Scientific Committee on Emerging and Newly Identified Health Risks

SCENIHR

Opinion on

The safety of dental amalgam and alternative dental restoration materials for patients and users

The SCENIHR adopted this opinion at the 10th plenary meeting on 29 April 2015

About the Scientific Committees

Three independent non-food Scientific Committees provide the Commission with the scientific advice it needs when preparing policy and proposals relating to consumer safety, public health and the environment. The Committees also draw the Commission's attention to the new or emerging problems which may pose an actual or potential threat.

They are: the Scientific Committee on Consumer Safety (SCCS), the Scientific Committee on Health and Environmental Risks (SCHER) and the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) and are made up of external experts.

In addition, the Commission relies upon the work of the European Food Safety Authority (EFSA), the European Medicines Evaluation Agency (EMEA), the European Centre for Disease prevention and Control (ECDC) and the European Chemicals Agency (ECHA).

SCENIHR

This Committee deals with questions related to emerging or newly identified health and environmental risks and to broad, complex or multidisciplinary issues requiring a comprehensive assessment of risks to consumer safety or public health and related issues not covered by other Community risk assessment bodies. Examples of potential areas of activity include potential risks associated with interaction of risk factors, synergic effects, cumulative effects, antimicrobial resistance, new technologies such as nanotechnologies, medical devices including those incorporating substances of animal and/or human origin, tissue engineering, blood products, fertility reduction, cancer of endocrine organs, physical hazards such as noise and electromagnetic fields (from mobile phones, transmitters and electronically controlled home environments), and methodologies for assessing new risks. It may also be invited to address risks related to public health determinants and non-transmissible diseases.

Scientific Committee members

Michelle Epstein, Igor Emri, Philippe Hartemann, Peter Hoet, Norbert Leitgeb, Luis Martínez Martínez, Ana Proykova, Luigi Rizzo, Eduardo Rodriguez-Farré, Lesley Rushton, Konrad Rydzynski, Theodoros Samaras, Emanuela Testai, Theo Vermeire

Contact:

European Commission
DG Health and Food Safety
Directorate C: Public Health
Unit C2 – Health information and Scientific Committees
Office: HTC 03/073 L-2920 Luxembourg

SANTE-C2-SCENIHR@ec.europa.eu

ISSN: 1831-4783 ISBN: 978-92-79-35605-6
doi: 10.2772/42641 ND-AS-14-002-EN-N

The opinions of the Scientific Committees present the views of the independent scientists who are members of the committees. They do not necessarily reflect the views of the European Commission. The opinions are published by the European Commission in their original language only.

http://ec.europa.eu/health/scientific_committees/emerging/opinions/index_en.htm

ACKNOWLEDGMENTS

Members of the working group are acknowledged for their valuable contribution to this opinion. The members of the working group are:

SCENIHR members:

Prof. Eduard Rodríguez-Farre (Chair since April 2013) - Barcelona Institute of Biomedical Research (IIBB), CSIC, and CIBER of Epidemiology and Public Health (CIBERESP). Barcelona, Spain.

Dr. Emanuela Testai - Istituto Superiore di Sanità, Environment & Primary Prevention Dept., Mechanisms of Toxicity Unit, Roma, Italy.

External experts:

Dr Ellen Bruzell – NIOM, Nordic Istitute of Dental Materials, Oslo, Norway.

Dr Wim De Jong (co-rapporteur) - Department for Product Safety Centre for Health Protection, National Institute for Public Health and the Environment, Bilthoven, The Netherlands.

Prof. Arne Hensten (chair until March 2013 and rapporteur) – UiT, the Arctic University of Norway, Tromsö, Norway.

Prof. Dr Gottfried Schmalz - University Hospital Regensburg, Department of Operative Dentistry and Periodontology, Germany and School of Dental Medicine (ZMK Bern) , University of Bern, Switzerland.

Dr Mogens Thomsen - Emeritus Research Director of Inserm (Institut National de Santé et Recherche Médicale), Toulouse, France.

The additional contribution of the following experts is gratefully acknowledged:

Prof. Dr Jan van Dijken – Umeå University, Sweden for his valuable contribution in elaborating the preliminary opinion.

Prof. Dr Prof. Wolfgang Dekant (University of Wurzburg, Germany) for his contribution at an early stage of development of the preliminary opinion.

Prof. Dr Philippe Grandjean (University of Southern Denmark, Odense, Denmark) for the advice provided to the working group in different phases of the preliminary opinion.

All Declarations of Working Group members and supporting experts are available at the following webpage:
http://ec.europa.eu/health/scientific_committees/emerging/members_wg/index_en.htm

ABSTRACT

In the light of new developments and studies on dental amalgam a request was submitted by the Commission to update the SCENIHR opinion produced in 2008 on the safety and performance of both dental amalgam and possible alternatives, such as resin-based composites, glass ionomer cements, ceramics and gold alloys.

This updated 2015 Opinion evaluates the scientific evidence on the potential association between amalgam and possible alternatives and adverse health effects, such as allergies and neurological disorders.

The SCENIHR recognises that dental amalgam is an effective restorative material and is a material of choice for specific restorations.

Currently in the EU, there is a shift away from the use of dental amalgam in oral health care towards an increased use of alternative materials. Because dental amalgam is neither tooth-coloured nor adhesive to remaining tooth tissues, alternative tooth-coloured filling materials have become increasingly used. The change is indicated by trends in education on dental treatment towards an increased use of alternative materials instead of amalgam. This reduction is in line with concern about the use of mercury, the metallic element used in dental amalgam and the general aim to reduce mercury use within the European Union.

The exposure of the general population to mercury is mainly due to fish consumption (organic mercury, methyl mercury) and dental amalgam (elemental mercury, inorganic mercury). The present Opinion reviews only the toxicology of elemental and inorganic mercury being relevant to amalgam safety considerations.

Local adverse effects in the oral cavity are occasionally seen with dental amalgam fillings, including allergic reactions and an association with clinical features characteristic of lichen planus, but the incidence is low (< 0.3% for all dental materials in general) and usually readily managed. Regarding systemic effects, elemental mercury is a well-documented neurotoxicant, especially during early brain development. Inorganic mercury also constitutes a hazard to kidney function. In some scientific reports the presence of dental amalgam has been suggested to be associated with a variety of systemic adverse effects, particularly developmental neurotoxicity as well as neurological and psychological or psychiatric diseases. However, the evidence for such effects due to dental amalgam is weak.

The most recent *in vitro* evidence provides new insight into the effects of mercury on developing neural brain cells at concentrations similar to those accumulated in human brain and found in post mortem specimen. The effects of genetic polymorphism concerning mercury kinetics may influence the degree of individual susceptibility with regard to mercury internal exposure and consequently toxicity. This may raise some concern for possible effects on the brain of mercury originating from dental amalgam. However, so far such effects have not been documented in humans, although some evidence on alteration of mercury dynamics have been reported.

Placement and removal of dental amalgam fillings results in transient short-time exposure to the patients compared to leaving the amalgam intact. There is no general justification for unnecessarily removing clinically satisfactory amalgam restorations, except in those patients diagnosed as having allergic reactions to one of the amalgam constituents. However, as with any other medical or pharmaceutical intervention, caution should be exercised when considering the placement of any dental restorative material in pregnant women.

The mercury release during placement and removal also results in exposure of dental personnel. Recent studies do not indicate that dental personnel in general, despite somewhat higher exposures than patients, suffer from adverse effects that could be attributed to mercury exposure due to dental amalgam. However, exposure of both patients and dental personnel could be minimised by the use of appropriate clinical techniques.

The alternative materials also have clinical limitations and toxicological hazards. They contain a variety of organic and inorganic substances and may undergo chemical reactions within the tooth cavity and adjacent soft tissues during placement. The SCENIHR Opinion "The safety of the use of bisphenol A in medical devices" (2015) concluded that release of BPA from some

dental materials was associated with only negligible health risks. A similar detailed risk assessment has not been performed for other compounds released from other alternative dental materials. Some of the monomers used are cytotoxic to pulp and gingival cells *in vitro*. There is *in vitro* evidence that some of these alternatives are also mutagenic although long-term health consequences are unclear. Allergies to some of these substances have been reported, both in patients and in dental personnel. However, information on the toxicological profile of alternative materials and clinical data on possible adverse effects of alternatives are very limited.

The SCENIHR concludes that current evidence does not preclude the use of either amalgam or alternative materials in dental restorative treatment. However, the choice of material should be based on patient characteristics such as primary or permanent teeth, pregnancy, the presence of allergies to mercury or other components of restorative materials, and the presence of impaired renal clearance.

The SCENIHR recognises that there is a need for further research, particularly relating to (i) evaluation of the potential neurotoxicity of mercury from dental amalgam and the effect of genetic polymorphisms on mercury toxicity and (ii) to expand knowledge of the toxicity profile of alternative dental restorative materials. Furthermore, there is a need for the development of new alternative materials with a high degree of biocompatibility.

Keywords: Dental amalgam, mercury, toxicology, exposure, resin-based composites, glass ionomer cements, allergy, systemic health effects, SCENIHR.

Opinion to be cited as: SCENIHR (Scientific Committee on Emerging and Newly-Identified Health Risks), Scientific opinion on the Safety of Dental Amalgam and Alternative Dental Restoration Materials for Patients and Users (update), 29 April 2015.

TABLE OF CONTENTS

EXECUTIVE SUMMARY

In the light of new developments and studies on dental amalgam, a request was submitted to update the previous Opinion of SCENIHR from 2008 on the safety and performance of both dental amalgam and possible alternatives, such as resin-based composites, glass ionomer cements, ceramics and gold alloys.

This updated 2015 Opinion evaluates the scientific evidence about any links that may exist between either amalgam or possible alternatives and allergies, neurological disorders or other adverse health effects.

The SCENIHR recognises that dental amalgam is an effective restorative material for the general population. From the perspectives of longevity, mechanical performance and economics, amalgam has long been considered the material of choice, especially for certain types of restorations in posterior teeth, including replacement therapy for existing amalgam fillings. However, dental amalgam is neither tooth-coloured nor can it adhere to remaining tooth tissues. It is retained in the tooth by mechanical means, such as undercuts in the cavity preparation. Its use has been decreasing in recent years and the alternative tooth-coloured filling materials are increasingly used. There is a trend towards minimal interventional, adhesive, techniques in dentistry, which are based on adhesion to tooth structure by chemical interaction and/or micromechanical retention. At the same time, the quality and durability of alternative materials have improved.

The exposure of the general population to mercury is mainly due to fish consumption (organic mercury, methyl mercury) and dental amalgam (elemental mercury, inorganic mercury). Mercury is the metallic element of concern used in dental amalgam. Mercury is a well-documented toxicant, with reasonably well-defined characteristics for the major forms of exposure, involving elemental mercury as well as organic and inorganic mercury compounds. This Opinion does not address the issues of organic mercury or methyl mercury.

Local adverse effects in the oral cavity are occasionally seen with dental materials in general, including allergic reactions and an association with clinical features characteristic of lichen planus. These reactions occur at an incidence below 0.3% and are usually readily managed.

Regarding systemic effects, elemental mercury is a well-documented neurotoxicant, especially during early brain development, and inorganic mercury also constitutes a hazard to kidney function. EFSA (2012) has recently evaluated inorganic mercury in food and recommended a tolerable intake limit (tolerable weekly intake of inorganic mercury of 4 µg/kg body weight, expressed as mercury). Several studies have explored the possible association of mercury derived from dental amalgam with a variety of adverse effects, particularly neurological and psychological or psychiatric diseases, including Alzheimer's disease, Parkinson's disease, and multiple sclerosis as well as kidney diseases. The causality evidence for such effects due to dental amalgam is weak because of contradictory reports and major challenges in exposure assessment, which is generally expressed as total mercury in body fluids (mainly urine), without differentiating between organic vs. inorganic forms as well as between sources (dietary vs. dental amalgam or others).

Mercury concentration in the adult brain is associated with the number of amalgam fillings. In the foetus, mercury concentration in the kidney (but not in the foetal brain) has a tendency to be associated with the mothers' number of amalgam fillings. Because the estimated elimination half-life for inorganic mercury in the brain exceeds 10 years, mercury is likely to accumulate in the central nervous system. The accumulated concentrations in brain tissue (as measured in post-mortem specimen) may reach values that are similar to those inducing neurochemical changes in experimental models *in vitro*. Such effects have not been convincingly demonstrated in humans as caused by dental amalgam.

So far, studies in children of school age did not convincingly demonstrate amalgam-associated neuropsychological deficits. However, recent studies suggest that genetic polymorphisms may influence the degree of individual susceptibility with regard to mercury internal exposure and consequently toxicity in children as well in adults.

The transient mercury release during placement and removal will result in transient exposure to the patients (resulting in a transient increase in plasma mercury levels) and also to the dental personnel. There is no justification for removing clinically satisfactory amalgam restorations as a precaution, except in those patients diagnosed as having allergic reactions to amalgam constituents.

Recent studies do not indicate that dental personnel in general, despite somewhat higher exposures than patients, suffer from adverse effects that could be be attributed to mercury exposure due to dental amalgam. Exposure of both patients and dental personnel could be minimised by the use of appropriate clinical techniques.

Respiratory air concentrations, blood levels and urinary excretion of mercury in individuals with amalgam fillings indicate that the levels of exposure encountered are 5 to 30 times lower than those permitted for occupational exposure. Tolerable limits for dietary exposures to mercury are relevant to amalgam safety considerations, as inhaled elemental mercury may add to the body burden of inorganic mercury. Recently the European Food Safety Agency reported that the tolerable weekly intake for inorganic mercury might be exceeded due to the additional inhalation exposure in people with a high number of amalgam fillings. However, evidence is weak and the data are mainly derived from model-based calculations. Studies on large patient collectives did not show any clear correlation of health effects with the number of dental amalgam restorations.

