12 artigos foram incluídos e 8 foram excluídos. A análise dos artigos seleccionados indicou que foram detectados baixos níveis de monómeros na saliva e na urina dos participantes, o que sugere uma toxicidade local e sistémica relativamente baixa.

Conclusões: Em todos os estudos seleccionados, as alterações observadas não foram consideradas suficientemente significativas para afetar substancialmente a saúde de um paciente, no entanto, a adesão às instruções do fabricante é importante. Se as medidas preventivas forem seguidas, incluindo a fotopolimerização adequada para o tempo de exposição apropriado, pode ser garantido um maior grau de conversão de monómeros. Isto, por sua vez, resulta numa menor libertação de monómeros livres e, consequentemente, numa menor probabilidade de ocorrência de qualquer tipo de toxicidade.

Palavras-chave: toxicidade; monómeros de resina; BPA; compósito de matriz resinosa; medicina dentária

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List of abbreviations

BPA – Bisphenol A

MMA – Methylmethacrylate

TEGDMA - triethyleneglycol dimethacrylate

EGDMA - ethyleneglycol dimethacrylate

Bis-GMA - 2,2-bis-[4-(2-hydroxy-3-methacrylo-xypropoxy)phenyl]-propane

2-HEMA - 2-hydroxy-ethylmethacrylate

THFMA- Tetrahydrofurfuryl Methacrylate

UDMA – Urethane-dimethacrylate

Bis-DMA -bisphenol A dimethacrylate

BADGE - BPA diglycidylether

DUDA - diurethane dimethacrylate

Bis-EMA- Ethoxylatedbisphenol-A-dimethacrylate

DMDMA – Decamethylendimethacrylate

PC-BisGMA - polycarbonate bisphenol A glycerol dimethacrylate

HDDMA - 1,6 hexanediol dimethacrylate

PEM-665- cycloaliphatic dimethacrylate

Gamma-GT - Gamma-glutamyl transpeptidase

HPLC - high-performance liquid chromatography

LCU – Light-curing unit

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Introduction

1. Introduction

Resin-matrix composites are widely used as restorative materials, due to their mechanical, optical properties and esthetic properties. However, resin-matrix composites have shown some drawbacks, such as polymerization shrinkage, the release of monomers from the organic matrix due to the incomplete conversion of the monomers during the polymerization reaction coupled with material degradation over time (1,2).

1.1 Toxicity of resin-matrix composites

Concerns have thus arisen regarding potential toxicity associated with materials used in resin-matrix composites restorations, which may include derivatives of bisphenol A (BPA). Specifically, compounds such as bisphenol A diglycidyl methacrylate (Bis-GMA), along with bisphenol A dimethacrylate (Bis-DMA), polycarbonate modified Bis-GMA (PC Bis-GMA), ethoxylated bisphenol A glycol dimethacrylate (Bis-EMA), and 2,2-bis[(4-methacryloxy polyethoxy) phenyl] propane (Bis-MPEPP) have raised concerns (3).

Moreover, several studies have highlighted cytotoxic effects associated with both triethylene glycol dimethacrylate (TEGDMA) and urethane dimethacrylate (UDMA) (4).

The release of monomers and BPA is contingent upon the chemical composition and quantity of the organic matrix present in resin matrix composites (5).The cytotoxicity assessment of these monomers was conducted using the bromodeoxyuridine (BrdU) assay and the lactate dehydrogenase (LDH) assay. This study presented the following results from lowest to highest degree of toxicity:HEMA < TEGDMA < UDMA < Bis-GMA (6).

Research has shown the presence of microgram quantities of HEMA and TEGDMA monomers in saliva within minutes to hours, after the placement of a composite restoration. Furthermore, these monomers have been detected in the dentine and pulp after hours to days of the composite placement (7).

This degradation of the resin-matrix composites, resulting from light-curing, thermal, mechanical or chemical influences, is intrinsically governed by multifaceted factors within the oral cavity. This includes the impact of saliva constituents via hydrolytic and enzymatic pathways, occlusal forces during

mastication, temperature fluctuations and the acidic by-products synthesised by oral microbial activity (8,9).

Resin-matrix composites microstructure is composed by an organic matrix, inorganic filler particles, a photoinitiator system, and silane coupling agents. The mechanical characteristics of resin-matrix composites are predominantly ruled by the filler component, where the reinforcing phase commonly involves a silane-coupled inorganic filler. The intricate microstructural features of resin-matrix composites play a substantial role in shaping their properties, with materials exhibiting higher filler content demonstrating enhanced resistance to property degradation in solvent environments (5,10).

The potential toxicity of specific components in resin-based materials requires ongoing, comprehensive evaluation of their physicochemical properties and biological reliability for complete clinical acceptance. Prior research has highlighted the toxicity risks associated with resin-matrix composites at both local and systemic levels, underscoring the critical importance of continued investigation in this area (1,11).

1.2 Toxic constituents of resin-matrix composites

1.2.1 BPA

BPA, a constituent integral to the organic matrix, is deemed as having the potential for toxicity. The characteristics of the organic matrix and the employed polymerization method dictate the degradation of resin-matrix composites and subsequent release of this molecule into the oral cavity (12).

The effects of BPA are largely akin to those of the estrogens diethylstilbestrol and ethinyl estradiol, albeit with a lower potency level (13). Due to their ability to simulate the actions of the estrogen hormone within living cells, BPA exhibits an affinity for binding to nuclear receptors, including estrogen receptors , androgen receptors,and estrogen-related receptors. Furthermore, it exerts adverse effects on the thyroid hormone receptor. Additionally, BPA interacts with membrane receptors, potentially instigating endocrine-disrupting effects (12,14).

These monomers can infiltrate not only the dental pulp, but also the gingivae and various organs through the bloodstream after absorption from saliva, potentially inducing both local and systemic toxicity (6,12).

Evidence suggests that BPA toxicity can significantly impact health, even at low doses (15).

1.2.2 Bis-GMA

Bisphenol A-glycidyl methacrylate (Bis-GMA) exposure has been demonstrated to induce the expression of specific isoforms in human pulp cells, such as carboxylesterases (CES), namely CES2, with lesser amounts of the CES1A1 and CES3 isoforms. This monomeric compound holds the potential to adversely impact dental pulp vitality and cause pulpal inflammation by disrupting the normal differentiation processes of pulpal fibroblasts. Additionally, Bis-GMA influences the migration and tenascin expression in keratinocytes and human gingival fibroblasts, potentially interfering with the healing of oral tissues post-injury. Furthermore, its biodegradation product, methacrylic acid (MMA), significantly diminishes the expression of intercellular adhesion molecule-1 (ICAM-1) in cells stimulated with Tumor necrosis factor alpha (TNF-α), consequently reducing leukocyte recruitment to inflammatory sites and influencing macrophage response, thereby impacting the immune system (9,16).

At heightened concentrations, Bis-GMA has shown to potentially induce cellular apoptosis or necrosis. Notably, prior investigations have evidenced that Bis-GMA does not liberate BPA, ostensibly attributed to the chemical structure of Bis-GMA, which purportedly inhibits hydrolysis at the ester bond (16,17).

1.2.3 Bis-DMA

A study conducted by Atkinson et al. compared two commonly employed BPA derivatives in dental materials: bisphenol A dimethacrylate (Bis-DMA) and Bis-GMA. In contrast to Bis-GMA, BPA has been detected in saliva following resin-matrix composites placement, arising from the hydrolysis of bisphenol dimethacrylate (Bis-DMA) catalyzed by salivary esterases. This occurs due to the inherent structural differences between these compounds. However, it is noteworthythat Bis-DMA is not widely used in dental materials (14,17). At 37°C,

Bis-DMA underwent rapid and substantial transformation into BPA. Within just 24 hours, the concentration of Bis-DMA significantly decreased from 200 ng/ml to 21.8 ng/ml. Intriguingly, under the tested conditions, Bis-GMA did not release any BPA. Instead, the conversion of Bis-DMA into BPA was identified as the primary responsible for BPA release from this sealant. This conversion occurred gradually over 24 hours, exhibiting a consistent increase in the conversion rate during this timeframe. These findings suggests that the inference that the bis-DMA monomer undergoes hydrolysis to BPA in saliva (4,18).

1.2.4 TEGDMA

TEGDMA is a genotoxic monomer known to impact DNA even at low concentrations, causing its fragmentation and destruction, with more pronounced effects at higher concentrations. Apoptosis induction leads to DNA fragmentation and activation of caspases -3, -8, and -9. Lipopolysaccharide-induced apoptosis decreases the levels of Interleukin-1β (IL-1β) and TNF-α release. High apoptosis/necrosis rates, combined with reduced TNF-α, are related with decreased IL-6 and IL-10 levels, and with the activation of several pathways that regulate physiological processes including inflammatory response, cell differentiation, cell proliferation, cell dead and survival and expression of proteins (for example the pathways p38MAPK, JNK, and ERK1/2). TEGDMA also suppresses cytokine release and alters cell surface antigen expression. It exhibits cytotoxic properties, inhibiting cell proliferation proportionally to its concentration. Despite this, its low molecular weight has been associated with reduced glutathione levels, since glutathione neutralises reactive oxygen radicals, reducing glutathione increases the amount of reactive oxygen radicals and oxidative stress (4,16).

1.2.5 HEMA

Prolonged exposure to HEMA, even at low concentrations, may result in immune suppression and clastogenic effects. Higher concentrations of this compound have been associated with significant reductions in IgG1 and IgM production and adverse effects on cell proliferation. HEMA exhibits genotoxic effects and toxicity on pulp cells and human gingival fibroblasts. Studies on RAW

264.7 macrophage cells indicate that HEMA induces apoptosis by increasing reactive oxygen species (ROS) levels or activating p53-dependent and ATM-dependent pathways, leading to cell cycle delays and DNA damage. In investigations involving human monocytes (THP-1) and peripheral blood mononuclear cells (PBMCs), HEMA exposure reduced IL-1β levels for up to 24 hours, disrupting post-transcriptional processes and cytokine release induced by lipopolysaccharide (LPS). Furthermore, HEMA impacts the innate immune system by markedly increasing IL-1β and IL-18 levels and promoting NLRP3 inflammasome formation (16,18).

1.3 Factors that influence toxicity

1.3.1 Light-curing

Light curing initiates a chemical reaction leading to the formation of a polymer network throught interaction acrylate resin monomers. However, the presence of oxygen in the oral cavity serves as a factor inhibiting this conversion of monomers to polymers, leading to the formation of oxygen inhibition layer (OIL) on the surface of resinous materials. Polymerization conversion consequently leads to the release of free monomers into the polymer network (5,14).

The factors that influence the amount of released monomers encompass the photopolymerization distance, wavelength, intensity, mode, and time. The degree of conversion typically varies between 50 and 70% during light-curing, reaching its maximum after 24 hours. Notably, in the first hour after light-curing, the conversion rate was 40%, indicating a greater likelihood of degradation in the oral cavity and subsequent release of toxic monomers to dentine, pulp, mucosa and periodontal tissues (12).

Research has been carried out to assess toxicity levels in relation to the power and type of polymerization light used. This study revealed that with increased exposure to radiation (ER >25 J/cm^2), the degree of conversion increased, promoting greater cell adhesion and a subsequent decrease in cytotoxicity, particularly observed during polymerization with blue and violet light. Previous studies have indicated that the cytotoxicity of these monomers diminishes when employed at high intensity for a short duration (12,19).

Regarding photopolymerization distance, evidence suggests that closer proximity yields superior biocompatibility and an increase in the curing distance between the composite surface and the light cure tip results in a reduction in radiant exposure of the light (20,21).

1.3.2 Type and size of the filler

Studies have shown that the size of fillers used in resin-matrix composites can impact toxicity. Over time, filler particle size has decreased within resin-matrix composites microstructure and currently filler particles size average 20-60nm up to 1-2 mm . Typically, fillers undergo treatment with a coupling agent to enhance bonding and stress transfer between fillers and the matrix. To investigate how particle size affects toxicity, a study was conducted using two different resin-matrix composites: Filtek™ Z250 and Filtek™ Z500 from 3M™ ESPE. Turbine drills with varying grit sizes were placed in a glass chamber to simulate drilling. The focus was on observing particle concentration, particularly very fine particles, released during drilling. Particle size distribution was analyzed using a Scanning Mobility Particle Sizer (SMPS). Particles were collected on a filter and characterized using dynamic light scattering (DLS) and scanning electron microscopy (SEM). Human bronchial epithelial cells (HBEC-3KT) were exposed to these loose particles to establish a dose-response relationship, and toxicity was assessed using the lactate dehydrogenase (LDH) assay and cell count kit-8 (CCK8). The study found that very fine particles released ranged between 15-35 nm in size, with most particles having a minimum size of 1 μm. Dynamic light scattering analysis revealed that these particles were larger and could agglomerate (4,22).

Various transparent mineral fillers are used to enhance the resin-matrix composites, to decrease shrinkage during the curing process and minimizing thermal expansion. Polymerization shrinkage is closely associated with the volumetric quantity of filler particles within the resin-matrix composite. Consequently, ongoing efforts are directed towards exploring new alternatives to enhance the performance of resin-matrix composites, with a primary focus on reducing polymerization shrinkage. Theoretically, increasing filler content has the

potential to alleviate polymerization shrinkage and its consequential contraction stress by reducing the volume of the organic phase (22–24).

1.3.3 Organic matrix composition

The quantity of BPA derivatives released, specifically the elution of this monomer, is dependent upon the organic matrix composition.The study with various resin-matrix composites with different organic matrix found that, Estelite Sigma Quick and SDR Plus Bulk Fill Flowable, exhibited highest concentration of eluted Bis-GMA under conditions simulating those found in oral cavity. Since resin-matrix composites vary in composition, the release of toxic monomers will also differ. For example, flowable resin-matrix composites, characterized by a higher proportion of organic matrix compared to packable resin-matrix composites, or higher amount of diluent monomers offer a larger surface area for contact, making them more susceptible to erosion and wear, and consequently leading to a higher potential release of these monomers (12,14).

1.4 Objetives

Knowing the high impact of the toxicity of the widely used resin-matrix composites, the aim of the present study is to perform a systematic review on the local and systemic toxicity of resin composites in humans.

The hypothesis of the present study was that the organic matrix monomers cause higher levels of toxicity within humans.

Materials and Methods

2. Materials and Methods

A systematic review aims to synthesize all existing and published literature on a given topic, consolidating information into a single document to facilitate the study and research of other professionals in the field. Through meticulous research utilizing outlined keywords and centered around a specific research question, we were able to produce a reasoned systematic review.

2.1 Registration protocol

This systematic review was registered on the PROSPERO (International Prospective Register of Systematic Reviews) platform, following the fundamental methodology outlined by the guidelines presented in PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) (25) with the registration number 480689 (Appendix 1).

2.2 Research question

A research question has been formulated with adherence to the PICO strategy, designated by P (population), I (intervention), C (comparison) and O (outcome).

The main objective of this systemic review is to understand:

Which factors(C) influence the toxicity (O) of dental composite resins (I) in humans (P), and what are their local or systemic adverse effects (O)?

2.3 Search strategy

The literature search was conducted across three scientific platforms, including Pubmed, Web of Science and Cochrane Central. The search filters employed specifically targeted articles in English, published in the last 20 years and studies conducted on humans.

In the PubMed search, both keywords and MeSH terms were used to refine and enhance the precision of the search. The tables below display the search terms employed in the different databases. Boolean operators, such as “AND”, “OR” were utilised.

Table 1- Keywords applied on Pubmed database

Table 1 Pubmed	
#1	(toxicity) OR (toxic)) OR (cytotoxicity)) OR (toxicology)) OR (BPA)) OR (Bis-GMA)) OR (Bisphenol-A)) OR (Bis-DMA)) OR (UDMA)) OR (TEGDMA)) OR (polymers)) OR (comonomers)) OR (copolymers)) OR (resin monomers)) OR (resin conversion)) OR (component release)) OR (bpa-derivatives)) OR (bisphenol a-glycidyl methacrylate)) OR (2,2-di(4-methacryloxyphenyl) propane)) OR (2,2-bis-(4-(2-methacryloxyethoxy)phenyl)propane)) OR (toxicology[MeSH Terms])) OR (Bisphenol A-Glycidyl Methacrylate[MeSH Terms])) OR (2,2-di(4-methacryloxyphenyl) propane[MeSH Terms])) OR (2,2-bis-(4-(2-methacryloxyethoxy)phenyl)propane[MeSH Terms])) OR (polymers[MeSH Terms])
#2	((((((((((resin-matrix composite) OR (resin composite)) OR (resin-based composites)) OR (dental composite resin)) OR (composite resins)) OR (resin-matrix composition)) OR (different composition resins)) OR (flow resin)) OR (bulk-fill resin composites)) OR (resin nanocomposites)) OR (conventional composite resin)) OR (composite resins[MeSH Terms])
#3	(((((Dentistry) OR (oral)) OR (mouth)) OR (dental)) OR (dentistry[MeSH Terms])) OR (mouth[MeSH Terms])
#4	((((((((((health) OR (oral health)) OR (human's health)) OR (biosafety)) OR (immunological effect)) OR (immune system)) OR (allergy)) OR (hypersensitivity)) OR (toxicity)) OR (cytotoxicity)) AND (release)
	#1 AND #2 AND #3 AND #4

Table 2 - Keywords applied on Web of science database

Table 2 Web of Science	
#1	ALL=(toxicity OR toxic OR cytotoxicity OR toxicology OR BPA OR Bis-GMA OR Bisphenol A OR Bis-DMA OR UDMA OR TEGDMA OR polymers OR comonomers OR copolymers OR resin monomers OR Resin conversion OR component release OR bpa-derivatives OR bisphenol a-glycidyl methacrylate OR 2,2-di(4-methacryloxyphenyl) propane OR 2,2-bis-(4-(2-methacryloxyethoxy)phenyl)propane)
#2	ALL=(resin-matrix composite OR resin composite OR resin-based composites OR dental composite resin OR composite resins OR resin-matrix composition OR different composition resins OR flow resin OR bulk-fill resin composites OR resin nanocomposites OR conventional composite resin)
#3	ALL=(Dentistry OR Dental OR oral OR mouth)
#4	ALL=(*in vitro* OR animal study OR cell culture OR tissue culture OR animal model OR laboratory animals OR cell cultivation OR *In Vitro* Techniques OR Animal Experimentation OR Cell Culture Techniques OR Tissue Culture Techniques
#5	ALL = (adhesive OR cement OR fillings OR orthodontic adhesive OR sealants OR adhesives OR Cements, dental OR Dental Sealants OR Pit and Fissure Sealants OR Root Canal Filling Materials
	#1 AND #2 AND #3 NOT #4 NOT #5

Table 3- Keywords applied on Cochraine database

Table 3 – Cochraine Central	
#1	toxicity OR toxic OR cytotoxicity OR toxicology OR BPA OR Bis-GMA OR Bisphenol A OR Bis-DMA OR UDMA OR TEGDMA OR polymers OR comonomers OR copolymers OR "resin monomers" OR "resin conversion" OR "component release" OR bpa-derivatives OR "bisphenol a-glycidyl methacrylate"
#2	"resin-matrix composite" OR "resin composite" OR "resin-based composites" OR "dental composite resin" OR "composite resins" OR "resin-matrix composition" OR "different composition resins" OR "flow resin" OR "bulk-fill resin composites" OR "resin nanocomposites" OR "conventional composite resin"
#3	dentistry OR Dental OR oral OR mouth
#4	"in vitro" OR "animal study" OR "cell culture" OR "tissue culture" OR "animal model" OR "laboratory animals" OR "cell cultivation" OR "In Vitro Techniques" OR "Animal Experimentation" OR "Cell Culture Techniques" OR "Tissue Culture Techniques"
#5	adhesive OR cement OR fillings OR "orthodontic adhesive" OR sealants OR Adhesives OR "Cements, dental"OR "Dental Sealants" OR "Pit and Fissure Sealants" OR "Root Canal Filling Materials"
	#1 AND #2 AND #3 NOT #4 NOT #5

2.4 Search filters

In the Web of Science and the Cochrane Central databases and search filters were English language and “human studies”. In the PubMed database a filter of “Randomised Controlled Trial (RCT)” was also used.