The SCENIHR notes that alternative materials to amalgam are chemically very complex and also have clinical limitations and may represent toxicological risks. They contain a variety of substances including organic solvents, may undergo chemical reactions within the tooth cavity and adjacent soft tissues during placement and may also degrade *in situ*. The SCENIHR Opinion "The safety of the use of bisphenol A in medical devices" (2015) concluded that release of BPA from some dental materials was associated with only negligible health risks. A similar extensive risk assessment has not been performed for other compounds released from alternative dental materials. Non-mercury containing alternatives are not free from any concerns about adverse effects. With respect to resin composite restorative materials and hybrid systems that incorporate polymerisable resins, there is *in vitro* evidence that some of the monomers used are highly cytotoxic to pulp and gingival cells. There is also *in vitro* evidence that some monomers are mutagenic although it is not known whether this has any clinical significance. Allergic reactions to some of these substances have been reported, and to a higher degree, both in patients and in dental personnel. Similar to treatment with dental amalgam, the use of these materials in pregnant women is discouraged.

It is noted that there are very limited scientific data available concerning exposure of patients and dental personnel to substances that are used in alternative restorative materials. Many of the monomers and other organic solvents used in them are volatile and need to be better identified and quantified. Further toxicological research on the various components of these alternative dental materials is warranted.

Alternative materials have now been in clinical use for well over thirty years, initially in anterior teeth and more recently also for restorations in posterior teeth. Existing clinical experience has revealed little evidence of clinically significant adverse events. It is also important to note that the composition of available materials has changed substantially in recent years with reduced bioavailability of harmful components from use of improved polymerisation processes and particular improvement in the adhesive systems and the filler parts. There is no evidence that infants or children are at risk of adverse effects arising from the use of alternatives to dental amalgam. However, similar to mercury, genetic polymorphisms may also exist for toxicokinetics of some constituents of these alternative materials. Cellular reactions towards resin monomers are regulated by the genes that are also involved in the reaction towards mercury and therefore genetic variability is also relevant for resin-based materials.

The SCENIHR notes that the full chemical specification of these alternative restorative materials is not always divulged, and it may be difficult to know exactly what they contain. As a result, there are limited toxicological data publicly available for these materials. Dental restorative materials are defined as medical devices according to the Council Directive

93/42/EEC concerning medical devices and belong to class IIa. Consequently, the certification process does not include examination of the design dossier and, therefore, the chemical specification does not have to be revealed to the third party. Although manufacturers are obliged to assess biocompatibility and the risk from unintended side effects, accessible information on the toxicity of the constituents of the materials as well as relevant exposure data is lacking. Therefore, the SCENIHR notes that it is not possible to provide a scientifically sound statement on the safety of these materials.

As a general principle, the relative risks and benefits of any dental treatment need to be explained to patients to assist them to make informed decisions. Better information concerning the relative risks of dental restorative materials requires more data. Therefore, it is recommended that manufacturers should provide this information.

More publicly available research data are also needed to have a broader basis for risk evaluation. In view of the controversial nature of this subject, it would also be beneficial for the community in general to be better informed of the recognised benefits and risks.

In the light of the above comments the SCENIHR concludes that dental amalgam already in place is not considered a health risk for the general population. Consequently, pre-existing amalgam restorations should not be removed as a preventive measure. As far as dental personnel are concerned, it is recognised that they may be at greater risk with respect to higher mercury exposure from dental amalgam than the general population, although the incidence of reported adverse effects seems to be in the same order of magnitude.

Information on exposure, toxicity and clinical outcomes for alternative materials is much scarcer than for dental amalgam. There is some evidence that some of the low molecular weight substances used in their preparation are associated with local allergic reactions. There are insufficient data to draw firm conclusions about associations between these alternative materials and neurological or other health disorders. The continuing evolution of these materials suggests that caution should be exercised before new variations are introduced into the market. As far as dental personnel are concerned, there are reports of small numbers of cases of induced allergies to these materials. Their volatile organic solvent species that are pervasive in dental clinics should be identified and quantified to enable proper risk assessment.

The SCENIHR concludes that dental restorative treatment can be adequately ensured by amalgam and alternative types of restorative material. The longevity of restorations of alternative materials in posterior teeth has improved with the continuing development of these materials and the practitioner's familiarity with effective placement techniques, but is in certain clinical situations (e.g. large cavities and high caries rates) still inferior to amalgam.

The choice of material should be based on patient characteristics such as primary or permanent teeth, pregnancy, presence of allergies to mercury or other components of the restorative materials, and presence of decreased renal clearance. The clinical trend towards the use of adhesive alternatives is considered advantageous as it implies that a sustained reduction in the use of dental amalgam in clinical practice will continue across the European Union.

The SCENIHR recognises a lack of knowledge and a need for further research, in particular in regard to genetic susceptibility related to mercury effects and to the constituents of alternative restorative materials. Furthermore, there is a need for the development of new alternative materials with a high degree of biocompatibility.

1. BACKGROUND

Dental amalgam and its substitutes are regulated under Council Directive 93/42/EEC concerning medical devices, according to which they must comply with the essential requirements laid out in the directive, in particular in relation to the health and safety of the patients.

Dental amalgam has been used for over 150 years for the treatment of dental cavities and is still used, in particular in large cavities, due to its excellent mechanical properties and durability. Dental amalgam is a combination of alloy particles and mercury that contains about 50% of mercury in the elemental form. Overall, the use of alternative materials such as composite resins, glass ionomer cements, ceramics and gold alloys, is increasing, either due to their aesthetic properties or alleged health concerns related to the use of dental amalgam.

In January 2005, the Commission adopted a proposal for a Community Strategy concerning Mercury in order to reduce mercury levels in the environment and human exposure. Pursuant to Action 6 of the Strategy, the use of dental amalgam should be evaluated with a view to considering whether additional regulatory measures are appropriate.

In view of the above, the Commission requested the Opinion of the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) on the safety of dental amalgam and alternative dental restoration materials. According to the SCENIHR Opinion adopted in May 2008, dental amalgam is a safe material to use in restorative dentistry for patients. No health risk other than allergic reaction in certain individuals can be associated with the use of dental amalgam. The alternatives are not without clinical limitations and toxicological risks, and less is known about these alternatives for which available scientific data are more limited.

In 2010 a report of the meeting convened by WHO on "Future Use of Materials for Dental Restoration" was published, in which a 'phase-down' of the use of dental amalgam at the global level was suggested. According to the report, this may be achieved effectively by strengthening the prevention of dental caries and by encouraging better use of quality alternatives to dental amalgam. More quality studies and systematic reviews are needed in the case of dental materials alternatives to amalgam. A recent "Study on potential for reducing mercury pollution from dental amalgam and batteries" (May 2012) addresses the environmental impacts of dental amalgam use (http://ec.europa.eu/environment/chemicals/mercury/pdf/BIO_Draft%20final%20report.pdf). The study did not evaluate the health aspects.

2. TERMS OF REFERENCE

In the light of recent developments and studies on dental amalgam we would like to ask the SCENIHR to update, if appropriate, the Opinion adopted in 2008. In view of possible safety concerns linked to the use of dental amalgam and its substitutes, it is essential to review and evaluate available scientific data related to the safety of these substances for patients and in particular for high risk groups.

In particular, the SCENIHR is asked the following questions:

1. Is there any new scientific evidence that justify reasons for concern from the health point of view in the use of dental amalgam as dental restoration material?

2. In view of the above, is the use of dental amalgam safe for patients and users, i.e. dental health professionals? Are certain populations particularly at risk, e.g. pregnant women or children? Is it possible to recommend certain practices to minimise patient's and user's exposure to dental amalgam?

3. Is there new scientific evidence on the safety and performance of alternative materials?

4. Is it possible to recommend alternative materials and certain practices related to these materials to reduce potential risks for patients and users?

5. In case there is not enough scientific data to answer these questions, the SCENIHR is asked to formulate recommendations for research that could help to provide the necessary data.

3. SCIENTIFIC RATIONALE

3.1. Introduction

This Opinion is an update of the safety issues of dental amalgam and alternative materials that have been previously considered in an Opinion published in 2008. Since then, additional information has been published, including clinical epidemiological studies. The present document therefore highlights new information, and it supplements and updates the previous opinion. While occupational exposures are included, this Opinion does not consider environmental aspects of amalgam use. It is recognised that both at European and United Nations level there are on-going efforts to reduce the exposure to mercury. The Scientific Committee on Health and Environmental Risks (SCHER) has recently adopted an Opinion regarding the contribution of dental amalgam to the environmental burden of mercury and the possible health effects deriving from environmental exposure to Hg coming from dental amalgam (SCHER, 2014).

One of the major components of the dental amalgam restoration is elemental mercury. The essential metallurgical principles of dental amalgam are fairly straightforward. Liquid mercury is able to react with many other metallic elements to produce a series of multi-phase alloys that are solid at room temperature. The present Opinion will focus on these mercury species. In the body, elemental mercury is oxidised to inorganic mercury, which also occurs as a food contaminant. EFSA (2012) has recently evaluated inorganic mercury in food and recommended a tolerable intake limit (tolerable weekly intake of inorganic mercury of 4 µg/kg body weight, expressed as mercury). Thus, the present Opinion will review the toxicology of elemental and ionic (mercuric) mercury as deemed appropriate in regard to amalgam safety considerations. Once released into saliva, inorganic mercury might be methylated by bacteria in the periodontal pocket and gastrointestinal tract, but the rate is not clear (Langendijk *et al.,* 2001, Leistevuo *et al.,* 2002, van der Hoeven *et al.,* 2007). However, the contribution of this reaction when compared to the intake of methyl mercury from the food is expected to be limited.

The alternatives for dental amalgam in dental restorations include resin-based composite materials, glass ionomer cements, ceramics, gold-based and other alloys, and a variety of hybrid structures. Many of them have been in use only for a limited number of years, and the toxicological database is limited, also in regard to reaction products. Thus, the data base is much more limited in regard to these dental materials, and some conclusions regarding toxic risks and long-term stability must therefore be tentative at this point. As amalgams are phased out, further documentation on new dental restoration materials must be secured so that the present high quality of care and high degree of safety can be maintained.

A changing scenario

Placing restorations due to dental caries is still a commonly performed treatment, but there are great variations in decision-making about the threshold for intervention with restorative treatment. This is a global issue.

Questionnaire surveys have been carried out, asking the practitioners whether they would operatively treat an occlusal lesion confined to the enamel in a patient with low risk of developing caries. In Iran 32 % (Ghasemi *et al.,* 2008), in France more than one half (Doméjean-Orliaguet *et al.,* 2004) and in the USA 63 % would do so (Gordan *et al.,* 2010). Of the Scandinavian respondents only 2.6% said that they would intervene that early (Gordan *et al.,* 2010). A survey based on questionnaires revealed that in 2009, 7 % of Norwegian dentists would restore approximal lesions confined to enamel, compared with (in similar studies) 18% in 1995 and 66 % in 1983. These changes in treatment threshold criteria indicate that many dentists have taken into account that caries is a slowly progressing disease and that especially initial carious lesions can be arrested (Vidnes-Kopperud *et al.,* 2011).

3.2. Methodology

This Opinion of the SCENIHR is concerned with the analysis of the evidence for the potential for either amalgam or alternatives to amalgam to have adverse effects on human health, from the perspectives of both scientific plausibility as well as experimental, clinical and epidemiological data. Recent scientific evidence is reviewed to determine whether it justifies any reason for concern in regard to health risks associated with the use of dental amalgam and currently available alternative materials. In this context SCENIHR refers to the definition of risk as mentioned in different ISO-EN standards (ISO EN 10993-1 and ISO-EN 14971).

The SCENIHR has considered evidence derived from a wide variety of sources, including peer-reviewed scientific and medical literature and published reports of institutional, professional, governmental and non-governmental organisations. In coherence with the usual practice of the SCENIHR, less weight has been given to work not freely available in the public domain.

The SCENIHR has reviewed as much evidence as possible and, especially where the available data on alternatives is limited, attention has been given to some less well-controlled studies where no other information was available. During the course of the deliberations of the Working Group, a Call for Information was issued by the Commission (8 August 2012 to 10 October 2012) and all of the responses have been considered. An extensive literature search was performed in 2012 (covering the period 2008-2012) by an external contractor with the following search terms:

Dental amalgams/mercury amalgams implants/fillings and:

- mercury exposure/levels/ blood/body burden/brain
- leaching/ loss/release/mobilisation/stability
- risk assessment/hazard/adverse effects/disorders/ neuro* effects/safety/risk benefits
- removal, health effects/implications/risk/risk benefit/safety
- cremation
- life cycle analysis/ manufacturing/use/disposal

Non –mercury/ceramic/implants/fillings and:

- leaching/ loss/release/mobilisation/stability
- risk assessment/hazard/adverse effects/disorders/ neuro* effects/safety/risk benefits
- removal, health effects/implications/risk/risk benefit/safety
- life cycle analysis/ manufacturing/use/disposal

In addition, during the writing of the Opinion, additional relevant literature up to 2014 was provided by both members of the working group and of SCENIHR. Literature published before 2008 that was not included in the previous Opinion but was considered relevant was also assessed. Furthermore relevant references provided via the public consultation were included as well.

In a review of the evidence for or against causation of disease, it is necessary to take into account the generally accepted criteria for causation. The SCENIHR published a memorandum on the weight-of-evidence approach to the evaluation of risks and hazards (SCENIHR, 2012). The criteria considered are: (i) the establishment of temporal relationship between exposure and outcome; (ii) the statistical evaluation of an effect; (iii) the evidence of a dose-response relationship; (iv) the plausibility and specificity of any association; and (v) the coherence of any putative association with existing knowledge.

On the other hand, these criteria, which build upon Hill's original 'aspects' are not symmetrical. That is, if one of the conditions is fulfilled, then it supports causality, but it does not necessarily speak against it if not (or not yet) fulfilled (Kaufman and Poole, 2000).