2.5 Inclusion and exclusion criteria

The inclusion criteria were *in vivo studies*, randomized controlled trial (RCT) and toxicity studies. The exclusion criteria were *in vitro* and animal studies, non-resin-matrix composite materials, systematic reviews with or metanalyses, narrative reviews clinical case reports and case series.

2.6 Selection process

The publications obtained from the databases (PubMed, Web of science and Cochraine Central) were exported to the Rayyan bibliography manager software(26), where duplicates were excluded.

Results

3. Results

A total of 1261 articles were obtained from the three databases: PubMed, Cochraine Central and Web of Science. Data was exported to the Rayyan bibliography manager software, where duplicates were excluded resulting in 1227 articles. After reading the titles and abstracts, 1206 articles were excluded, resulting in 20 articles selected for full reading. Of these, 12 were included and 8 excluded, as shown in Figure 1. Data was retrieved on the 10 of January 2024.

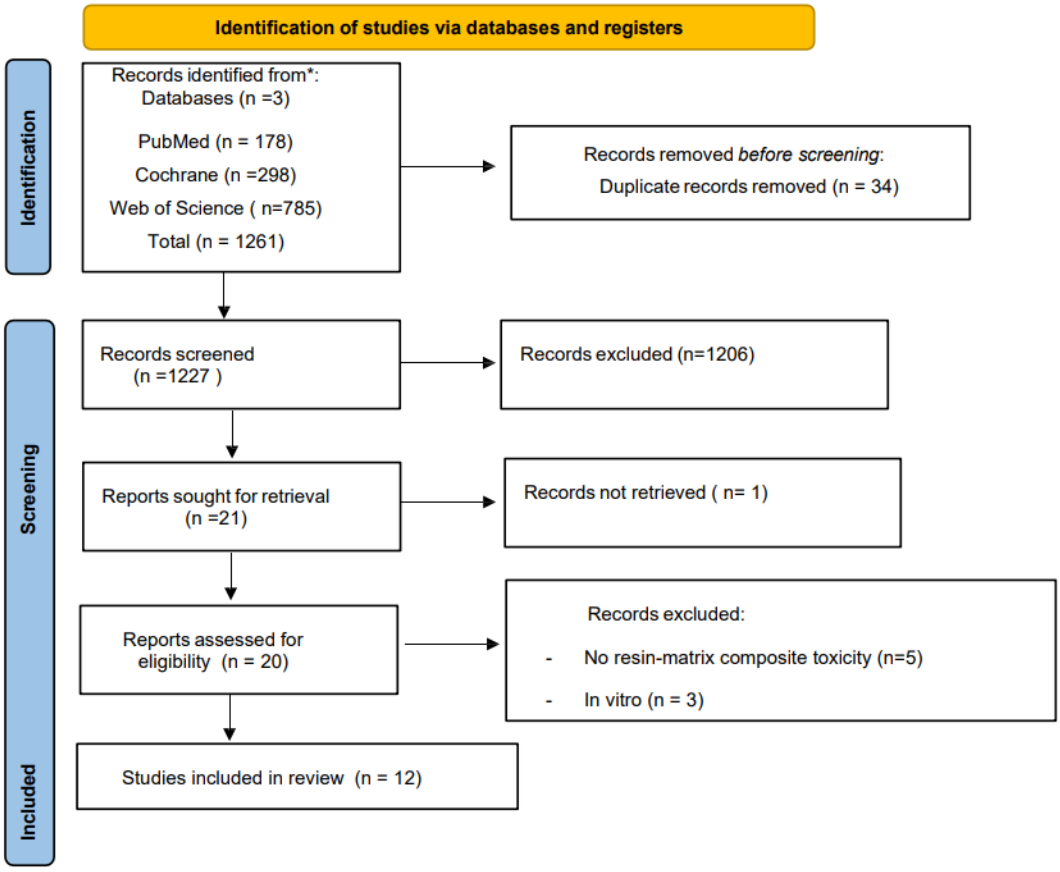


Figure 1- PRISMA flow diagram with research data

3.1 Data extraction

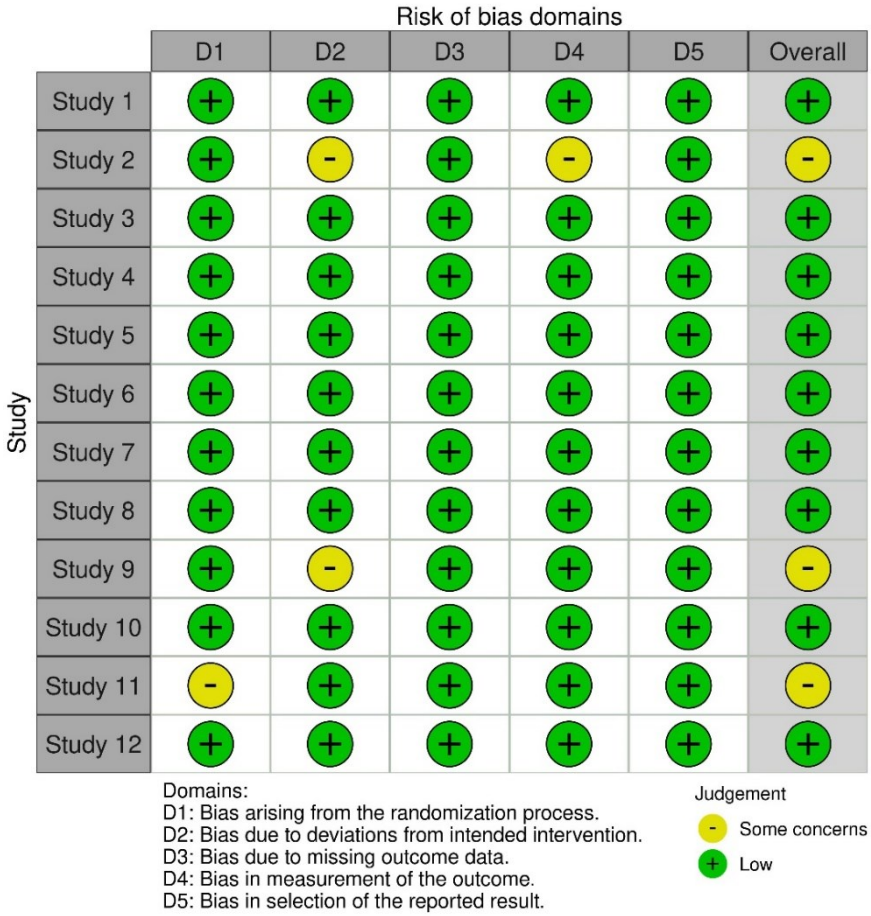
The study characteristics and numerical data were extracted using a predefined Excel file. The characteristics of the study (author, design, clinical setting, country, year), the characteristics of the patients (sample size, age) and the results were evaluated and listed on Table 5.

3.2 Inter-rater analysis and risk of bias Assessment

Cohen's kappa coefficient was used to assess the vagreement among researchers, giving in the first part of the selection (title and abstract) Cohen's k = 0.99 Almost perfect agreement (99.8%) and in the second part of the selection (full reading) Cohen's k = 0.89 Almost perfect agreement (95%).

The Cochrane Risk of Bias tool was used to assess risk of bias in the studies (Appendix 2).

Table 4 - The Cochrane Risk of Bias tool



In the present systematic review, all included articles investigate specific monomers toxicity attributed to resin-matrix composites. To evaluate the quantity of detected monomers and their associated toxicity levels, were performed analyses of saliva, urine and blood.

Regarding the analytical methodologies, two articles (16.66%) focused solely on urine analysis, three (25%) exclusively on saliva analysis, and another two (16.66%) concentrated on blood analysis (27–33). One article (8.33%)

examined both urine and saliva, while another (8.33%) evaluated both urine and blood (34,35).

The remaining three articles (23%) employed distinct toxicity assessment methods. One employed skin-patch testing, another employed neuropsychological tests, and the third applied tests to evaluate the physical development of children (36–38).

For these studies, various materials were used for analysis, such as resin-matrix composites, pit and fissure sealants, flowable composites, compomers and adhesives.