In the weight of evidence approach, lines of evidence or hypothesis for causality are evaluated based on the supportive studies. When a line of evidence is consistently supported by various studies (i.e. evidence is independently reproduced in different studies) causality is likely

between the observed effect and exposure to the substance. Strength and weaknesses of the studies evaluated are considered. The weight of evidence can be categorised as follows:

Strong overall weight of evidence: Coherent evidence from human and one or more other lines of evidence (in particular model/ mechanistic studies) in the absence of conflicting evidence from one of the other lines of evidence (no important data gaps).

Moderate overall weight of evidence: Good evidence from a primary line of evidence but evidence from several other lines is missing (important data gaps).

Weak overall weight of evidence: Weak evidence from the primary lines of evidence (severe data gaps).

Uncertain overall weight of evidence: Due to conflicting information from different lines of evidence that cannot be explained in scientific terms.

Weighing of evidence not possible: No suitable evidence available.

A major problem in many of the reviewed epidemiological studies was the quantitative evaluation of the contribution of mercury exposure coming from dental amalgam.

The evidence for the presence of a causal relationship between exposure to dental amalgam and/or alternative restoration material, and adverse health effects are discussed in the chapters below.

3.3. Dental Amalgam

In this Chapter, the essential and relevant characteristics of dental amalgam and the evidence concerning the general exposure and toxicity of mercury-based substances are explained and discussed. This is followed by an assessment of the reported adverse effects in individuals with amalgam restorations, the epidemiological and clinical evidence concerning adverse effects in dental personnel, and general observations about the clinical usefulness of dental amalgam restorations.

3.3.1. Metallurgical principles and physical-chemical properties

The principles and physical-chemical properties of dental amalgams are described in the previous Opinion (2008). The SCENIHR is not aware of new developments in amalgam metallurgy.

Mercury is a metallic element that occurs naturally and also in the form of several types of ore, the mercury burden of the environment being derived in part from natural sources, in part from accumulated anthropogenic emissions.

3.3.1.1. Major Forms of Mercury

Each form of mercury has its own toxicological profile and shows major differences in toxicokinetics.

3.3.1.2. Background exposure to mercury

<u>Exposure to Mercury in Adults</u>

As described in the previous Opinion (SCENIHR, 2008), background exposure to mercury by inhalation is very low in the general population. The main source for mercury inhalation is dental amalgam as indicated by relatively old data published by WHO in 1990.

The major sources of mercury intake in the diet is methyl mercury, essentially in fish and also inorganic mercury coming from non-fish diet sources. Table 1 shows current estimates for dietary exposures to inorganic mercury (EFSA, 2012).

Table 1: Summary statistics of the chronic dietary exposure to inorganic mercury (µg mercury /kg b.w. per week) by age class (gyEFSA, 2012)

Age group	Minimum			Median			Maximum		
	LB	MB	UB	LB	MB	UB	LB	MB	UB
Mean dietary exposure in total population									
Toddlers	0.27	0.79	1.31	0.37	1.13	1.71	0.59	1.36	2.16
Other children	0.24	0.59	0.89	0.38	0.84	1.24	0.76	1.13	1.75
Adolescents	0.16	0.39	0.59	0.25	0.44	0.68	0.51	0.73	0.94
Adults	0.14	0.26	0.38	0.23	0.41	0.55	0.40	0.53	0.70
Elderly	0.13	0.23	0.33	0.22	0.35	0.48	0.30	0.42	0.55
Very elderly	0.14	0.25	0.35	0.19	0.33	0.47	0.24	0.38	0.52
P95 dietary exposure in total population									
Toddlers	0.67	1.35	2.18	0.84	1.77	2.83	1.07	2.30	4.06
Other children	0.50	1.12	1.66	0.86	1.62	2.20	1.85	2.27	3.37
Adolescents	0.31	0.71	1.00	0.62	0.88	1.26	1.70	1.85	2.33
Adults	0.36	0.53	0.72	0.59	0.78	1.02	1.52	1.66	1.83
Elderly	0.25	0.40	0.55	0.54	0.72	0.92	0.77	0.94	1.12
Very elderly	0.25	0.40	0.54	0.47	0.62	0.82	0.64	0.81	1.01

The minimum, median and maximum of the mean and the 95th percentile exposure values across European countries and dietary surveys are shown.
LB, UB, MB, respectively lower bound, upper bound and middle bound exposure estimates.

In line with JECFA, the EFSA CONTAM Panel (2012) established a tolerable weekly intake (TWI) for inorganic mercury of 4 µg/kg b.w., expressed as mercury. TWI for methyl mercury of 1.3 µg/kg b.w., expressed as mercury was established, which is somewhat lower than the TWI JECFA level of 1.6 µg/kg b.w. It was concluded that mean dietary exposure across age groups does not exceed the TWI for methyl mercury, with the exception of toddlers and other children in some surveys. The 95th percentile dietary exposure is close to or above the TWI for all age groups. High fish consumers may exceed the TWI by up to approximately six-fold. Unborn children constitute the most vulnerable group. The EFSA stated that dietary inorganic mercury exposure in Europe does not exceed the TWI. Inhalation exposure of mercury vapour from dental amalgam is likely to increase the internal inorganic mercury exposure. The TWI might be exceeded when a high number of dental amalgam fillings is present, but no further indication is given.

Exposure during pregnancy and breast-feeding

Mercury vapour, like methyl mercury, is capable of passing the placental barrier. Thus, in a study of 99 mother-child pairs, a strong positive correlation between maternal and cord blood total Hg levels was found (ρ=0.79; P<0.001). Levels of Hg in the cord blood were significantly associated with the number of maternal amalgam fillings (ρ=0.46, P<0.001) and with the number of years since the last filling (ρ=−0.37, P<0.001); these associations remained significant after adjustment for maternal age and education. The median values of total Hg

concentrations were 0.63 µg/L (range 0.14-2.9 µg/L) and 0.80 µg/L (range 0.15-2.54 µg/L) for maternal and cord blood, respectively (Palkovicova *et al.,* 2008).
Mercury is usually present in amniotic fluid. In one study of 72 pregnant women (Luglie *et al.,*2005) there was an overall mean mercury concentration in amniotic fluid of 0.37 ± 0.49 ng/ml. The women were divided into those with a low concentration of less than 0.08 ng/ml, the detection limit of their analytical method (26.4% of the subjects) and those with a concentration of greater than 0.08 ng/ml, mean 0.49 ±- 0.52 ng/ml (73.6% of subjects). A dependence of mercury concentration in amniotic fluid on number of amalgam fillings ($p=0.03$) and fish consumption ($p=0.04$) was observed, but not significant at their preset level ($p<0.01$).

Björnberg *et al.,*(2005) reported that infant blood inorganic mercury was similar to maternal blood mercury at delivery (median =0.09 µg/L) but decreased until the end of follow-up at 13 weeks of age (0.05 µg/L), while remaining unchanged in maternal blood. The exposure of the infants to inorganic mercury was low being higher at birth than during the breast-feeding period. In breast milk the authors could not differentiate between inorganic and organic mercury. They concluded that the exposure to both forms of mercury is higher before birth than during the breast-feeding period, and that methyl mercury seems to contribute more than inorganic mercury to postnatalnfant exposure via breast milk.

In addition, mercury has been detected in foetal brain and kidneys. The concentrations in the kidneys (but not in the brain) showed a tendency to increase with the number of amalgam fillings of the mother, with no statistical significance. Brain levels were in the range of 2-23 µg/kg wet weight, and kidney levels in the range of 5-34 µg/kg (Lutz *et al.,* 1996).

Brain tissue obtained from 18 foetuses and 35 children below 5 years of age showed mercury concentrations up to 6 and 20 µg/kg, respectively. A significant correlation ($p< 0.05$) with the mother's number of amalgam fillings (grouped as less than 2 or more than 10 fillings), was evident only for older children and not for foetuses. In foetuses and older infants significantly higher mean mercury concentrations in the liver and the renal cortex were found, if the mothers had more than 10 teeth with dental amalgam (Drasch *et al.,* 1994).

Da Costa *et al.,*(2005) reported on a correlation between breast milk mercury and the number of amalgam surfaces. However, Drasch *et al.*(1998), compared mercury in breast milk and in cow's-milk-based formulas and concluded that even for mothers with large numbers of dental amalgam, these fillings should pose little danger to breast-feeding infants. Indeed, during the first 2 mo, it is uncertain if any correlation between milk mercury concentrations and maternal amalgam filling exists.

Drexler and Schaller (1998) concluded that Hg exposure in breast-fed babies from maternal amalgam is of no significance to foetal and neonatal Hg blood. Stoz *et al.,*(1995) also reported that newly made tooth fillings during pregnancy had no influence on Hg concentrations in newborns.

Overall, the evidence provided by the available studies seems not to indicate a strong relationship between amalgam fillings and mercury concentration in breast milk.

3.3.1.3. Intake estimates for mercury from dental amalgams

Mercury vapour is released from silver amalgam restorations during chewing, tooth brushing, and parafunctional activities including bruxism. The parameters of this release of mercury vapour by amalgam depends on the number of fillings, the filling size and placement, chewing habits, food texture, grinding and brushing teeth, nose-mouth breathing ratio, inhalation, ingestion and body weight, and the surface, composition and age of the amalgam restorations. Therefore, there are large variations in the estimation of daily mercury release from the restorations. Accordingly, exposure assessment is complicated and inherently imprecise. Feasible assessment of the recent mercury exposure from amalgam restorations is routinely recorded as dose parameters in terms of mercury concentrations in urine and blood (EFSA,

2012; Grandjean and Yorifuji, 2012).Although mercury is also released in saliva, due to the low gastrointestinal absorption, the mercury uptake through saliva was considered to be low (0.2 and 3 µg/kg b.w. per week) (Björkman *et al.,*1997).

As discussed in the previous Opinion, the World Health Organization (WHO) reported a consensus average estimate of 10 µg/day of amalgam derived mercury (range: 3-17 µg/day) (WHO 1991). The daily uptake of mercury from amalgam fillings has been estimated by other Authors to range between 3.8 and 17 µg/day, and results in a steady-state level of mercury in body fluids (Sandborgh-Englund 1998a and 1998b). In case of individuals with a large number of amalgam fillings, dental amalgam may account for 87% of the absorbed inorganic mercury (WHO 1991). In individuals with only a few amalgam fillings, this source may account for about 50% of the absorbed inorganic mercury (summarised in ATSDR, 1999). Unfortunately, many of the older papers only use the arbitrary system of "few" and "large" numbers of restored teeth or surfaces. There are 20 teeth (premolars and molars) with 100 surfaces that may potentially be restored with dental filling materials.

More recently the assessment of exposure from dental amalgam was estimated as 0.2 to 0.4 µg/day per amalgam-filled tooth surface or 0.5 to 1 µg/day per amalgam filled tooth (Richardson *et al.,* 2011); each amalgam-filled surface results in an increase of mercury in urine of 0.1 µg Hg/L or 0.06 to 0.07 µg Hg/g creatinine (summarised in Richardson *et al.,* 2011). However, this calculation has been criticised by Nicolae *et al.,*(2013), arguing that not every aspect of mercury exposure, toxicity or pharmacokinetics was considered in the calculations made by Richardson *et al.,*(2011). Data obtained by measuring urinary mercury levels in the Canadian population show values of 0.12 µg Hg/L and 0.31 µg Hg/L (Nicolae *et al.,* 2013). It was estimated that for the vast majority of the Canadian population (up to 98.23%) this mercury level was below levels associated with any health risks. For the same exposure level the absorption values of inorganic mercury from dental amalgam was estimated six times lower compared to the absorption of organic mercury from food (Jones 1999; Nicolae *et al.,* 2013).

Similar results for blood and urine concentrations have been obtained for amalgam-bearers in the UK (Eyeson *et al.,* 2010) and Canada (Dutton *et al.,* 2013). Dye *et al.,*(2005) found that the average urinary mercury level in women of childbearing age was 1.3 µg/L and an increase of 1.8 µg/L was seen for each ten dental surfaces restored with amalgam. Levels of 1-5 µg/L were described as the normal range for non-occupational groups (Hørsted-Bindslev 2004). Similarly, in a study of 1127 healthy males, Kingman *et al.,*(1998) found an average total mercury urinary concentration of 2.55 µg/L. There was a significant correlation between this level and amalgam exposure equivalent to an increase of 1 µg/L of urine for each 10 amalgam surfaces. Substantially elevated urine levels, i.e. approximately five times higher than controls, have been reported in individuals who regularly used nicotine chewing gums (Sällsten *et al.,*1996).

In a prospective study of adolescents in the Casa Pia study in Portugal, the urinary mercury excretion was averaged approximately 3 µg/L in those with amalgam fillings, compared to 2 µg/L in controls at age 18 years. There was a statistically significant dose-dependent correlation between cumulative exposure to Hg from dental amalgams and urinary Hg levels, after covariate adjustment. When urine values in children of 8 years with amalgam and without were compared, they found 2.77 µg Hg/L without and 3.28 µg Hg/L with amalgam restorations (Geier *et al.,* 2012).

Due to the reduction of use of dental amalgam in children, the mercury levels in that population are significantly decreased as indicated by a study in Germany (Link *et al.,* 2012).

The removal of amalgam fillings causes an additional transient Hg-exposure and results in a transient increase in plasma Hg levels. The mercury-dose from removal of 16 amalgam filled surfaces is estimated to be around 40 µg mercury, based on data from Sandborgh-Englund (1998a and 1998b). This single-dose exposure is equal to the integrated chronic mercury dose from amalgam restorations over 2.3-10 days.

Greater plasma Hg-peaks have been shown in conjunction to amalgam removal in the studies by Molin *et al.,*(1990) and Berglund and Molin (1997), whereas later studies show plasma peaks in parity with Sandborgh-Englund *et al.,*(1998b) (Halbach *et al.,* 1998; Halbach *et al.,*

2000; Kremers *et al.,*1999). The number of fillings removed and the working technique (water spray, suction efficiency, rubber dam use) affects the amount of mercury released.

Retention data are available from analyses of autopsy specimens. Brain tissue generally shows average total mercury concentrations below 10 µg/kg, with a highly significant association between number of amalgam fillings and surfaces on the one hand and the mercury concentration in occipital cortex and pituitary gland. In a study of mercury in Swedish autopsy samples from 30 subjects, with an average of 13.2 amalgam surfaces, the median concentrations of methyl mercury and inorganic mercury in the occipital lobe cortex were 4 and 5 µg/kg wet weight, respectively. In one of the samples from occipital cortex the concentration of inorganic mercury (164 µg/kg) was 9 times higher than the concentration of the second highest case and fulfilled the criteria of an "extreme outlier" from a statistical point of view. The subject was found to have been employed as a dental assistant in the past (Björkman *et al.,* 2007).

Another study from Italy showed that cerebral cortex concentrations averaged about 200 µg/kg in subjects with more than 12 amalgam fillings, i.e. being over 10-fold higher than in subjects with three fillings or less (Guzzi *et al.,* 2006). Mercury levels were significantly higher in brain tissues compared with thyroid and kidney tissues in subjects with more than 12 occlusal amalgam fillings but not in subjects with 3 or less occlusal amalgams. However, no information was available on the fish consumption, therefore it was not possible to estimate the relative contribution of diet vs. dental amalgam. For comparison, adult victims who died from methyl mercury poisoning in Japan had mercury concentrations in the brain that averaged about 10 mg/kg, while much lower concentrations, about 1 mg/kg, were found in victims of foetal Minamata disease (Takeuchi and Eto, 1999). Based on these data, the total amount of mercury that must reach the brain to cause a condition commensurable with severe clinical disease or fatal poisoning would therefore be 1 mg/kg brain or more (that is 5 fold higher than the ones measured in individuals bearing more than 12 amalgam fillings). However, in a recent assessment of the neurological problems from Minamata poisoning described by Takeuchi and Eto (1999), methylmercury uptake was re-evaluated. Regarding the neurological symptoms no dose response relationship was established, limiting the interpretation of the earlier described results (Maruyama *et al.,* 2012).

In living kidney donors, the kidney mercury concentration increased by 6% for every additional amalgam surface, but was not associated with fish consumption, thus suggesting that amalgam fillings constitute a main source of inorganic mercury exposure (Barregard *et al.,* 2010). Since the major part of mercury in the kidneys has a half-life of about 2 months (Sallsten *et al.,* 1994), the kidney mercury concentrations likely reflect exposures during the most recent year or so. While some sex difference in kidney mercury retention has been reported, animal studies suggest that genetic factors may substantially affect mercury excretion in the urine and mercury accumulation in the kidneys (Ekstrand *et al.,* 2010). This notion is supported by human epidemiological evidence on differences in elimination associated with gene variants (Goodrich *et al.,* 2011), as described below.

3.3.1.4. Exposure to mercury in dental personnel

The mercury body burden of dental personnel is usually higher than in the general population. The mean urine mercury levels in dental personnel has been variously reported to range from 3 µg/L to 22 µg/L, compared to 1-5 µg/L as the normal range for non-occupational groups (Hørsted-Bindslev 2004). The increased body burden is attributed to dental personnel mixing and applying dental amalgam and removing amalgam restorations.

Ritchie *et al.,*(2004) showed that dentists had, on average, urinary mercury levels over 4 times that of control subjects. All but one dentist had urinary mercury below the UK Biological Monitoring Guidance Value of 20 µmol mercury /mol creatinine. Over 67% of the 180 surgeries visited had environmental mercury measurements in one or more areas above the Occupational Exposure Standard (OES) in UK. In the majority of these surgeries the high levels

of mercury were found at the skirting and around the base of the dental chair. In 45 surgeries (25%) the personal dosimetry measurement (i.e. in the breathing zone of dental staff) was above the OES.

Correlations have been found amongst dentists between urinary mercury levels and the number of hours worked in the surgery ($r=0.22$, $P=0.006$) and the number of amalgam restorations placed ($r=0.38$, $P<0.001$) and removed ($r=0.29$, $P<0.001$) in a week, with urine mercury levels in dentists ranging from 0.02 to 20.90 (mean 2.58) nmol mercury per nmol creatinine. A contributing and thus confounding factor in such investigations is the number of amalgam surfaces dentists have in their own mouths (Ritchie *et al.,*2002, Ritchie *et al.,*2004).

Dental personnel may now be exposed to much less mercury than in the past, in view of the increased use of encapsulated dental amalgam, improvements in amalgam capsule design, the heightened awareness and practice of appropriate dental mercury hygiene measures, and the increasing use of alternative, non-mercury-containing materials (Hørsted-Bindslev 2004). However, despite trends to reduce exposure to mercury, large, highly statistically significant differences ($P<0.0001$) may be found between dental personnel (in particular dentists) and controls, with respect of mean urinary, hair (head and pubic) and nail (finger and toe) mercury levels (Morton *et al.,* 2004). Nevertheless, according to head hair mercury data acquired over 35 years in Scottish dental practice (Duncan *et al.,* 2011) median concentrations were reduced from 8.6 µg/g in the period 1975-1979 to 0.5 µg/g in the period 2005-2009. The reduction was attributed to preparation techniques and increased awareness. In comparison, mean hair mercury concentration in the U.S. population of women in childbearing age is 0.20 µg/g (McDowell *et al.,* 2004).

High levels of exposure can also occur during preclinical training of students. A study in the Dental Simulation Laboratory in a dental school in Puerto Rico revealed substantially higher exposure levels for mercury vapour than otherwise typical for dental clinics. Thus, eight-hour averages exceeded a level of 100 µg/m^3 by several-fold. In contrast, mercury bound to particulate matter (PM10) was low (0.1 – 1.2 µg/m^3). In the Dental Clinic itself the levels were below 100 µg/m^3 (Gioda *et al.,* 2007). In a more recent study in Canada it was observed that mercury vapour exposure during dental training on amalgam removal remained below occupational exposure limits (Warwick *et al.,* 2013).

Since most dental chair-side personnel do not touch dental amalgam during mixing and placement anymore, it is considered that the main sources of mercury exposure are aerosols, created in the immediate working environment during placing and in particular the removal of restorations of dental amalgam, and the exhaust air from dental vacuum systems. In a study with three different dental clinics, one clinic with 30 dental chairs had about 1.5 times the concentration of Hg directly at the vacuum outlet than NIOSH recommendation (Stone *et al.,* 2007). Interestingly, another clinic with 100 dental chairs and a 15 times larger number of amalgam fillings placed per day was well below the NIOSH level. Immediate working environment aerosols and exhaust air from dental vacuum systems may be inhaled. The wearing of face masks provide little, if any, respiratory barrier to mercury vapour.

In a recent study in Canada it was observed that mercury vapour exposure during dental training on amalgam removal remained below occupational exposure limits (Warwick *et al.,* 2013).

3.3.1.5. Considerations on exposure

All exposure measurements are subject to imprecision and may not reflect the true mercury concentrations in the target organs. Mercury exposure is generally expressed as total mercury in body fluid or tissues, without differentiating between organic vs. inorganic forms as well as between sources (dietary vs. dental amalgam or other minor sources). As a general caveat, exposure imprecision tends to bias study findings towards the null hypothesis, i.e. the dose-related toxic effects may be underestimated (Grandjean 2008; Grandjean and Budtz-Jørgensen, 2010)

The use of chelating agents (e.g. DMPS) was found to be of no added diagnostic value (Vamnes *et al.,* 2000) and chelating substances may be associated with notable side effects (Schuurs *et al.,* 2000).

There may be differences in internal exposure since mercury excretion may differ between boys and girls 8-18 years of age, treated with dental amalgam (Woods *et al.,*2007). Mercury is eliminated as glutathione (GSH) conjugates (Custodio *et al.,* 2005). Goodrich *et al.,*(2011) suggest that polymorphisms in selenoproteins and glutathione-related genes may influence elimination of mercury in the urine and hair or mercury retention following exposures to inorganic mercury (via dental amalgams) and methyl mercury (via fish consumption). (See paragraph 3.3.2.1)

While several common mutations of the catalase gene (*CAT*) are known, their impact on the mercury toxicokinetics is unknown. Alcohol intake may inhibit this enzyme. Experimental studies in guinea pigs suggest that combined ethanol and mercury vapour exposure will lead to increased mercury retention in the brain, heart and kidney when compared to exposure only to mercury vapour (Yoshida *et al.,*1997).

Sherman *et al.,*(2013) suggested that Hg isotopes can be used to differentiate between exposure to fish-derived inorganic mercury and elemental mercury inhaled from dental amalgams. A large part of the urinary mercury was found to be derived from methyl mercury due to fish consumption. Demethylation of methyl mercury from seafood gave a major contribution to the mercuric mercury excreted in the urine in North American seafood-consumers with fewer than 10 amalgam fillings. Only for individuals with more than 10 amalgam restorations did a large percentage of the mercury derive from exposure to elemental mercury.

3.3.1.6. Conclusions on mercury exposure from dental amalgam

Exposure of individuals to mercury from dental amalgam fillings has been estimated based on assumptions regarding relative exhalation/inhalation of elemental mercuryvaporised in the oral cavity and ingestion of Hg dissolved in saliva. Exposure assessments based on such considerations have a significant variation due to differences in systemic availability of Hg after inhalation and ingestion. Moreover, individual factors influencing mercury-release from dental amalgam fillings (such as gum chewing, tooth brushing, bruxism, dietary habits, and different rates of Hg releases from different amalgam types) are difficult to consider in such assessment.

The SCENHIR therefore performed the exposure assessment based on urinary excretion of Hg in individuals with and without amalgam fillings. Data on urinary excretion of mercury are available on a large number of subjects from several surveys. Urinary excretion of mercury is considered a suitable biomarker of systemic exposures to elemental and inorganic mercury, though some of this may have been derived from organic mercury that was demethylated. In addition, attention must be paid to the fact that urinary mercury excretion is affected by several other factors other than absorption of elemental mercury from amalgams. Fish and seafood consumption has a major influence on mercury body burden in the general population and few studies have been designed to separate the contribution from the various sources. Data on total urinary excretion indicate that dental amalgam restorations are currently considered the main source of inorganic mercury exposure.

However, recently results obtained by using mercury isotopes to differentiate between exposure to fish-derived or amalgam derived-mercury in the urine indicate that a large part of the urinary inorganic mercury was found to be derived from fish consumption and only for fish-consumer-individuals with more than 10 amalgam restorations a large percentage of mercury derives from exposure to elemental mercury from amalgam. Consequently at low levels of exposure from amalgam, the urinary mercury excretion will provide an imprecise indication of that source of exposure for inorganic mercury exposure. Unfortunately no other non-invasive

biomarker is available, nor is any non-invasive way to estimate the levels possibly accumulated in different tissues.
Estimated daily absorption of inorganic mercury from dental amalgam ranges from 3 – 17 µg/day. It also has been estimated that in urinary excretion of mercury each amalgam filling will contribute to an increase of 0.1 µg Hg/L. The mean urine mercury levels in non-occupational groups range from 0.1 to 5 µg/L, while in dental personnel reported ranges are between 3 µg/L and 22 µg/L.

3.3.2. Mercury toxicology

In general, the toxicology of mercury is highly dependent on the route of administration, the exposure conditions and the speciation of mercury. Since human exposure to mercury from dental amalgams may occur by inhalation of mercury vapour released from the dental fillings into the oral cavity, by ingestion of the released inorganic mercury, or swallowing small pieces of amalgam releasing mercury in the alimentary tract, this discussion focuses on the toxicology of inorganic mercury. The ECHA website indicates the following classification for mercury (http://apps.echa.europa.eu/registered/data/dossiers/DISS-9d872986-171c-222a-e044-00144f67d249/AGGR-995f8d07-ac73-4081-8ad0-ed8b5616e7ee_DISS-9d872986-171c-222a-e044-00144f67d249.html).

Table 2: Hazard statements

Hazard statements	Risk phrases	Safety phrases
H330: fatal if inhaled	R26: very toxic by inhalation	S45: in case of accident or if you feel unwell, seek medical advice immediately
H360: may damage fertilityor the unborn child	R61: may cause harm to unborn child	S53: avoid exposure - obtain special instructions before use
H372: causes damage to organs	R48/23: Toxic: danger of serious damage to health by prolonged exposure through inhalation	S60: this material and its container must be disposed of as hazardous waste
	R50/53: - Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment	S61: avoid release to the environment

It should be noted that classification is a hazard based process, referring to the intrinsic toxicological potential, with no reference to the doses able to elicit the effects. The dose-response is a concept related to risk assessment.

3.3.2.1. Toxicokinetics

General toxicokinetics

Mercury vapour is lipophilic and can pass biological membranes, including the blood-brain barrier and placenta, thus resulting in deposition in the central nervous system, including the foetal brain. The vapour dissolved in the blood and tissues rapidly becomes oxidised due to catalase activity. Ionic Mercury becomes bound to some extent to metallothionein and accumulates in the kidneys. Excretion takes place mainly through the urine and some is eliminated through faeces and sweat (Sanfelieu, 2003).