3.3 Resin composite

In five studies, Z100™ by 3M ESPE™ was used, which contained Bis-GMA and TEGDMA monomers (5-10 wt% each), except for one that only contained Bis-GMA (31,32,35,37,38). The resin-matrix domposite Z250™ from 3M ESPE™ was investigated in one study, which specified the Bis-GMA, Bis-EMA, TEGDMA and Urethane-dimethacrylate (UDMA) monomers. The study specifies Bis-GMA and TEGDMA at 1-5% and Bis-EMA and UDMA at 5-10% (33).

Only one study used Tetric Evoceram by Ivoclar-Vivadent™, however the complete composition details were not provided (34).

In one of the studies, Charisma™ by Heraeus Kulzer™ was evaluated, and the microstructure contained monomers such as, Bis-GMA and TEGDMA at a volumetric concentration of 58 v/v% and 78 wt% of inorganic fillers including barium aluminum, fluoride glass, and silicon dioxide. Additionally, Grandio™ by VOCO™ was investigated and included Bis-GMA, TEGDMA, and UDMA 71.4 v/v% and of 87 wt% of glass-ceramic particles (30).

One of the studies used seven different resin-matrix composites from the others studies. They were as follows: Progress™ by Kanebo™ and Metafil Flo™ by Sun Medical™ with UDMA and TEGDMA; Palfique Toughwell™ by Tokuyama™ and Xeno CFII™ by Sankin Kogyo™ only with Bis-GMA; Beautifil™ by Shofu™, Prodigy™ by Kerr™, and Clearfil ST™ by Kuraray™ with Bis-GMA and TEGDMA in their composition (31).

3.4 Flowable composite

In two studies, a Revolution™ by Kerr™ flowable resin-matrix composite was investigated, containing solely Bis-GMA monomers in its composition (32,35).

3.5 Compomer

In three studies, a Dyract™ by Dentsply™ compomer was employed. Its composition was different between studies. In one, UDMA and TEGDMA were the monomers contained in the composition , while in the other, only UDMA was registered. However,both studies contained 72 wt% of inorganic fillers (strontium-fluorosilicate-glass). In the third study it was specified composition of UDMA and trimethacrylate resins (32,37,38).

3.6 Pits and fissures sealant

A pits and fissures sealant was analyzed in two studies, using Ultraseal XT™ by Ultradent™. In both studies, Bis-GMA was present in their composition. The only difference was that in one of them also contained the diurethane dimethacrylate (DUDA) monomer (32,35).

3.7 Adhesive

In addition those materials, adhesives were also incorporated into two studies, each study employed a different adhesive. One applied Clearfil S3 Bond™ by Kuraray™, containing Bis-GMA and TEGDMA monomers at 71.4 vol% and 87 wt%, respectively, alongside glass-ceramic particles and colloidal silica as inorganic fillers. The other study employed Unifil™ by GC™, containing solely the UDMA monomer (30,31).

In the skin patch testing study, the following monomers were analysed MMA, TEGDMA, EGDMA, Bis-GMA, 2-hydroxy-ethylmethacrylate (2-HEMA) (0.2 % pet, Chemotechnique Diagnosis) and formaldehyde (0.1 %aq. Art. No. F002A, Chemotehnique Diagnostics) (36).

However, in three studies there were no specifications regarding the brands or types of materials used. Among these, two studies solely indicated the analysis of BPA concentration without specifying which monomers were analyzed. The

third study explicitly stated the analysis of Bis-GMA, but did not mention the specific material (27–29).

3.8 Light-curing

Three of the selected articles investigated the influence of light-curing on local or systemic toxicity. One study reported a light-curing duration of 20-30 seconds using the Satelec Mini LED light-curing unit, emitting light at 600-700 mW/cm² intensity within the 440-460 nm range (34). Another study employed a light-curing duration of 40 seconds with the Elipar Freelight II™ by 3M-ESPE™, emitting light at 1200 mW/cm² intensity within the 430-480 nm range (30).

Another study mentioned a light-curing duration of 60 seconds without specifying the light-curing unit or intensity used (31).

The study conducted by Lyapina et al. investigated skin tests involving different monomers. Their findings suggest that third and fourth-year dental students represent a demographic potentially susceptible to sensitization to MMA and TEGDMA alongside cross-sensitization between MMA and formaldehyde. This susceptibility is attributed to their systematic exposure to resin-matrix composites and adhesives. Furthermore, the study suggests that dental patients may also face a risk of cross-sensitization to formaldehyde and certain methacrylic monomers (36).

In the Maserejian et al. Study examining alterations in children's immune responses following resin-matrix composite treatment, important findings were observed. Significant alterations in B-cell reactivity were noted, while monocyte reactivity exhibited a decline within the initial six-month period. Moreover, these alterations were particularly pronounced in children with a higher prevalence of BisGMA-based composite restorations (32).

The study by Yıldız et al. investigated the correlation between resin-matrix composites and the release of substances into the oral cavity, assessing their impact on lipid peroxidation and DNA oxidation through blood circulation monitoring. Results revealed a notable increase in the 8-OHdG/106 dG ratio within the composite filling cohort. Furthermore, Bis-GMA and TEGDMA were found to heighten markers indicative of lipid peroxidation and DNA oxidation (33).

Across the array of selected studies, concentrations of both BPA and monomers were not observed to reach levels deemed sufficiently high to elicit either local or systemic toxicity. Consequently, it was deduced that exposure to BPA within resin-matrix composites restorations remains low, thus limiting the potential risk of adverse effects. Furthermore, no significant association was found between concentration levels and the quantity of resin restoration surfaces, nor substantial alterations were noted in the variables evaluated across the studies (27–31,34,35,37,38).

The summary of the relevant studies included in this systematic review can be consulted in Table 5.

Table 5 - Summary of the relevant studies included in this systematic review

Authors Year Country	Sample size /Age range	Main objectives	Resin-based materials	Methodology	Monomers source materials	Local or systemic toxicity	Main outcomes
Lyapina et al. 2014, Bulgaria (36)	139 individuals divided into four groups: dental professionals (mean age 52.32 years), 3rd and 4th-year dental students (mean age 22.05 years), 6th-year students (mean age 26.39 years), and patients with no acrylate exposure (mean age 47.43 years).	Investigate the prevalence and risk of sensitization to methacrylate monomers and formaldehyde among different dental groups.	Resin based materials (composite resins and dental adhesives), not specified.	Employed a questionnaire and skin patch testing for MMA, TEGDMA, EGDMA, Bis-GMA, 2-HEMA, and formaldehyde. Patch tests used hypoallergenic patches and followed ICDRG standards	-MMA TEGDMA,EGDMA Bis-GMA , (2-HEMA), THFMA and formaldehyde	Local – oral administration (resin-matrix restorations) Systemic - allergic reactions	Risk of sensitization to MMA and TEGDMA in dental students; dental patients may risk cross-sensitization to formaldehyde and methacrylic monomers.
Berge et al.,2016 Norway (29)	Forty individuals aged between 20 and 35	Measure the concentration of BPA in saliva to determine the impact of dental composite fillings	Resin-matrix composites, though detailed compositions were unspecified	BPA levels were measured using LC/MS from saliva samples stored at -80ºC.	It doesn't specify which monomers it is analyzing, only BPA (bisphenol A)	Local - oral administration (resin-matrix restorations)	Exposure to BPA is low Potential risk of adverse effects is limited.

Authors Year Country	Sample size /Age range	Main objectives	Resin-based materials	Methodology	Monomers source materials	Local or systemic toxicity	Main outcomes
Berge et al. 2019 Norway (34)	20 patients requiring restorations involving 2 or more surfaces (mean age 16-40 years).	Examine BPA levels in saliva and urine pre- and post-dental treatment with resin-matrix composites.	Resin Composite Tetric EvoCeram by Ivoclar-VivadentTM, A2, Schaan,Liechtenstein. Complete composition details were not provided.	-Rubber dam was not used -Light-curing: 20-30 s, Satelec Mini LED; Aceton, Meriganac, France, 600–700 mW cm2,440–460 nm Samples were collected before, 10 min, 1,24 h nd 1 week post-treatment and analyzed using LC/MS.	Bis-GMA	Local – oral administration (resin-matrix restorations) Systemic - BPA concentration in the urine.	Increase in BPA concentration in saliva after resin matrix restorations (initially). A decrease in BPA concentration exponentially over time. No changes in urine.
Chung et al. 2012 South Korea (27)	495 children Mean age 8-9 years	Explore the correlation between urinary BPA levels and the presence of composite dental restorations and sealants.	Unspecified composite materials were used, resin-matrix composite and pit sealant	The number of surfaces classified as: 'none', '1-5', '6-10' and '11 or more'. Urine was collected and adjusted to creatinine by dividing the amount of BPA in the urine by the amount of creatinine, stored at 80ºC. BPA was determined with liquid chromatography.	It doesn't specify which monomers it is analyzing, only BPA	Local – oral administration (resin-matrix restorations) Systemic -BPA concentration in the urine	Higher BPA concentration with more than 11 surfaces restored; no significant association with the number of surfaces restored.

Authors Year Country	Sample size /Age range	Main objectives	Resin-based materials	Methodology	Monomers source materials	Local or systemic toxicity	Main outcomes
Trachtenberg et al. 2014 United States of America (35)	5116 children screened, 534 included (mean age 6-10 years).	Investigate whether increased exposure to BPA from resin-based composites is linked to adverse renal function outcomes.	Resin composite Z100, 3M ESPE™, St.Paul, MN. Composition: Bis-GMA (5-10 wt%), TEGDMA(5-10 wt%) Sealant of pits and fissures Ultraseal XT by Ultradent ™ (South Jordan, UT) Composition: Bis-GMA Flowable composite Revolution by Kerr ™ (Orange, CA) Composition: not specified	Samples of urine and blood wereinspected for mercury and renal function were discarded. The analysis was preformed for the following renal markers:Gamma-GT, Albumin, Low-grade albuminuria and NAG.	Bis-GMA TEGDMA	Local - oral administration (resin-matrix restorations) Systemic - evaluate BPA related to with adverse renal function	No significant changes in renal function markers at a 5-year follow-up.
Maserejian et al. 2012 United States of America (37)	5116 children screened, 534 included (mean age 6-10 years).	To assess if exposure to resin-based composites affects neuropsychological development.	Resin composite Z100 composite by 3M ESPE™ (St. Paul, Minn) Composition: bis-GMA and TEGDMA Compomer Dyract by Dentsply™ Caulk, Milford,Del) Composition: UDMA and TEGDMA with 72 wt % of inorganic fillers (strontium-fluorosilicate-glass)	Both number of teeth with caries (2-4 vs.5) or geographic location were evaluated. Children underwent neuropsychological testing and physical measurements, with data analyzed for any developmental changes.	Bis-GMA UDMA TEGDMA Bis-DMA BADGE	Local - oral administration (resin-matrix restorations) Systemic - evaluate BPA associated with neuropsychological development	No significant associations between composite resins and changes in neuropsychological tests at a 4/5-year follow-up. There may be small adverse effects when there is greater exposure to these materials.

Authors Year Country	Sample size /Age range	Main objectives	Resin-based materials	Methodology	Monomers source materials	Local or systemic toxicity	Main outcomes
Maserejian et al. 2012 United States of America (38)	534 children (mean age 6-10 years).	To determine if resin-based composites influence physical development.	Resin Composite Z100 composite by 3M ESPE™(St. Paul, MN, USA) Composition: Bis-GMA Compomer Dyract AP, by Dentsply Caulk ™ (De Trey, Konstanz, Germany) Composition: UDMA with 72 wt % of inorganic fillers (strontium-fluorosilicate-glass)	Stratified by number of teeth with caries (2-4 vs. ≥ 5) and rural/urban location. Through data collector (NECAT) height, weight and body fat percentage were investigated annually. BMI and BMI z-score were calculated.	Bis-GMA BADGE TEGDMA UDMA	Local - oral administration (resin-matrix restorations) Systemic - evaluate BPA associated with physical development	No significant changes in the variables studied.
Maserejian et al. 2014 United States of America (32)	For immune sub study: 257 children -198 children were excluded - 59 children were included (29 amalgam and 30 composite)	Examine changes in immune function related to resin composite treatments.	Resin Composite Z100 composite by 3M ESPE™(St. Paul, MN, USA) Composition:Bis-GMA and TEGDMA Compomer Dyract AP, by Dentsply Caulk ™ (Milford, Delaware) Composition: UDMA and trimethacrylate resins	Imune markers were measured at 5-7 days, 6, 12, 18 months and 5 years. Immune markers: white blood cell (WBC), T-cell, B-cell , neutrophil and monocyte.	Bis-GMA TEGDMA UDMA DUDA	Local - oral administration (resin-matrix restorations) Systemic - evaluate BPA associated with imune function.	More Bis-GMA based composite restorations during the first 6 months: Changes in B cell responsiveness were greater Monocyte responsiveness decreased

	Mean age 6-10 years		<u>Sealant of pits and fissures</u> Ultraseal XT by Ultradent ™ (South Jordan, UT) Composition: Bis-GMA and DUDA <u>Flowable composite</u> Revolution by Kerr ™ (Orange, CA) Composition: Bis-GMA				
Gul et al. 2017 Turkey (30)	18 male participants Mean age 19-24 (21.11 ± 1.32) years	To investigate BPA levels in saliva and serum and their association with hormonal levels.	<u>Resin Composite</u> Charisma by Heraeus Kulzer ™ GmbH (Hanau, Germany) Composition: Bis-GMA, TEGDMA with 58 vol%, 78 wt% of inorganic fillers Barium aluminum, fluoride glass Silicium dioxide Grandio by VOCO ™ GmbH Cuxhaven, Germany Composition: Bis-GMA, TEGDMA, UDMA with 71.4 vol%, 87 wt% of inorganic fillers:glass-ceramic particles <u>Adhesive</u> Clearfil S3 Bond by Kuraray™,Okayama, Japan Composition:	Light-curing: 40 s LCU :Elipar Freelight II, 3M-ESPE™ Dental Products emitted 1200 mW/cm2 light intensity at a range of 430-480nm Blood and urine samples were taken and stored at 80ºC. Liquid chromatography (HPLC) with a UV detection.	Bis-GMA TEGDMA UDMA HEMA	Local - oral administration (resin-matrix restorations) Systemic - evaluate BPA in saliva and serum association with hormone levels	BPA levels remained below harmful thresholds, with no impact on hormonal balance.

			Bis-GMA, HEMA with 71.4 vol%, 87 wt%, Glass-ceramic particles colloidal silica of inorganic fillers				
Authors Year Country	**Sample size /Age range**	**Main objectives**	**Resin-based materials**	**Methodology**	**Monomers source materials**	**Local or systemic toxicity**	**Main outcomes**
Yıldız et al. 2017 Turkey (33)	41 participants Mean age 17-23 years	To study the effects of resin materials on lipid peroxidation and DNA oxidation.	Resin Composite Filtek Z250 by 3M ESPE™ Dental Products St Paul, MN, USA) color A2, lot number N152614. Composition: TEGDMA 1-5%, Bis-GMA 1-5%, Bis-EMA 5-10%, and UDMA 5-10%.	Blood samples were analyzed pre and post-application- for Bis-GMA and TEGDMA detection HPLC was preformed HPLC with electrochemical (HPLC-ECD) was preformed to determine 8-OHdG (8-hydroxydeoxyguanosin and dG levels in the hydrolyzed DNA samples HPLC with fluorescent detection (HPLCFLD) was used to determine MDA (malondialdehyde) concentrations in blood samples .	Bis-GMA TEGDMA UDMA Bis-EMA	Local - oral administration (resin-matrix restorations) Systemic – effects in lipid peroxidation and DNA.	Significant increases in markers of lipid peroxidation and DNA damage post-treatment.

Authors Year Country	Sample size /Age range	Main objectives	Resin-based materials	Methodology	Monomers source materials	Local or systemic toxicity	Main outcomes
Sasaki et al. 2005 Japan (31)	21 participants No mention of participants' ages	To monitor changes in salivary BPA concentration post resin restoration.	<u>Resin composite</u> Z100 composite by 3M ESPE™(St. Paul, MN, USA) Composition: Bis-GMA and TEGDMA Progress by Kanebo™, Ltd., (Tokyo, Japan) Composition: UDMA and TEGDMA Palfique Toughwell By Tokuyama ™ Corp., (Tokyo, Japan) Composition: Bis-GMA Metafil Flo by Sun Medical ™ Co., Ltd., (Shiga, Japan) Composition: UDMA and TEGDMA Beautifil by Shofu™, (Kyoto, Japan) Composition: Bis-GMA and TEGDMA Xeno CFII by Sankin Kogyo™ (Tochigi, Japan) Composition: Bis-GMA	Each cavity was then filled with 0.1 g of composite resin. Light-curing: 60s Polishing: silicon points BPA levels were assessed using an ELISA kit after restoration	Bis-GMA TEGDMA UDMA	Local - oral administration (resin-matrix restorations)	Generally low BPA levels except with Beautifil, indicating low toxicity with Bis-GMA-free materials.

			Prodigy by Kerr™ Orange, CA) Composition: Bis-GMA and TEGDMA Clearfil ST by Kuraray™ (Okayama, Japan) Composition: Bis-GMA and TEGDMA <u>Adhesive</u> Unifil S by GC™ Corp., (Aichi, Japan) Composition: UDMA				
Authors Year Country	**Sample size /Age range**	**Main objectives**	**Resin-based materials**	**Methodology**	**Monomers source materials**	**Local or systemic toxicity**	**Main outcomes**
Lyapina et al. 2016 Bulgaria (28)	84 participants - 55 students of dental medicine (25 male and 30 female, mean age 25.8 ± 4.7 years) - 29 dental patients (13 male and 16 female, mean age 42.9 ± 15.3 years) – control group	To assess urinary BPA levels in dental students and patients treated with BPA-containing materials.	Resin composite containing ingredients based on Bis-GMA, not specified.	Sample first-morning urinary BPA levels was performed by Creative Diagnostics (USA) competitive Bisphenol A ELISA kit (detection limit, < 10 pg/ml, Prod. No.: DEIA493). It has been measured using human biomonitoring (HBM) for exposure and health risk assessment.	Bis-GMA	Local- oral administration (resin-matrix restorations)/handling of BPA-containing products in clinical practice Systemic - BPA concentration in the urine	Higher urinary BPA levels in patients, though not necessarily indicative of adverse health effects.

Discussion

4. Discussion

The present systematic review reported the major findings from previous studies regarding the toxicity of resin-matrix composites. This study gathered relevant information on the composition of resin-matrix composites and both local and systemic toxicity. It also covered *in vivo*, methodologies for measuring these toxicities identified factors associated with resin composites toxicity, such as lower light-curing time exposure, and collected preventive measures to reduce the potential toxicity of resin-matrix composites. The findings of the present research do not validate the study hypothesis.