Oral ingestion of liquid elemental mercury results only in a very limited absorption, typically <0.01 % of the dose (ATSDR, 1999; MAK, 1999; Klaassen, 2001). Dermal absorption of liquid elemental mercury is also very limited. In contrast, approximately 80% of the inhaled elemental mercury vapour is absorbed in the lungs. Due to the high lipid solubility, elemental mercury rapidly penetrates alveolar membranes and is then distributed to all tissues of the body. Elemental mercury is slowly oxidised in the blood in a saturable process to give Hg^{2+} probably by catalases. Due to the ease of saturation of the enzymatic oxidation of elemental mercury to Hg^{2+}, the proportion of inorganic mercury in blood increases with increasing dose of inorganic mercury. A small part of the elemental mercury vapour dose received is also eliminated by exhalation and a small part of the dose is delivered to the central nervous system.
Human toxicokinetic data are scant: it has been reported that after a single exposure to mercury vapour the half-time of distribution to the plasma compartment is approximately 5 hr (Sandborgh-Englund *et al.,* 1998). The amount of mercury in plasma at the time of the peak concentration was 4% of the inhaled dose (95% confidence limit, 3–5%). Approximately 7% of the initial dose is found deposited in the cranial region after a single exposure to nontoxic levels of the vapour. The kidney is the main depository.
When experimental toxicology data are considered, it appears that in squirrel monkeys, a 4-hour exposure to mercury vapour led to a brain retention of 0.27 % of the absorbed amount. In mice, a somewhat higher immediate retention of about 1.2 % was seen, with a decrease over several days to about 0.4 % (Berlin *et al.,* 1969). One can assume that up to 0.3-7% of the absorbed dose may be retained in the central nervous system. Thus, the daily inhalation of up to 10 µg from amalgam fillings may after almost complete absorption result in a brain retention of up to 0.03 – 0.7 µg per day, or an increase in the concentration up to 0.1 µg/kg per day assuming a brain of 1 kg. Although these crude estimates likely represent a worst-case scenario, they indicate an approximate order of magnitude for further consideration.

A recent review of pharmacokinetic modelling studies concluded that predictions using a long half-life of 27.4 years for mercury in the brain are consistent with autopsy findings, and that the evidence from such studies point to a half-life of inorganic mercury in human brains of several years to several decades (Rooney, 2014).

Within the brain, mercury vapour results in high concentrations in the cerebellum, especially in Purkinje cells (Sørensen *et al.,* 2000). Autoradiography studies of marmoset monkeys and mice exposed to radioactive $^{203}Hg^0$ vapour documented that the retention in the central nervous system includes specific accumulation in the anterior horn cells of the spinal cord (Roos and Dencker, 2012; Rooney, 2013).

Methyl mercury elimination in humans mainly occurs via the biliary route after conjugation with liver glutathione S-transferases (GSTs), which produce a stable glutathione–metal conjugate which is then, eliminated mainly via faeces (Ballatori and Clarkson, 1985). However, some mercury can be reabsorbed, thus contributing to the inorganic mercury circulating in the blood. Excretion of inorganic mercury takes place via both urine and faeces. Urinary mercury originates mainly from mercury in kidney tissue.

GSTs are present in all mammalian tissues. They are divided into several classes dependent on their cell location and structure.

GSTs are highly polymorphic in humans; e.g. GSTM1*∗0 (cytosolic mu (µ), subfamiliy 1, null genotype) and GSTT1*∗0 (cytosolic theta (θ, subfamily 1, null genotype) resulting in the

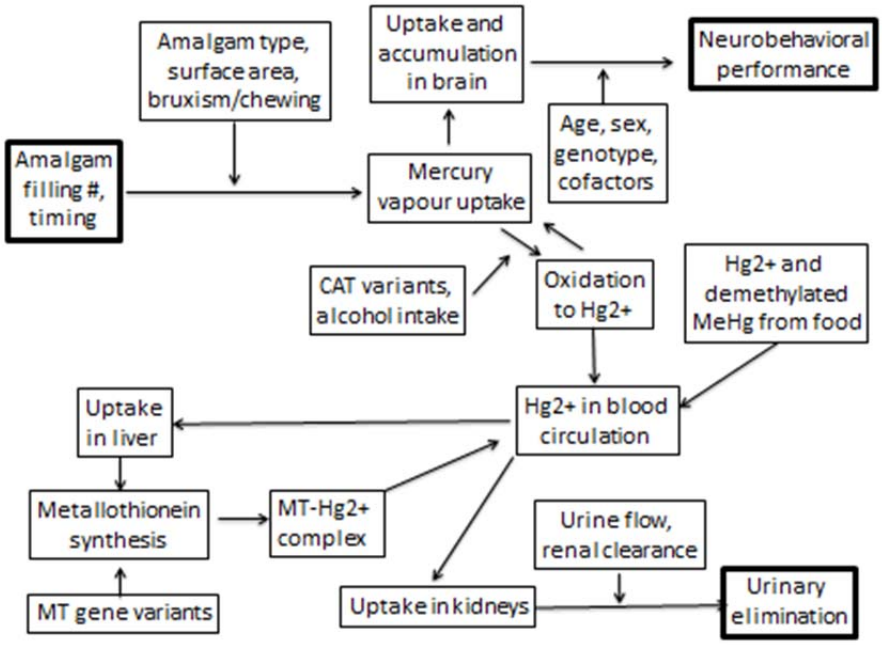
deletion of the entire gene. GST polymorphisms may be associated with methyl mercury detoxification (Mazzaron Barcelos *et al.,* 2012).

Demethylation of methyl mercury from seafood (mainly by gut microflora) may also contribute to the mercuric mercury excreted in the urine, as previously suggested by WHO (1990) by population studies (Johnsson *et al.,* 2005), and by recent studies on mercuryisotopes (Sherman *et al.,* 2013). Indeed, species involved in environmental mercury methylation are present in the human gut (Gibson *et al.,* 1993), and limited evidence supports the notion that human faecal and oral microorganisms can generate methyl mercury from inorganic mercury (Edwards and McBride, 1975; Leistevuo *et al.,* 2001). However, the extent and the rate to which this happens given rise to increased methyl mercury exposure due to dental amalgam is unclear.

Thus, the urinary mercury excretion may not solely originate from amalgam fillings and other sources of elemental and inorganic mercury have to be considered. Sherman *et al.,*(2013) reported that while hair-mercury from dental professionals reflect isotope ratios typical for seafood, the urinary mercury reflected mercury isotope content from dental amalgam. However, in urine also mercury isotope content was noted similar to ratios in seafood as well, though with a wide variability that probably reflect differences in dietary habits. The investigators calculated that, in North American seafood-consumers with fewer than 10 amalgam fillings, most of the mercury in urine comes from demethylation of methyl mercury absorbed from seafood. Accordingly, at low levels of exposure from amalgam, the urinary mercury excretion will provide an imprecise indication of the inorganic mercury exposure. At higher exposure levels, occupational exposure studies also document substantial variability in urinary excretion levels (Symanski *et al.,* 2001). Part of this variability may be related to additional factors such as sample contamination, diurnal variation in exposure and urine production, usage of spot samples, and routine laboratory variability. The authors conclude that in the use of random- and mixed-effects models that combine data across occupational groups, additional studies are warranted to evaluate whether it is reasonable to assume common variances and covariances among measurements collected on workers from different groups.

However, the extent to which this happens and results in increased methyl mercury exposure is unclear.

Figure 1: Fate of inorganic mercury and potential effects



Source: Philippe Grandjean

3.3.2.2. Toxicity of Elemental Mercury

The toxicity of elemental (mercury vapour) and inorganic mercury in animals was recently evaluated by the EFSA and by the JECFA. Both used the results of a 6-month repeated dose study performed in the 1990s as a basis to derive a tolerable weekly intake (TWI) based on effects on absolute and relative kidney weights in rats ($BMDL_{10}$ of 0.06 mg/kg b.w. per day) applying the standard safety factors.

The EFSA (2012) also evaluated some recent studies (Huang *et al.,* 2011; Lukačínová *et al.,* 2011, 2012) that reported ototoxicity and reproductive toxicity. Both studies used only a single dose level. In the Huang study, ototoxicity was observed at a dose equivalent to 0.37 mg/kg b.w. per day as mercury, which is a dose level approximately 6 fold above the $BMDL_{10}$ used as point of departure in the risk assessment. The multigeneration study by Lukačínová *et al.,*(2011) (single dose level of 0.022 – 0.029 mg/kg b.w. per day expressed as mercury) reported adverse effects on survival, lifespan and reproductive parameters at a lower daily dose of mercury exposure than that reported to induce kidney effects. The results of this study were not considered in EFSA's risk assessment due to significant limitations in study design and reporting (i.e. only one dose level tested), low number of animals/group and an unusually high survival (90 -100 %) in control rats as compared to 30 to 35 % in mercury-exposed rats. The SCENIHR supports this evaluation.

Recent toxicology studies have focused on developmental vulnerability to mercury vapour toxicity and the impact of genetic predisposition. In a study that involved postnatal exposure up to 20 days of age in mice, effects were assessed at 12 weeks (Yoshida *et al.,* 2011). Mercury concentrations in the brain were below 0.5 µg/g. Patterns of exposure-associated changes in gene expression in the brain were more extensive in metallothionein (MT)-I/II null mice, which also showed a decrease in locomotor activity in an open field test. In particular, decreases were detected in calcium-calmodulin kinase II (Camk2a) involved in learning and memory. The meaning and relevance of these changes for induction of adverse effects in humans are not clear yet.

3.3.2.3. Neurotoxicity of mercury in laboratory models

Several studies have demonstrated the *in vitro* toxicity of methyl mercury to neuronal cells. Rodent neuronal stem cells in culture showed increased cell death and inhibited differentiation at methyl mercury concentrations as low as 2.5-5 nM (Tamm *et al.,* 2006). Human neural crest cells derived from human embryonic stem cells were tested in a migration assay (Zimmer *et al.,* 2012). A 50% inhibition was seen at 50 nM but statistically significant effects were seen also at 5 nM, while effects at lower concentrations were not distinguishable from the background. In primary cultures of rat cerebellar granular cells (Hogberg *et al.,* 2010), gene expression of neuronal markers was determined from RNA assays after exposure to methyl mercury chloride. Changes in RNA expression and increased neuronal cell death were induced by 50 nM, while changes at 5 nM were equivocal. In a recent study, methyl mercury triggers pronounced effects ($p<0.05$) on proliferation of human amniotic fluid stem cells starting at concentrations as low as 30 nM (6 ng/mL). At higher concentrations, it induced apoptotic effects (Gundacker *et al.,* 2012).

Evidence from *in vivo* animal studies and human autopsies has shown that the most prominent feature after mercury exposure is neuronal loss and alteration of neuronal migration during brain development (Castoldi *et al.,* 2008; Costa and Giordano, 2012). *In vitro* studies have confirmed that mercury primarily targets neuronal cells with a greater affinity than glial cells (Gassó *et al.,* 2001, 2003; Suñol & Rodriguez-Farre, 2012; Costa and Giordano, 2012). The range of Hg concentrations that affect neuronal viability range from 0.4 to 2.9 µM (IC50) when using both primary cultures or neural cell lines, cerebellar granule cells (CGC) being the most sensitive to cytotoxicity (Costa and Giordano, 2012).

Cerebellar granule cells are targeted selectively by mercury compounds *in vivo* (Sanfeliu *et al.,* 2003). Despite the affinity of mercury for thiol groups present in all cells, the molecular determinant(s) of selective cerebellar degeneration remain to be fully elucidated, but neuronal glutamate transport is an important target to be taken into account when assessing mercury-induced neurotoxicity (Fonfria *et al.,* 2005).

These *in vitro* data need to be interpreted in light of the retained mercury concentrations in the brain following mercury vapour exposure, as the tissue distribution in squirrel monkeys exposed prenatally or postnatally to mercury vapour is quite similar to the distribution pattern after exposure to methyl mercury (Berlin *et al.,* 1969).

3.3.3. Toxicology of other metallic elements in amalgam

This has been assessed thoroughly in the former SCENIHR Opinion (2008). There does not seem to be any new information, except for the possibility of nanoparticles being formed by removal, normal wear and attrition of the dental amalgam fillings. This particular issue is discussed in the SCENIHR Opinion: Nanosilver: safety, health and environmental effects and role in antimicrobial resistance (2014). The elements other than mercury used in dental amalgam all have their own, different profiles in terms of essentiality and/or toxicology. There is no scientific evidence that any of those elements currently used in dental amalgam restorations constitute a risk of adverse health effects in individuals apart from allergic reactions to the individual elements.

3.3.4. Weight-of-evidence for a possible risk after exposure to dental amalgam

Regulatory limits for mercury exposures decreased over the years as adverse effects at lower levels of exposure have become better documented. As shown in table 3, inhalation of mercury at an occupational exposure limit results in an uptake of more than 60 µg of Hg per day, whereas inhalation of mercury from dental amalgams results in body burdens which are about

one-fourth or less than those considered acceptable from occupational exposures at present. Similarly, a biological exposure limit of 30 µg Hg/g creatinine in urine is 5-to-10-fold higher than that typically occurring in subjects with amalgam fillings. Thus, the margin between occupational and amalgam-related exposures is less than 10-fold. Tolerable limits for dietary exposures to mercury are relevant to amalgam safety considerations, as inhaled elemental mercury may add to the body burden of inorganic mercury. Recently, the EFSA reported that the tolerable weekly intake for methyl mercury might be exceeded due to fish consumption, while the TWI for inorganic mercury might be exceeded due to the additional inhalation exposure in people with a high number of amalgam fillings. However, evidence is weak as the data are mainly derived from model-based calculations. Studies on large patient collectives did not show any correlation of health effects with the number of amalgam restorations.

Table 3: Air concentrations, blood levels and urinary excretion of mercury in individuals with amalgam fillings compared to levels of mercury considered safe for occupational exposures.