4.1 Resin-matrix composites and toxicity

The quantity of monomers released by resin-matrix composites may be influenced by the polarity of the solvent, the polymerization degree of the material, and variations in the chemical structure and filler-monomer ratio, all of which significantly impact on monomer release and the cytotoxicity of the material. Additionally, the porosity of the material and the thickness of the sample play pivotal roles in the elution process (39).

However, in some studies high release of Bis-GMA was detected regardless of polymerization time, storage conditions or type of material, making it more difficult to prevent. Nonetheless, may offer potential protocols to mitigate their possible toxicity (39,40).

In the study conducted by Sideridou et al (41), it was observed that UDMA and Bis-GMA exhibited significantly greater initial polymerization reactivity compared to TEGDMA and Bis-EMA. Within 10 seconds of polymerization, UDMA and Bis-GMA promoted reaction percentages of 49.5% and 22.9% of their double bonds, respectively, whereas TEGDMA and Bis-EMA only reached 13.7% and 13.5%, respectively. However, as polymerization progressed, TEGDMA and Bis-EMA displayed a markedly higher rate of polymerization than UDMA and Bis-GMA, Figure 2. Ultimately, TEGDMA exhibited the highest conversion. This phenomenon may be due differences in the chemical structure of the linking group between the methacrylate groups. Consequently, the degree of conversion followed the sequence: Bis-GMA < Bis-EMA < UDMA < TEGDMA. It is noteworthy the distinct chemical properties and reactivity potential between Bis-GMA and

TEGDMA, with Bis-GMA being considered more reactive due to its steric structure, which hinders a higher degree of conversion. These findings align with the investigation of Geurtsen et al. (42) since TEGDMA exhibits the highest degree of monomer conversion, and for that reason is considered to be less prone to toxicity. Conversely, Bis-GMA, characterized by a lower degree of conversion, probably due to higher monomer viscosity, is associated with a heightened level of toxicity (33,39,41,43).

It has been established that the most likely monomer to be released is HEMA, probably due to its small size and low molecular weight. Following HEMA, TEGDMA is second more prone to released, whereas Bis-GMA is released in comparatively lesser concentrations, particularly in water-based formulations. The higher molecular weight of Bis-GMA, combined with its substantial dimensions and limited solubility in water, elucidates the variance in the observed values. Nevertheless, it has been observed that greater monomer release occurs in an organic solvent environment compared to a water-based solution (44). Cytotoxicity is also influenced by molecular weight, higher molecular weights correspond to greater cytotoxicity. Given the molecular weights of prevalent monomers as follows: HEMA (130 g/mol), TEGDMA (286 g/mol), UDMA (471 g/mol), and Bis-GMA (512 g/mol), the variance in molecular weight can be indicative of cytotoxic potential (33,42).

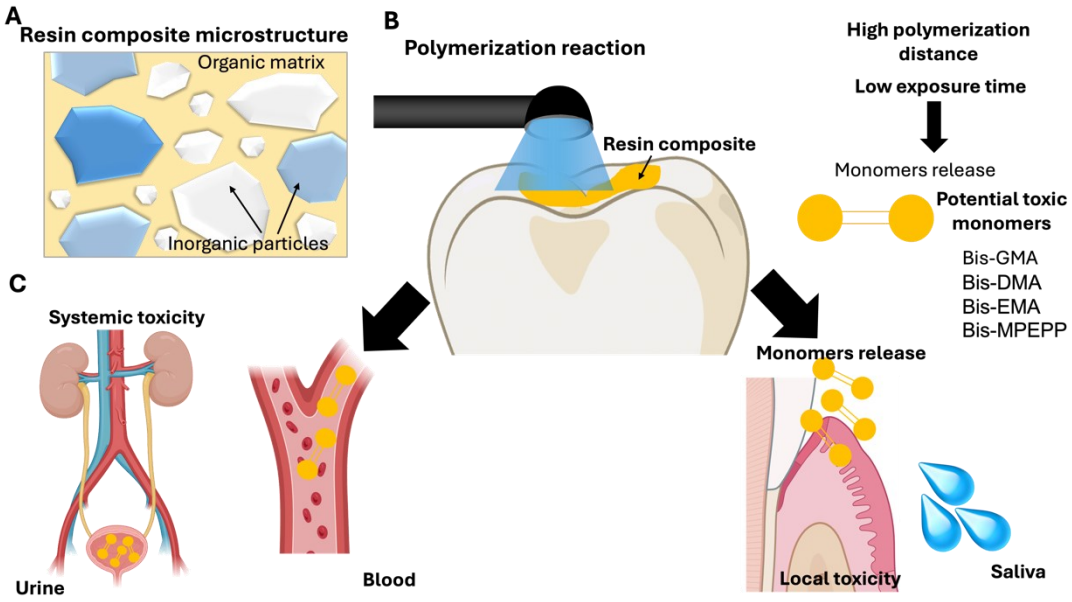


Figure 2- Schematics of monomers release potential toxicity (original image by Maria Cordeiro)

As demonstrated in the investigation by Wataha JC et al.(45), flowable resin-matrix composites characterized by reduced filler content and elevated monomer concentration in comparison to conventional materials displayed an increased in vitro cytotoxicity. This phenomenon may be attributed to the increased organic matrix content of flowable composites, and lower inorganic content comparatively to conventional resin-matrix composites that cause lower mechanical properties, facilitating increased degradation. However, conventional resin-matrix composites, when applied in thick layers, can achieve an incomplete polymerization, leading to increased release of residual monomers (45,46).

In the investigation conducted by Bakopoulou et al., that analyzed the impact on the induction of sister chromatid exchange (chromosome aberrations), as well as effects on cell cycle kinetics and mitotic indices of human peripheral lymphocytes, several conclusions were drawn. Notably, Tetric Ceram exhibited the highest frequency of SCE induction. Filtek Z250™ was associated with cell cycle delays and a reduction in the mitotic index. Furthermore, the genotoxicity and cytotoxicity rankings were observed as follows: Tetric Ceram™ > Filtek Z250™ > Simile™ > Adoro = Conquest Sculpture™ (47).

4.2 Analysis of the methodologies used to toxicity analysis

Toxicity analysis primarily centered on the quantification of BPA levels. However, methodologies varied across studies. Skin patch testing was conducted using adhesive patches containing methacrylic monomers and formaldehyde. Participants were free from antiallergic medication. Readings were taken on day 2 post-patch removal, with a follow-up on day 3. Reactions were interpreted according to the International Contact Dermatitis Research Group method, classifying them as negative, doubtful, weakly positive, strongly positive, extremely positive, or irritant (36).

To assess toxicity in saliva, several methodologies were adopted.

One involved using a triple quadrupole linear ion trap mass spectrometer (QTRAP 5500; AB Sciex, Foster City, CA, USA) coupled with liquid chromatography. Saliva was added to an isotopically labeled internal standard for BPA (D16-BPA) (29,34).

In another study, BPA was assessed by 'EIKEN' BPA ELISA kit (Eiken Chemical Co. Ltd., Tokyo, Japan), a competitive enzyme-linked immunosorbent assay (ELISA) specifically designed for quantifying BPA levels in biological samples such as serum and plasma (31).

In the three studies assessing toxicity regarding the immunological functions, physical and neuropsychological development of children, a consistent methodology was employed. Exposure levels were evaluated using ”surface years“ (years of restorative material present in the mouth), calculated for each material by weighting each treated surface by the years present in the mouth during follow-up. Sensitivity analyses considered total surface years and the number of existing filled surfaces to assess potential confounding by factors related to the severity of dental disease. Additionally, efforts were made to determine if results for resin-matrix composite treatment levels were influenced by confounders related to the severity of dental disease rather than the treatment material itself. Multivariable linear mixed-effects models with repeated measures of immune function were employed to estimate the association between exposure to resin-matrix composites and changes in immune function from baseline (32,37,38).

As previously indicated, urine analysis served as one of the methodologies employed to assess toxicity, with variations observed across the studies examined.

In a particular study aimed at total BPA analysis in urine samples, 200 µl aliquots were subjected to digestion with 10 µl of glucuronidase and supplemented with 1 ng of D16-BPA (34,48).

In another study, urinary BPA levels were adjusted for creatinine concentrations. The pretreated sample was analyzed using high-performance liquid chromatography triple tandem mass detector (HPLC-ESI MS/MS, Agilent 6410) (27,49).

A different methodology was also used in another study, Gamma-glutamyl transpeptidase (gamma-GT) levels were assessed at the beginning of study and annually over the five-year experimental period. Gamma-GT was quantified using the dimension reagent cartridge of Dade Behring's GGT Flex system. Albumin was quantified via an automated immunochemical nephelometric method

employing reagents and calibrators from Beckman Coulter. Total NAG was determined using an automated photometric method utilizing reagents and calibrators. Urinary creatinine was quantified using the Jaffe photometric method (35,50).

Another methodology involved utilizing the competitive Bisphenol A ELISA kit from Creative Diagnostics (USA) to quantify BPA levels in biological samples (detection limit, < 10 pg/ml, Production number: DEIA493). This method entailed measuring morning urinary BPA levels using a single sample (28).

In two studies, the HPLC (high-performance liquid chromatography) system was utilized. In one study, both saliva and blood samples were analyzed, while in the other study, only blood samples were analyzed. Additionally, in one of the studies, HPLC was used exclusively to detect Bis-GMA and TEGDMA (30,33,51,52).

4.3 Prevention of BPA-associated toxicity

Although in the selected studies BPA toxicity did not reach toxicity levels, it is crucial to prevent the resin-matrix composites toxicity during a dental restoration, via polymerization exposure time, polymerization distance that can lead to incomplete polymer chain formation increasing the hazerd of local and systemic toxicity (21).