Medium	Individual with dental amalgam fillings	Occupational limit
Air	3 – 17 µg Hg/day	70 µg Hg/day*
Urinary	1- 5 µg Hg/L	30 µg Hg/g creatinine
Blood	3 – 5 µg Hg/L	9 µg Hg/L

**Based on an alveolar ventilation of 9 L/min, a retention of 0.8 for elemental mercury. The EU recommended limit is 0.02 mg/m^3.*

3.3.5. Adverse effects in individuals with amalgam restorations

Mercury toxicity associated with methyl mercury, elemental (vapour) and inorganic mercury is well documented (EFSA 2012; ATSDR, 1999). The question remains whether mercury exposure from dental amalgams can cause adverse health effects, including neurological and kidney diseases, neuropsychological deficits and other less clearly defined conditions, such as chronic fatigue, memory impairment and depression.

The types of adverse effects may be local, systemic or psychological, and are discussed below.

3.3.5.1. Localized mucosal reactions

The possibility that restorative dental materials could be responsible for lesions within the mouth associated with direct contact between the material and the oral mucosa is obviously of importance. Such localised reactions are often discussed in the context of allergies and hypersensitivity.

In the dental clinic two reaction patterns are relevant: the delayed reaction (Type IV) and the immediate reaction (Type I). In the type IV reaction, the incomplete allergens (haptens) are brought in contact with tissue proteins by way of the oral mucosa to form complete allergens. Provided that previous sensitisation has taken place, specialised T-lymphocytes now produce inflammatory mediators causing tissue damage, seen as contact mucositis, i.e. intra-oral diffuse red zones, blisters, or ulceration with pain and burning sensation. The inflammation is not always limited to the exposure site. Contact dermatitis may be observed in the face or more distant locations as urticarial or eczematous reactions. An enhanced risk for atopic

patients to become sensitised against dental materials in general could not be established. However, for special materials like amalgam and composite resins (Bis-GMA; a methacrylate) there seems to be a higher risk for sensitisation for atopic patients (Rojas-Alcayaga *et al.,* 2012). A suspected Type IV reaction may be confirmed with an epidermal patch test (Roitt and Delves, 2006, Schmalz and Arenholt-Bindslev, 2009).

An immediate type (Type I) allergic reaction is based on the release of vasoactive humoral mediators from mast cells or basophilic granulocytes. These mediators are released from the cells upon contact with antigens binding to the IgE antibodies on their surface. The antigen specific IgE antibodies provide the specificity of the allergic response. The released mediators lead to increased capillary permeability and contraction of smooth muscles. The symptoms may consist of urticaria, asthmatic seizures, swelling of the mucosa of throat and eyes and even result in anaphylaxis, all seen within minutes. This immediate type of hypersensitivity is in general associated with allergic responses to protein allergens. Potential full allergens encountered in restorative dentistry are mainly limited to the accessories used, including residual proteins from natural rubber latex in gloves, rubber dam, polishing remedies or parts of anaesthetic cartridges and in seldom cases acrylates (Schmalz and Arenholt-Bindslev, 2009).

A chronic inflammatory response of the gingival tissue around restorations may be present, which appears as chronic gingivitis, recurrent necrotic gingivitis and periodontal pockets. When patients with self-diagnosed oral problems (142 women and 76 men) were examined, the mean concentration of mercury in the whole blood was 17.3 nmol/l and no value exceeded 50 nmol/l. Mental disorder was diagnosed in 93 cases (42.7%), including 41 cases of generalized anxiety disorder and 12 cases of panic disorder. A total of 82 patients (40%) did not work because of medical reasons or unemployment (Herrstrom and Hogstedt, 1993). However, no correlation could be demonstrated between the oral symptoms and a generalized toxic effect of amalgam fillings.

Amalgam tattoos, which are occasionally observed, are associated with the iatrogenic introduction of small particles of dental amalgam, inadvertently implanted into oral soft tissues during dental procedures. Tattoos are resistant to protracted conventional therapies. Most of the foreign bodies examined by light-microscopy and Energy-dispersive X-ray spectroscopy (EDS) methods contained amalgam (amalgam dusts) that appears either as fine granular or larger globular structures implanted in gingival tissues. There is no free mercury, but large globular pieces of amalgam, which induce metallothionein expression in adjacent histiocytes. There is no consequence to the presence of tattoos, except the unpleasant dark blue staining of the gingiva (Lau *et al.,* 2001) and currently there is no indication for the surgical removal of these tattoos.

Metals, including mercury, in close contact with skin and mucosa are well-recognised causes of contact dermatitis (Garner, 2004, Raap *et al.,* 2009). Oral lichen planus is associated with dental restorations and one of the causes may be contact allergy to constituents of dental amalgam (McPharland and Warnakulasuriya, 2012, Ahlgren *et al.,* 2013). Khamaysi *et al.,*(2006) examined 134 patients presenting with mucosal reactions, where the most frequent oral manifestations were cheilitis, peri-oral dermatitis, burning mouth, lichenoid reactions and orofacial granulomatosis. Patch testing showed several allergens in this group, including metals such as gold, cobalt, platinum, nickel and mercury. No specific association between any one metal and a specific clinical manifestation was found but mercury was not a significant factor contributing to the pathogenesis of oral lichenoid reactions. In another study on a patient group with Oral Lichen Planus (OLP) and on Oral Lichenoid Reactions, sensitisation towards amalgam was found to be more seldom than towards gold sodium thiosulfate, palladium chloride or nickel sulfate (Raap *et al.,* 2009).

When dental amalgam was removed in a subgroup of patients suspected of amalgam contact hypersensitivity lesions, considerable improvement was seen (Thornhill *et al.,* 2003). Seventy percent of these patients also showed a positive skin patch test for amalgam or mercury. Total or partial replacement of amalgam fillings following a positive skin patch test reaction to ammoniated mercury, liquid mercury, or amalgam is followed by significant improvement, when the lesions are confined to areas in close contact with amalgam fillings. Similar results have been reported in a more recent study (Luiz *et al.,* 2012) and in a review by McPharland

and Warnakulasuriya (2012). Even if there is no topographic relationship, improvement occurs in nearly all patch test-positive patients (Laeijendecker *et al.,* 2004) although there is no general evidence that either OLP or oral lichenoid lesions patients would routinely benefit from having *all* their amalgam restorations replaced (Baccaglini *et al.,* 2012). If mercury is the allergen, the removal of the filling should lead to complete remission after about 3 months. A total of 51 patients who had oral lichenoid lesions suspected to be related to the dental restorations were investigated. Fifty three per cent (n= 27) of the patients had positive patch test reactions, 24 of them for one or more mercury compounds. Nine months after the removal of the fillings, 42% of the patients were completely healed. Improvement was found in 47% especially when lesions were in close contact with restorations (Issa *et al.,* 2005). Contact with amalgams and positive patch testing are good but not absolute indicators of the beneficial effect of amalgam replacement (Montebugnoli *et al.,* 2012). This possible adverse effect of dental amalgam is widely recognised and reflected in contemporary contra-indications for the use of this material.

Burning Mouth Syndrome can occasionally be associated with a change in the appearance of the clinically normal oral mucosa but no significant association between the burning mouth patients and positive patch test reactions was found (Marino *et al.,* 2009). In some cases it may be associated with a strong allergy to mercury and a positive patch test supports the removal of the amalgam filling. Full recovery and complete remission of systemic dermatitis may occur after removal of a mercury-containing filling (Pigatto *et al.,* 2004). Patch-test analysis for the determination of mercury allergies was carried out by Wong and Freeman (2003) on a group of 84 patients with reticulate, lacy, plaque-like or erosive oral lichenoid lesions. Thirty-three (39%) of the patients had positive patch-test findings. The amalgam fillings were removed for thirty of them, and an improvement was seen within 3 months in 28 of them (87%).

3.3.5.2. Systemic effects

There are a number of epidemiological studies on the possible health effects of mercury released by dental amalgam fillings. The effects reported may affect the nervous and renal system, and also the immune, respiratory, cardiovascular, gastro-intestinal, haematological, and reproductive systems. A variety of study designs has been used, some of which are less than optimal, thus limiting the conclusions that can be drawn. Bates (2006) concluded that the available studies show little evidence of effects on general chronic disease incidence or mortality. On the other hand, although a number of new studies have been published after 2006, most of the studies reviewed were ecological, i.e. without individual exposure information, or based on proxy measures of exposure, such as number of amalgam fillings. Thus, because of exposure misclassification, such studies may overlook dose-response relationships, unless the linkage is strong.

In a New Zealand retrospective cohort study of 20.000 military personnel (84% males) followed up for 20 years, data on dental history was linked with national mortality, hospital discharge and cancer incidence databases. The study design was highly appropriate, but no association was found between dental amalgams and chronic fatigue syndrome or kidney diseases. Based on the ICD codes, amalgam exposure showed a significantly increased risk of mononeuritis of the upper limb and mononeuritis multiplex, while inflammatory and toxic neuropathy showed a decreased risk. The authors state that in the absence of supporting evidence, they regarded these results as hypothesis-generating. It is likely they have arisen as a result of the number of statistical tests that were carried out—the well-known 'multiple comparisons' issue. The number of cases for investigation of Alzheimer's or Parkinson's diseases was insufficient to draw any conclusion (Bates *et al.,* 2004).

Other population-based studies have focused on dentistry personnel in comparison with other occupational groups (Thygesen *et al.,* 2011). They are reviewed in 3.3.6.

Cross-sectional studies are less informative. For example, in 56 patients with perceived chronic mercury toxicity (various medical symptoms), mercury levels in blood and urine were within the

reference range (Eyeson *et al.,* 2010). However, the exposure assessment may not represent the causative exposure, thus preventing meaningful conclusions. Similar concerns can be raised in regard to several other studies of patient groups.

The available evidence for health effects due to mercury from amalgam fillings is discussed below in relation to specific organ systems.

Urinary system

Mortada *et al.,*(2002) investigated 49 healthy individuals with amalgam fillings and 51 matched controls. The mercury concentration in urine was correlated to the number of amalgam fillings. In the amalgam group, urinary excretion of NAG and albumin correlated with the number of fillings and albuminuria with blood and urine mercury levels. Other kidney biomarkers were not affected. Bellinger *et al.,*(2006) selected 534 children for a randomised clinical trial, comparing groups with amalgam restorations and alternative composite resins (New England Children's Amalgam Trial). After five years, renal data were obtained on 409 children. A significantly higher mean urinary mercury level was noted in the amalgam group, but the renal function was comparable in the two groups as measured by creatinine adjusted albumin levels. However, a follow up of the same group of children showed an increased prevalence of microalbuminuria among children with amalgam fillings (Barregard *et al.,* 2008), but no change in biomarkers for tubular function.

In the Casa Pia study, 507 children from Lisbon were randomised to amalgam or composite resin dental care groups and evaluated annually over a 7 year period. Analyses showed no significant association of amalgam with various renal biomarkers including microalbuminuria (DeRouen *et al.,* 2006, Barregard *et al.,* 2008). Later, some urinary porphyrins were reported to be increased in a subgroup of the youngest children in the amalgam group, but the levels were below those considered to be able to cause renal damage (Woods *et al.,* 2009). Other analyses of selected samples from the same study using different statistical methods (after data had been generated) suggest that Hg-associated urinary porphyrins are increased in amalgam treated children (Geier *et al.,* 2011) and that glutathione-S-transferases (GST)–α increased with time in amalgam treated children (Geier *et al.,* 2012). This study has been challenged by DeRouen *et al.,*(2014), authors of the original study, who draw the attention to the fact that Geier *et al.,*used a post-hoc evaluation with the potential of bias and that the statistical methods Geier *et al.,*used did not comply with current standards (e.g. no correction for multiple comparisons).

A cross-sectional study of 403 Chinese school children, about half of whom had amalgam fillings, showed a slight increase in urinary mercury concentration in children with amalgam fillings, but no difference in renal biomarkers was observed (Ye *et al.,* 2009).

A study from Saudi Arabia analysed a number of different renal biomarkers in 182 children. Only urinary NAG levels were significantly higher in children with dental amalgam fillings than in those without fillings (P=0.008). In contrast, both α1-MG and 8-OHdG levels were higher in the non-amalgam group than those with and P-values were 0.004 and 0, respectively. None of the biomarkers revealed a significant correlation with the number of dental amalgam fillings (Al-Saleh *et al.,* 2011, 2012). The authors state that confirmation of these data is needed.

Studies in rodents suggest that mercury elimination is compromised as a result of experimental kidney damage (Zalups, 1997). Systematic studies in humans have not been found.

Overall, the conclusion of available epidemiological studies is that only limited evidence suggests that mercury from dental amalgam fillings affect clinical kidney function, although any long-term risk of kidney disease in humans needs to be ascertained. The known accumulation of mercury in the kidneys and the observed effect on some porphyrin excretion and possible changes in special biomarkers are of some concern. However, additional data are necessary to evaluate whether such changes have long-term clinical significance.

Neurological System

Neurological diagnoses

Inorganic mercury is a neurotoxicant and it has therefore been suggested that it may play a role in the pathogenesis of neurodegenerative diseases such as Alzheimer's disease (Mutter *et al.,* 2010).

A cross-sectional study that found substantially elevated blood-mercury concentrations in Alzheimer patients, especially those with early-onset disease (Hock *et al.,* 1998), is difficult to evaluate, as the premorbid levels and sources of exposure are unknown. Also, this study found no association with the number of fillings as such. However, these findings have not been confirmed. A recent review of the literature reported some cases of increased mercury levels in brain tissue of patients with Alzheimer's disease but measurements in other tissues and body fluids were inconsistent. While retention in the brain would be considered most relevant, the data available do not allow a judgement on whether a relationship exists between dental amalgam and Alzheimer's disease (Mutter *et al.,* 2010).

A possible association between amalgam and multiple sclerosis has been suggested (Bates *et al.,* 2004), but the evidence is inconclusive. Thus, the small number of subjects, inadequate and imprecise exposure data, and inadequate control recruitment methods constitute limitations of the available studies (Aminzadeh and Etminan 2007).