Several factors have been identified that influence the degree of monomer conversion enhancing toxicity. These factors include the colour of the resin-matrix composite, the type of filler, the thickness of the increment, the duration of light curing, LCU (light-curing unit) distance and angulation, and the light unit system used. Adequate polymerization is considered the primary criterion for a successful restoration (21,53).

Regarding the colour of the resin-matrix composites, several studies have shown that translucency may influence polymerization time. Specifically, a longer polymerization time is recommended for darker shades. More translucent resin-matrix composites allow for greater light transmission and, consequently, greater monomer conversion. Therefore, for darker shades, a longer polymerization time is advisable to achieve adequate polymerization and enhance the degree of monomer conversion (54,55).

In dark shades resin-matrix composites regarding the duration of adequate curing time, it has been demonstrated that extending the exposure to light beyond the manufacturer's recommended curing time by an additional 10-20 seconds has positive effects on the degree of monomer conversion (21,56).

The thickness of the increment also influences the degree of conversion. An increment thickness greater than 2 mm results in a decrease in the degree of conversion and requires polymerization for 60 seconds at an irradiance of at least 400 mW/cm². However, it is important to note that in Bulk-fill resin-matrix composite, increments of 2 to 4 mm can be placed while maintaining the appropriate degree of conversion. This is due to the greater translucency and consequently greater light transmission compared to conventional resin-matrix composites (57,58).

It has been demonstrated that the type of monomer, filler type, filler content, and the refractive index of the polymer matrix and filler significantly influence the ability of light to transmit through the resin-matrix composites layers (21,59).

Bulk-fill resin-matrix composites exhibit larger filler sizes (> 20 μm), leading to a reduced filler-matrix interface, thereby enhancing the transmission of polymerization light and resulting in a higher degree of conversion. Additionally, these materials have lower filler quantities, which contribute to increased translucency and, consequently, greater monomer conversion compared to flowable and conventional resin-matrix composites (60,61).

- Employing rubber dams

One study found that employing rubber dams reduces BPA levels in saliva. Results indicated that concentrations of BPAHPE and Bis-GMA within one hour post-treatment were two to four times higher in participants without a rubber dam compared to those with one. However, it is noteworthy that between 1-8 hours post-treatment, BPA concentrations in saliva returned to pre-treatment levels (17,62).

- Application of pumice on a cotton ball or in a rotating rubber dental prophylaxis cup

In another investigation, the application of pumice on cotton or within a rotary rubber dental prophylaxis cup was found to be notably effective in mitigating the absorption of Bis-DMA, Bis-GMA, and TEGDMA compared to dry or moist cotton rubbing, or the use of an air/water spray (17).

- Light-curing exposure time > 40s

A study concluded that with a short polymerization time of no more than 40 seconds and an oral cavity temperature of approximately 37°C, resin-matrix composites are never fully polymerized. The propagation of the cross-linking reaction significantly reduces the mobility of monomers, preventing complete polymerization (41,44).

- Light-curing unit distance and angulation

The optimal distance is 0 mm, not exceeding a maximum of 7 mm, with the angle maintained at 90º, perpendicular to the resin-matrix composite surface. (21,63).

- Rinse for 30 seconds with water

Evidence suggests that rinsing with water for 30 seconds after polymerization decreases the amount of BPA released in saliva. For children unable to rinse or spit, it is recommended to use an air/water syringe immediately after applying resin-matrix composites (17,31).

- Adequate personal protective equipment

Enhanced risk management strategies and more effective occupational health and safety programs are advised to facilitate the use of appropriate personal protective equipment for individuals, including students and professionals, who are exposed to methacrylates. This is crucial for mitigating potential hazards associated with skin allergies triggered by the evaluated monomers (36).

Several limitations of the study can be identified. Firstly, the analysis was limited to specific monomers, excluding others that may also be potentially toxic. More high-quality studies, particularly randomized controlled clinical trials (RCTs), are necessary. Additionally, the heterogeneity of resin composite materials poses a challenge. There are confounding factors in measuring BPA levels, considering its presence in food and the environment. Furthermore, long-term assessments are essential to accurately confirm the analyzed results. Due to time constraints, other databases such as EMBASE and Scopus were not included in the analysis.

Conclusion

5. Conclusion

This systematic review shows that low levels of free monomers, among participants with resin-matrix composites restorations were detected .

In all the selected studies, the presence of toxicity did not affect the patient's health. However, some precautionary measures must be followed when using resin-matrix composites to increase the degree of monomer conversion: adequate photopolymerization and appropriate exposure time. Reducing the release of free monomers, leads to insignificant toxicity levels.

Human exposure to BPA has multiples sources, such as dietary and environmental, which act as confounding factors. Further research is needed to establish causality, particularly considering exposure to non-dental sources of BPA.

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7. Appendix

Appendix 1 - PROSPERO

PROSPERO
International prospective register of systematic reviews

NHS
National Institute for Health Research

UNIVERSITY of York
Centre for Reviews and Dissemination

Systematic review

*Fields that have an **asterisk (*)** next to them means that they **must be answered. Word limits** are provided for each section. You will be unable to submit the form if the word limits are exceeded for any section. Registrant means the person filling out the form.*

This record cannot be edited because it has been marked as out of scope

2. Original language title.

For reviews in languages other than English, give the title in the original language. This will be displayed with the English language title.

Toxicity of resin-matrix composites in dentistry - A systematic review

3. * Anticipated or actual start date.

Give the date the systematic review started or is expected to start.

16/01/2024

4. * Anticipated completion date.

Give the date by which the review is expected to be completed.

06/06/2024

5. * Stage of review at time of this submission.

This field uses answers to initial screening questions. It cannot be edited until after registration.

Tick the boxes to show which review tasks have been started and which have been completed.

Update this field each time any amendments are made to a published record.

The review has not yet started: No

Review stage	Started	Completed
Preliminary searches	Yes	No
Piloting of the study selection process	Yes	No
Formal screening of search results against eligibility criteria	No	No
Data extraction	No	No
Risk of bias (quality) assessment	No	No
Data analysis	No	No

Provide any other relevant information about the stage of the review here.

7. * Named contact email.

Give the electronic email address of the named contact.

9. Named contact phone number.

Give the telephone number for the named contact, including international dialling code.

11. * Review team members and their organisational affiliations.

Give the personal details and the organisational affiliations of each member of the review team. Affiliation refers to groups or organisations to which review team members belong. **NOTE: email and country now MUST be entered for each person, unless you are amending a published record. PLEASE USE AN INSTITUTIONAL EMAIL ADDRESS IF POSSIBLE.**

Miss Maria Cordeiro. Faculty of Dental Medicine - Universidade Católica Portuguesa
Professor Patrícia Correia. Faculty of Dental Medicine - Universidade Católica Portuguesa
Professor Rita Pereira. Faculty of Dental Medicine - Universidade Católica Portuguesa
Professor Ana Gomes. Faculty of Dental Medicine - Universidade Católica Portuguesa

13. * Conflicts of interest.

List actual or perceived conflicts of interest (financial or academic).
None

14. Collaborators.

Give the name and affiliation of any individuals or organisations who are working on the review but who are not listed as review team members. **NOTE: email and country must be completed for each person, unless you are amending a published record.**

16. * Searches.

State the sources that will be searched (e.g. Medline). Give the search dates, and any restrictions (e.g. language or publication date). Do NOT enter the full search strategy (it may be provided as a link or attachment below.)

PubMed, Scopus, Cochrane and Web of Science

18. * Condition or domain being studied.

Give a short description of the disease, condition or healthcare domain being studied in your systematic review.

There are several factors that increase the toxicity of composite resins, such as the type of light curing process, the type and size of filler, and the varying composition of the matrix.

20. * Intervention(s), exposure(s).

Give full and clear descriptions or definitions of the interventions or the exposures to be reviewed. The preferred format includes details of both inclusion and exclusion criteria.

The toxicity of resins varies due to multiple factors that enhance their potential harm. Concerns have been raised regarding the potential toxicity of composite resins, primarily due to the presence of free monomers capable of inducing toxicity. These factors include light curing conditions (such as distance, wavelength, intensity, and curing time), as well as the type of composite used, which can have various systemic implications, impacting various organs and significantly jeopardizing overall health.

22. * Types of study to be included.

Give details of the study designs (e.g. RCT) that are eligible for inclusion in the review. The preferred format includes both inclusion and exclusion criteria. If there are no restrictions on the types of study, this should be stated.

Inclusion criteria: primary research studies, such as randomized controlled trials, case-control studies and cohort studies.

24. * Main outcome(s).

Give the pre-specified main (most important) outcomes of the review, including details of how the outcome is defined and measured and when these measurement are made, if these are part of the review inclusion criteria.

There are several factors affecting the toxicity of dental resin composites. Some studies have found that the type of light-curing process used, the size and charge of the particles and the different types of resin matrix. Light-curing is a chemical reaction that ultimately results in a polymer network of interacting acrylate resin monomers. These materials contain some monomers that were not able to complete the light-curing process and have been shown that not only the type of light curing, but also the type and size of filler material affect toxicity.

Toxicity of the particles was demonstrated, but only 48 to 72 hours after initial exposure and at the highest doses. With regard to the different types of resin matrix, it was concluded that the amount of free and residual monomers released by Bis-GMA depends on the type of composite, and therefore there will be a higher level of toxicity in some and a lower level in others.

26. * Data extraction (selection and coding).