In regard to amyotrophic lateral sclerosis (ALS), the evidence suffers from the same weaknesses as indicated above. It is thought that an interaction between mercury exposure and an individual's genetic makeup is required to produce epigenetic changes that may ultimately lead to the disease (Callaghan *et al.,* 2011).

Parkinson's disease is suggested to be linked to mercury exposure, but the disease has a multifactorial etiology. In workers exposed to mercury vapour, single-photon emission computed tomography examination revealed decreased dopamine innervation in the striatum, caudate and putamen, and a negative association with urinary mercury and simulated exposure levels (Lin *et al.,* 2011). Such findings reflect early changes that may be part of the Parkinson's disease pathogenesis. However, a nation-wide register-linkage study of dentists and dental assistants, as compared to professionals and secretaries in general practitioners' and lawyers' offices, did not show any increased risk of Parkinson's disease associated with dentistry employment although a small excess risk could not be excluded (Thygesen *et al.,* 2011). Thus, overall, the current evidence does not allow any judgment on whether mercury exposure from amalgam fillings is associated with the development of degenerative diseases of the nervous system.

A large American study of 452 2-to-5-year-old children with autism or autism spectrum disorders did not show any difference in current blood mercury concentrations in patients compared to controls (Hertz-Picciotto *et al.,* 2010). The blood levels of mercury depended both on the number of amalgam fillings and fish consumption, but they may not necessarily reflect premorbid or causative exposures.

A prospective blinded study on 100 patients with autism showed a correlation between the number of amalgam fillings in the mother during pregnancy and the severity of autism (Geier *et al.,* 2009). The patients were recruited at outpatient genetic consultations at the Genetic Centers of America. Patients whose mother had 6 or more amalgam fillings had 3.2 times greater risk of having a severe autism compared to patients with mild autism where the mother had 5 or less amalgam fillings. However, this paper shows serious limitations in methodology used (e.g. the estimation of the number of amalgam fillings present during pregnancy in the past, no adjustment for diet and socio-economic status).

In conclusion, the overall available data do not show a correlation between autism and blood mercury levels in small children. However, although causality was not demonstrated, one paper indicated a a possible association between the severity of autism in autistic children and the number of dental amalgam fillings in their mothers during pregnancy, thus suggesting a need for further research.

Neurological function tests

In the Casa Pia study (DeRouen *et al.,* 2006), annual neurological examinations were performed on 507 children. There were no significant differences between the amalgam and resin-based composite groups and it was concluded that exposure to mercury from dental amalgam does not adversely affect the neurological status of children (Lauterbach *et al.,* 2008, Mackert 2010). In the parallel study performed in the US (Bellinger *et al.,* 2006), a total of 534 children aged 6 to 10 years at baseline with no prior amalgam restorations and 2 or more posterior teeth with caries were randomly assigned to receive dental restoration of baseline and incident caries during a 5-year follow-up period using either amalgam (n=267) or resin composite (n =267) materials. The primary neuropsychological outcome was a 5-year change in full-scale IQ scores. Secondary outcomes included tests of memory and visuomotor ability. In this study, there were no statistically significant differences in adverse neuropsychological effects observed over the 5-year period in children whose caries were restored using dental amalgam or composite materials.

In a cross-sectional study of 403 Chinese school children, neurobehavioral and neuropsychological performance could not be shown to be associated with the presence of amalgam fillings (Ye *et al.,* 2009).

In cross-sectional studies of U.S. air force personnel, no significant associations were found between amalgam exposure and clinical neurological signs of abnormal tremor, coordination, station or gait, strength, sensation, or muscle stretch reflexes or for any level of peripheral neuropathy among the study participants. However, a statistically significant association was detected between amalgam exposure and the continuous vibrotactile sensation response in non-diabetic participants (Kingman *et al.,* 2005). No adjustment was made for multiple tests and the authors conclude "Overall, we found no association between amalgam exposure and neurological signs or clinically evident peripheral neuropathy". No follow-up studies have been published.

Auditory thresholds were measured in 39 non-smoking women aged 40-45 years. There was a significant positive correlation between the number of amalgam fillings and the decline in hearing thresholds, the strongest association was found at 14 kHz (Rothwell and Boyd 2008). No correlation was found for non-amalgam fillings. This has not been confirmed by other studies so far.

The visual system may also be vulnerable to mercury exposure, but the studies usually do not include the sensory test outcomes that would have revealed such deficits. In one study, visual contrast sensitivity was examined in relation to exposure from dental amalgam. A decline was shown at increasing urinary mercury excretion (geometric mean, 0.16 µg/24 h in connection with an average of 1.15 amalgam fillings per child) in 384 German children at age 6 years. According to the authors, this decline could not be classified as a disease (Altmann *et al.,* 1998).

In conclusion, there are some publications that indicate that exposure to mercury may be associated with some decline in the auditory and visual system.

Neurobehavioral functions

During the past decades, mercury and other metals have been claimed to be responsible for a series of mental health problems, with a variety of symptoms (Bratel *et al.,* 1997a,b), not limited to neurobehavioural ones.

A series of patients with various health complaints were referred to the Dental Biomaterials Adverse Reaction Unit in Bergen, Norway (Lygre *et al.,* 2005). The complaints were heterogeneous. Many individuals displayed multiple subjective symptoms associated with several organ systems. The most common were fatigue, muscle and joint pain, dizziness and headache. Intra-oral symptoms were related to burning sensations, taste disturbances and dry mouth. After removal of the mercury-containing fillings, a small decrease in the intensity of different symptoms was noted. Intra-oral symptoms were decreased and the decrease was statistically significant for taste disturbances (p=0.001), dry mouth (p=0.034), and stiffness/paraesthesia (p=0.05). However, the symptoms were still higher than in a reference group sampled from the general population in Norway.

Follow-up studies on the above-mentioned patient study were recently published (Sjursen *et al.,* 2011, Lygre *et al.,* 2012). Three years after removal of amalgam fillings most of the health complaints decreased, being statistically significant for taste disturbances, pain from muscles and joints, gastrointestinal complaints, complaints from ear/nose/throat and fatigue. Interestingly, serum levels of several Th1 cytokines were slightly but significantly increased in the patient group before removal of the fillings and some of them were normalised one year after (Björkman *et al.,* 2012). It is unclear if raised cytokine levels may explain some of the symptoms.

Another study from Germany compared three strategies in 90 patients with health complaints attributed to amalgam fillings. The individuals were randomly assigned to either removal of amalgams fillings, removal combined with doses of vitamins and trace elements, or participation in a health promotion program without removal of dental amalgam. In all three groups clinically relevant improvements were observed after 1 year, with no statistically significant difference between the groups (Melchart *et al.,* 2008).

Two longitudinal studies were carried out on a Swedish population including patients with amalgam related complaints. The first one evaluated cognitive functions in 342 patients and 342 matched controls (Sundström *et al.,* 2010). None of the cognitive tests showed any difference between the groups. The second study involved 337 patients with self-reported amalgam complaints and the same number of matched controls (Sundström *et al.,* 2011). Many of the patients with complaints had experienced negative life events as somatic illness, death of a very close family member or financial problems. It was concluded that adverse negative life events could play a vital role in understanding and explaining amalgam-related complaints.

A German study analysed two different databases. In the first, 90 patients attributed their health complaints to dental amalgam, and in the second 116 patients from an outpatient unit for environmental medicine attributed their symptoms to environmental sources other than amalgam. The results showed some differences in symptomatology, while general psychological distress was similar in both groups, indicating no strong evidence for an amalgam-specific syndrome (Weidenhammer *et al.,* 2009).

In conclusion, patients with self-reported symptoms attributed to amalgam fillings constitute a heterogeneous group; the study design presents possible selection bias, not having defined inclusion/exclusion criteria, thus limiting the validity of data, which are difficult to interpret. Negative life events and environmental factors may also play a role.

Neuropsychological development

The developing brain is known to be uniquely sensitive to neurotoxic damage, but exposures in early life generally result in non-specific deficits that may be difficult to document in the presence of multiple risk factors (Grandjean and Landrigan, 2014).

Two randomised, controlled clinical trials have been carried out on the neuropsychological and renal effects of dental amalgam in children (Bellinger *et al.,* 2006 and 2007, DeRouen *et al.,* 2006).

In the first study 534 children aged 6 to 10 years living in the New England area (USA), were randomly assigned to receive dental restorations using either amalgam (n=267) or resin composites (n=267). They were selected from a background population almost 10 times larger and re-examined after 5 years. No difference appeared in full-scale IQ. No difference was found in the general memory index. It was concluded that the exposure to mercury from dental amalgam at this age, on average, was not associated with any detectable adverse neuropsychological effects over a five year period and that the use of dental amalgam is not associated with an increase in children's risk of experiencing neuropsychological dysfunction. The findings suggest that the health effects of amalgam restorations in children need not be the basis of treatment decisions when choosing restorative dental materials. Another follow-up study showed no evidence that exposure to mercury from dental amalgams was associated with adverse psychosocial outcomes over the five-year period following initial placement of amalgams. All significant associations favoured the amalgam group (Bellinger *et al.,* 2008).

In the other randomised clinical trial ("The Casa Pia study"), annual follow-up for 7 years was carried out on 507 children in Lisbon, Portugal (DeRouen *et al.,* 2006). The children received either amalgam restorations (n=253) or resin composites (n=254). The creatinine-adjusted urinary mercury levels were 1.8µg/g in the amalgam group, and 1.9 µg/g in the composite group. No statistically significant difference was found in measures of memory, attention, visual function, or nerve conduction velocities over all the 7 years of follow-up. The authors also noticed that the need for additional restorative treatment was approximately 50% higher in the composite group. These data suggest that exposure to dental amalgam restorations within this age range has no important adverse effect on average psychological development, with the superior performance of the amalgams compared to alternatives being noteworthy.

However, further examination of the data, with assessment of the heterogeneity of the coproporphyrinogen oxidase gene (CPOX) gene (CPOX is an enzyme responsible for the conversion of coproporhyrinogen III to protoporphyrinogen III in the haeme biosynthetic pathway), showed decreased neurobehavioral test performance correlated with increased urinary mercury level in boys with the CPOX4 variant (Woods *et al.,* 2012). This enzyme defect causes hereditary coproporhyria (HCP), in which one third presents with neurological symptoms. The disease is latent before puberty although a few homozygous cases with onset in early childhood have been reported (Sassa, 2006). HCP can be induced by drugs, environmental stressors and diet changes.

Examination of other genetic polymorphisms in the genes of metallothionein and catechol-O-methyltransferase also showed that certain variants increased the susceptibility of boys to adverse neurobehavioral effects of mercury (Woods *et al.,* 2013, 2014). It is important to note that the three articles by Woods *et al.,*(2012, 2013, 2014) do not compare amalgam versus alternative treatment, but evaluate the association between mercury levels in urine and outcome of the neurobehavioral tests. The authors estimate that only about 17 % of the urinary mercury level variation was due to amalgam (15 % in girls), indicating considerable background mercury exposure unrelated to dental amalgam. They therefore conclude that the findings do not support an association between mercury in dental amalgam and adverse neurobehavioral outcome observed (Woods *et al.,* 2013, 2014).

A retrospective study of 587 mother-child pairs from the Seychelles evaluated the association between prenatal exposure from maternal amalgam restoration status and the results of six neurodevelopmental tests at the age of 66 months. None of the tests showed an adverse association with the number of amalgam fillings in the mothers during gestation (Watson *et al.,* 2011). This cohort also failed to show any clear evidence of adverse neurotoxic effects of methyl mercury exposure (Karagas *et al.,* 2012).

Likewise, in a cross-sectional study of 403 Chinese school children, neurobehavioral and neuropsychological performance could not be shown to be associated with the presence of amalgam fillings (Ye *et al.,* 2009).

In conclusion, there is no evidence that amalgam negatively influences the neuropsychological development of children.

Immune System

Mercury is able to induce autoimmunity in susceptible strains of rodents and so the question arises as to whether such effects are seen in humans with respect to amalgam related mercury exposure.

In 24 patients heavily exposed to amalgam and showing various adverse effects, none developed autoimmunity to glomerular basement membrane, even in patients showing allergy to mercury (Guzzi *et al.,* 2008).

The susceptibility to sensitisation to dental materials was compared in 40 atopic and 40 non-atopic patients. Among the atopic patients, 67 % were sensitised to one or more allergens, including amalgam and ammoniated mercury, while 55 % of the non-atopic patients were sensitised (Rojas-Alcayaga *et al.,* 2012). The difference is not significant ($p>0.05$) and thus suggests the need for further studies.

A subpopulation of the participants in the New England study were tested for *in vitro* manifestations of immunotoxic effects of dental amalgam. T-cell and monocyte responses were slightly diminished 5-7 days after amalgam restorative treatment, but no differences were observed at follow-up at 6, 12 or 60 months (Shenker *et al.,* 2008).

In a Norwegian study of immune markers in patients with self-reported health complaints associated with amalgam fillings, an increased level of Th1 type proinflammatory cytokines was found in the patients. Twelve months after removal of the fillings, the cytokine level was normalised for most of the cytokines (Björkman *et al.,* 2012) along with a decrease of the symptoms (Sjursen *et al.,* 2011). It is unknown if the increased level of proinflammatory cytokines might have played a role for the health complaints.

In conclusion, inorganic mercury exposure may cause adverse effects on the immune system. However, there is no evidence that autoimmune disease is provoked in humans by mercury exposure from amalgam fillings. In some patients with allergy to mercury, clinical improvement is seen after removal of amalgam fillings. There is some evidence that exposure to mercury influences proinflammatory cytokine levels, but the clinical implications are not clear.

Reproductive system

Although reproductive effects have been addressed in several of the studies discussed in this Opinion, there is very little data available on this subject. There is no evidence of any association between amalgam restorations and either male of female fertility or obstetric parameters. One study that attempted to examine the question of fertility in detail failed to show any correlation between the mercury burden from amalgam restorations and male fertility disorders (Hanf *et al.,* 1996).