Describe how studies will be selected for inclusion. State what data will be extracted or obtained. State how this will be done and recorded.

Study characteristics and numerical data will be extracted using predefined excel file. Study characteristics (design, clinical setting, country), patient characteristics (number of dental composite resins-treated teeth, sex, age) and outcomes will be assessed.

28. * Strategy for data synthesis.

Describe the methods you plan to use to synthesise data. This but should be and describe how the proposed approach will be applied to your data. If meta-analysis is planned, describe the models to be used, methods to explore statistical heterogeneity, and software package to be used.

The data will be presented according to the papers/study design, as stated previously, in a table format and a narrative summary will be conducted.

30. * Type and method of review.

Select the type of review, review method and health area from the lists below.

Type of review

Cost effectiveness

No

Diagnostic

No

Epidemiologic

No

Individual patient data (IPD) meta-analysis

No

Intervention

No

Living systematic review

No

Meta-analysis

No

Methodology

No

Narrative synthesis

No

Network meta-analysis

No

Pre-clinical
No

Prevention
No

Prognostic
No

Prospective meta-analysis (PMA)
No

Review of reviews
No

Service delivery
No

Synthesis of qualitative studies
No

Systematic review
Yes

Other
No

Health area of the review

Alcohol/substance misuse/abuse
No

Blood and immune system
No

Cancer
No

Cardiovascular
No

Care of the elderly
No

Child health
No

Complementary therapies
No

COVID-19
No

Crime and justice

No

Dental
Yes

Digestive system
No

Ear, nose and throat
No

Education
No

Endocrine and metabolic disorders
No

Eye disorders
No

General interest
No

Genetics
No

Health inequalities/health equity
No

Infections and infestations
No

International development
No

Mental health and behavioural conditions
No

Musculoskeletal
No

Neurological
No

Nursing
No

Obstetrics and gynaecology
No

Oral health
Yes

Palliative care

No

Perioperative care

No

Physiotherapy

No

Pregnancy and childbirth

No

Public health (including social determinants of health)

No

Rehabilitation

No

Respiratory disorders

No

Service delivery

No

Skin disorders

No

Social care

No

Surgery

No

Tropical Medicine

No

Urological

No

Wounds, injuries and accidents

No

Violence and abuse

No

32. * Country.

Select the country in which the review is being carried out. For multi-national collaborations select all the countries involved.

34. Reference and/or URL for published protocol.

If the protocol for this review is published provide details (authors, title and journal details, preferably in Vancouver format)

Not applicable.

Add web link to the published protocol.

Or, upload your published protocol here in pdf format. Note that the upload will be publicly accessible.

No I do not make this file publicly available until the review is complete

Please note that the information required in the PROSPERO registration form must be completed in full even if access to a protocol is given.

36. Keywords.

Give words or phrases that best describe the review. Separate keywords with a semicolon or new line. Keywords help PROSPERO users find your review (keywords do not appear in the public record but are included in searches). Be as specific and precise as possible. Avoid acronyms and abbreviations unless these are in wide use.

37. Details of any existing review of the same topic by the same authors.

If you are registering an update of an existing review give details of the earlier versions and include a full bibliographic reference, if available.

38. * Current review status.

Update review status when the review is completed and when it is published.New registrations must be ongoing so this field is not editable for initial submission.

Please provide anticipated publication date

Review_Ongoing

40. Details of final report/publication(s) or preprints if available.

Leave empty until publication details are available OR you have a link to a preprint (NOTE: this field is not editable for initial submission). List authors, title and journal details preferably in Vancouver format.

Give the link to the published review or preprint.

Appendix 2 – Risk Of Bias tool 2

Risk of bias assessment

Responses underlined in green are potential markers for low risk of bias, and responses in red are potential markers for a risk of bias. Where questions relate only to sign posts to other questions, no formatting is used.

Domain 1: Risk of bias arising from the randomization process

Signalling questions	Comments	Response options
1.1 Was the allocation sequence random?		Y / PY / PN / N / NI
1.2 Was the allocation sequence concealed until participants were enrolled and assigned to interventions?		Y / PY / PN / N / NI
1.3 Did baseline differences between intervention groups suggest a problem with the randomization process?		Y / PY / PN / N / NI
Risk-of-bias judgement		Low / High / Some concerns
Optional: What is the predicted direction of bias arising from the randomization process?		NA / Favours experimental / Favours comparator / Towards null /Away from null / Unpredictable

Domain 2: Risk of bias due to deviations from the intended interventions (*effect of assignment to intervention*)

Signalling questions	Comments	Response options
2.1. Were participants aware of their assigned intervention during the trial?		Y / PY / PN / N / NI
2.2. Were carers and people delivering the interventions aware of participants' assigned intervention during the trial?		Y / PY / PN / N / NI
2.3. If Y/PY/NI to 2.1 or 2.2: Were there deviations from the intended intervention that arose because of the trial context?		NA / Y / PY / PN / N / NI
2.4 If Y/PY to 2.3: Were these deviations likely to have affected the outcome?		NA / Y / PY / PN / N / NI
2.5. If Y/PY/NI to 2.4: Were these deviations from intended intervention balanced between groups?		NA / Y / PY / PN / N / NI
2.6 Was an appropriate analysis used to estimate the effect of assignment to intervention?		Y / PY / PN / N / NI
2.7 If N/PN/NI to 2.6: Was there potential for a substantial impact (on the result) of the failure to analyse participants in the group to which they were randomized?		NA / Y / PY / PN / N / NI
Risk-of-bias judgement		Low / High / Some concerns
Optional: What is the predicted direction of bias due to deviations from intended interventions?		NA / Favours experimental / Favours comparator / Towards null /Away from null / Unpredictable

Domain 2: Risk of bias due to deviations from the intended interventions (*effect of adhering to intervention*)

Signalling questions	Comments	Response options
2.1. Were participants aware of their assigned intervention during the trial?		Y / PY / PN / N / NI
2.2. Were carers and people delivering the interventions aware of participants' assigned intervention during the trial?		Y / PY / PN / N / NI
2.3. [If applicable:] If Y/PY/NI to 2.1 or 2.2: Were important non-protocol interventions balanced across intervention groups?		NA / Y / PY / PN / N / NI
2.4. [If applicable:] Were there failures in implementing the intervention that could have affected the outcome?		NA / Y / PY / PN / N / NI
2.5. [If applicable:] Was there non-adherence to the assigned intervention regimen that could have affected participants' outcomes?		NA / Y / PY / PN / N / NI
2.6. If N/PN/NI to 2.3, or Y/PY/NI to 2.4 or 2.5: Was an appropriate analysis used to estimate the effect of adhering to the intervention?		NA / Y / PY / PN / N / NI
Risk-of-bias judgement		Low / High / Some concerns
Optional: What is the predicted direction of bias due to deviations from intended interventions?		NA / Favours experimental / Favours comparator / Towards null /Away from null / Unpredictable

Domain 3: Missing outcome data

Signalling questions	Comments	Response options
3.1 Were data for this outcome available for all, or nearly all, participants randomized?		Y / PY / PN / N / NI
3.2 If N/PN/NI to 3.1: Is there evidence that the result was not biased by missing outcome data?		NA / Y / PY / PN / N
3.3 If N/PN to 3.2: Could missingness in the outcome depend on its true value?		NA / Y / PY / PN / N / NI
3.4 If Y/PY/NI to 3.3: Is it likely that missingness in the outcome depended on its true value?		NA / Y / PY / PN / N / NI
Risk-of-bias judgement		Low / High / Some concerns
Optional: What is the predicted direction of bias due to missing outcome data?		NA / Favours experimental / Favours comparator / Towards null /Away from null / Unpredictable

Domain 4: Risk of bias in measurement of the outcome

Signalling questions	Comments	Response options
4.1 Was the method of measuring the outcome inappropriate?		Y / PY / PN / N / NI
4.2 Could measurement or ascertainment of the outcome have differed between intervention groups?		Y / PY / PN / N / NI
4.3 If N/PN/NI to 4.1 and 4.2: Were outcome assessors aware of the intervention received by study participants?		NA / Y / PY / PN / N / NI
4.4 If Y/PY/NI to 4.3: Could assessment of the outcome have been influenced by knowledge of intervention received?		NA / Y / PY / PN / N / NI
4.5 If Y/PY/NI to 4.4: Is it likely that assessment of the outcome was influenced by knowledge of intervention received?		NA / Y / PY / PN / N / NI
Risk-of-bias judgement		Low / High / Some concerns
Optional: What is the predicted direction of bias in measurement of the outcome?		NA / Favours experimental / Favours comparator / Towards null /Away from null / Unpredictable

Domain 5: Risk of bias in selection of the reported result

Signalling questions	Comments	Response options
5.1 Were the data that produced this result analysed in accordance with a pre-specified analysis plan that was finalized before unblinded outcome data were available for analysis?		Y / PY / PN / N / NI
Is the numerical result being assessed likely to have been selected, on the basis of the results, from...		
5.2. ... multiple eligible outcome measurements (e.g. scales, definitions, time points) within the outcome domain?		Y / PY / PN / N / NI
5.3 ... multiple eligible analyses of the data?		Y / PY / PN / N / NI
Risk-of-bias judgement		Low / High / Some concerns
Optional: What is the predicted direction of bias due to selection of the reported result?		NA / Favours experimental / Favours comparator / Towards null /Away from null / Unpredictable

Overall risk of bias

Risk-of-bias judgement		Low / High / Some concerns
Optional: What is the overall predicted direction of bias for this outcome?		NA / Favours experimental / Favours comparator / Towards null /Away from null / Unpredictable