The fecundability of 558 female dental surgeons was examined vs. 450 high school teachers. Occupational exposure had no clear adverse effects on fertility among female dental surgeons, except for a possible effect in the last pregnancy of multiparous dental surgeons. However it should be noted that beside mercury, dentists were occupationally exposed also to chloroform, ethanol, benzene, which could act as confounding factors (Dahl *et al.,* 1999).

Other effects

A study of 75 mother-child pairs from Slovakia showed that exposure to mercury from amalgam and the environment influences thyroid hormone status with e.g. lower thyroxine levels in the mothers. However, in this study, mercury exposure of children did not correspond with the cord or maternal blood mercury at the time of delivery. Mercury exposure status of children at age of 6 months depended more likely on other sources than prenatal exposure. (Ursinyova *et al.,* 2012). The relationship between blood mercury levels and antithyroid antibodies was reported (Gallagher & Meliker, 2012). A higher frequency of autoantibodies towards thyroglobulin was noted in women with the highest mercury levels. Although in the latter study dental amalgam presence was not considered, the findings appear meaningful even if the clinical implications are not clear.

Bergdahl *et al.,*(2007) and Naorungroj *et al.,*(2013) found that edentulism was correlated with lower cognitive status. Tooth loss and gingival bleeding were markers of poorer executive function among dentate people. The association of lower cognitive scores with edentulism suggests that past oral diseases may be a risk indicator for cognitive decline, whereas the association with gingival inflammation indicates a possible effect of cognitive decline on oral health.

The relationship between mastication and cognitive function remains unclear, but both animal and experimental human studies suggest a possible causal relationship (Hansson, 2013). They hypothesised that natural teeth are of importance for hippocampus-based cognitive processes, such as episodic long-term memory. A population-based sample of 273 participants (55-80 years of age; 145 women) was investigated in a cross-sectional study. The participants underwent health assessment, completed a battery of cognitive tests, and took part in an extensive clinical oral examination. The number of natural teeth contributed uniquely and significantly to explaining variance (3-4%) in performance on measures of episodic memory and semantic memory over and above individual differences in age, years of education,

gender, occupation, living conditions, and medical history. The number of natural teeth did not have an influence on the performance of measures of working memory, visuospatial ability, or processing speed. Within the limitations of the current study, a small, but significant, relationship between episodic memory and number of natural teeth is evident.

The influence of other, sometimes confounding, parameters in investigating possible relationships between dental amalgam exposure and biochemical or psychological alterations need to be addressed.

Occupational studies have contributed evidence that prolonged exposure (approximately 15 years) to mercury vapour can affect sensory perception in regard to the visual system, resulting in sub-clinical color vision impairment (Urban *et al.,* 2003). Thus, permanent impairment of contrast sensitivity has been documented in former workers from a lamp manufacturing facility (Costa *et al.,* 2008). Furthermore, in workers with exposure to mercury vapour at least one year ago and a current urinary mercury excretion average of 1.4 µg/g creatinine, deficits were detected in colour vision (Feitosa-Santana *et al.,* 2008). Later follow-up supported the conclusion that the deficits may be permanent (Feitosa-Santana *et al.,* 2010). In contrast, another study from Poland showed less clear differences in colour vision in currently exposed workers (Jedrejko and Skoczyńska, 2011). These data are of importance, as vision is usually not included in neurobehavioral assessment batteries, although vision could well be a particularly sensitive target for mercury vapour.

The earlier, now banned use of mercury as antimicrobial agent was reported to induce antibiotic resistance. (Hall *et al.,* 1970, Joly *et al.,* 1975 and Poiata *et al.,* 2000). For the induction of antibiotic resistance in relation to the use of dental amalgam, contradictory studies were reported (Summers *et al.,* 1993, Ready *et al.,* 2007, Roberts *et al.,* 2008). However, in the positive studies the increase in antibiotic resitance did not seem to influence the health of the individual patients.

In general, the intestinal exposure to mercury from dental amalgam seems to be extremely low; as a consequence an effect on intestinal flora is not anticipated.

General conclusion

The exposure of the general population to mercury is mainly due to fish consumption (methyl mercury plus inorganic mercury to a lower extent) and dental amalgam (elemental mercury vapour, inorganic mercury). Elemental, organic and inorganic mercury is toxic to humans and experimental animals, the mechanisms and the degree of toxicity being different depending on the mercury forms. Individual variation in response has been reported especially in determining exposure; age also plays a role in susceptibility, in that the developing brain is more prone to the toxic effects of mercury.

The EFSA (2012) reported that the tolerable weekly intake for inorganic mercury might be exceeded due to the additional inhalation exposure in people with a high number of amalgam fillings. This information is derived from mainly model-based calculations. However, in direct patient studies from Ahlqwist *et al.,*(1993, 1995) no correlation of possible health symptoms for cardiovascular disease, diabetes, cancer and early death in Swedish women with the number of existing amalgam filling was found. In a further study on a large population of 4,787 patients claiming health effects from amalgam (Melchart *et al.,* 1998) no significant correlation between the intensity of complaints or particular groups of symptoms and the number of amalgam-filled surfaces was found. Therefore, no conclusions related to restrictions of the number of amalgam fillings can be drawn.

Concerning the urinary system, several studies show that parameters of kidney function may be influenced by mercury from amalgam, but there is no convincing evidence that dental amalgam is associated with a clinically decreased kidney function in the patients in the short or long term. On the other hand, decreased kidney function (decreased renal clearance) is likely to decrease the ability to eliminate mercury and other substances via the urine.

For the neurological system, there is no clear evidence for an increased risk for Alzheimer's disease, Parkinson's disease or amyotrophic lateral sclerosis associated with amalgam fillings. The data are inconclusive for multiple sclerosis.

Likewise, a possible association between amalgam fillings and clinical signs of peripheral neuropathy (paraesthesia) has not been replicated in more recent studies.

The visual and auditory system may be influenced by mercury from amalgam fillings. There is some evidence that indicates that exposure of the mother in early pregnancy to mercury from amalgam may promote the development of autism in the child. Large studies have been carried out to evaluate the neuropsychological development in children with amalgam fillings or alternative treatments. These studies do not give convincing evidence for a negative effect on the children.

A special patient group is constituted by individuals that attribute various health complaints to amalgam restorations. Some of these patients have a psychiatric or psychological disorder and in some cases a negative life event has been experienced by them. In general, the symptoms seem to improve after removal of the amalgam fillings, but symptoms also resolve after a health promotion program without removal of dental amalgam (Melchart *et al.,* 2008).

The immune system is influenced by mercury exposure in experimental animals and humans. There is no evidence for an increased risk for autoimmune disease due to amalgam fillings, but it seems that the level of Th1 type cytokines may be increased by mercury exposure. The main adverse immune reactions in patients are local reactions near the amalgam restorations, which mainly resolve after removal of the amalgam fillings. In addition, some patients may develop an allergic response to mercury or the dental amalgam.

The local effects of dental amalgam are well established as well as the possibility for individual patients to show allergy to mercury, but they occur at low frequency. Regarding the systemic effects, several papers have suggested effects of dental amalgam exposure on the central nervous system. Since contrasting results have also been published, further studies are needed in order to confirm or negate these findings.

Unfortunately, many of the studies reviewed have imprecise exposure assessment, incomplete adjustment for covariates, and genetic polymorphism has not been considered.

3.3.6. Epidemiological and clinical evidence concerning adverse effects of dental amalgam in dental personnel

Long-term retention in brain and kidneys is impossible to measure in clinical studies (see 3.3.2.2), and mercury concentrations in blood and urine samples may not be sufficiently informative in regard to cumulated past mercury exposures from different origins. As an example, measurement of mercury in autopsy samples showed a case of brain cortex with a mercury concentration of 164 µg/kg, i.e. 9 times higher than the concentration of the second highest case; the subject was later found to have been employed as a dental assistant in the past (Björkman *et al.,* 2007). Mercury concentrations in urine and blood may therefore be misleading as they reflect more recent exposures to mercury. Thus, many studies have used occupational status as a proxy for mercury vapour exposure (Hørsted-Bindslev, 2004). When reviewing past studies of dental personnel, exposure conditions must be considered, in particular the handling of both silver and copper amalgam filling materials without protective gloves and without a proper ventilation system. However, even recent studies support the notion that dental assistants have more frequent neurological symptoms, although the association to mercury vapour exposure is uncertain, as the symptoms are generally non-specific, and other chemical risk factors may have been present (Ngim *et al.,* 1992, Moen *et al.,*2008, Hilt *et al.,* 2009).

No clear association has been detected between mercury exposure and negative health effects in dentists, although their mercury blood level is higher than in a control population. The life span of dentists was shown to be three years greater than that for a control non-dentist group. The same type of effect was seen with many other parameters, indicating that the general health of dentists is good (McComb, 1997). The data do not allow for appropriate adjustment for beneficial factors associated with the dental profession, but these

factors at least appear to exceed any perceived disadvantageous effects due to mercury exposure.

Heggland *et al.,*(2011) investigated whether women who have worked as dental personnel in Norway, a group with possible previous exposure to mercury vapour, have had an excess risk of having children with congenital malformations or other adverse pregnancy outcomes compared to the general population. A cohort of female dental personnel was identified from the archives of the public dental healthcare and the national trade unions in Norway. Data on births and pregnancy outcomes during 1967–2006 were obtained from the Medical Birth Registry of Norway (MBRN). The final cohort of dental personnel consisted of 4482 dental assistants and 1011 dentists. All other women registered in the MBRN were assigned to the control group, in total 1 124 758. Excess risks of several adverse pregnancy outcomes for dental personnel compared to the general population were estimated. Analyses were conducted for the whole time period as well as stratified by 10-year periods.

Female dental personnel had no observed increased occurrence of congenital malformations (including malformations of the central nervous system, dysplasia of the hip, clubfoot, malformations of the heart and great vessels), low birth weight, preterm birth, small for gestational age, changed gender ratio, multiple birth, stillbirth, or prenatal death. On a group level, they did not observe any excess risks of congenital malformations or other adverse pregnancy outcomes among female dental personnel in Norway during 1967–2006 compared to the general population. Svendsen and Hilt (2011) emphasised that assessment and classification of exposure is essential in epidemiological studies and questionnaires might not be the best method to estimate exposure. They found a marked difference between the pairs of employees working in the same clinic regarding the start and termination years for the different preparation methods, and this was partly independent of their occupation. Kappa values for using different preparation methods in the questionnaire and at the interview varied between 0.41 (moderate) to 0.88 (very good). The results of this study indicated that a mailed questionnaire will cause misclassification of exposure.

The observed occurrence of false positive exposure classifications from the questionnaire compared to the interview was higher than for false negative. This is important and may result in serious bias if the prevalence of exposure is low. Due to missing information, detailed questionnaires may also be inefficient if the goal is to construct exposure measures from combinations of several answers in the questionnaire.

Dentists were significantly more likely than control subjects to have suffered from disorders of the kidney (6.5 % vs. 0.6 %) but these self-reported symptoms were not significantly associated with their level of mercury exposure as measured in urine (Ritchie *et al.,* 2004). This difference between dentists and controls remained significant after correcting for multiple comparisons and after adjusting for age and sex using logistic regression (adjusted odds ratio of kidney disorders for dentists: 15.2 (95% CI = 1.8 to 126.3; p = 0.01). As exposure was assessed cross-sectionally, it is possible that the kidney disease resulted in a decreased urinary mercury excretion.

A US study of dentists and dental assistants suggested that an increased prevalence of symptoms of depression, anxiety, and memory was associated with two genetic polymorphisms thought to convey hypersusceptibility to mercury vapour toxicity (Heyer *et al.,* 2009).

More recent epidemiological studies have utilised registry information and therefore avoided problems associated with self-selection and other biases. Still, such studies assumed that all subjects with the same occupational title have the same exposure, thereby introducing possible misclassification. A Danish nation-wide registry study of hospital admissions of 122,481 workers, including 5731 dentists and 33,858 dental assistants, as compared to professionals and secretaries in general practitioners' and lawyers' offices, did not show any increased risk of Parkinson's disease, neurological disease, or kidney disease, associated with dentistry employment (Thygesen *et al.,* 2011).

A US study using pharmacy utilisation data examined a representative sample of dentists and a matched control group and found increased prescription utilization of specific illness medications for neuropsychological, neurological, respiratory, and cardiovascular disease

(Duplinsky and Cicchetti, 2012). However, the link of adverse outcomes to mercury exposure from amalgam work in either of the two latter studies is not clear.

Neurobehavioural tests in 98 dentists (mean age 32, range 24-49) and 54 unexposed controls (mean age 34, range 23-50) consisting of motor and visual function tests showed a deficient performance of the dentists compared to the controls. The performance decreased at increased dose, calculated as the product of the average air mercury concentrations and years of exposure. The dentists were exposed to an average personal air concentration of 0.014 (range 0.0007-0.042) mg/m^3 for a mean period of 5.5 (range 0.7-24) years (Ngim *et al.,* 1992).

Clinical neurological findings

Sletvold *et al.,*(2012) investigated whether dental personnel with previous exposure to metallic mercury (vapour) have later developed disturbances in cognitive function. Ninety-one female participants who had been selected from a previous health survey of dental personnel were investigated neuropsychologically within the following domains: motor function, short-term memory, working memory, executive function, mental flexibility, and visual and verbal long-term memory. The scores were mainly within normal ranges. Relationships between an exposure score, the duration of employment before 1990, and previously measured mercury in urine as independent variables and the neuropsychological findings as dependent variables, were analysed by multiple linear regression controlling for age, general ability, length of education, alcohol consumption, and previous head injuries. The only relationship that was statistically significant in the hypothesised direction was between the previously measured urine mercury values and visual long-term memory, where the urine values explained 30% of the variability. As the study had a low statistical power and also some other methodological limitations, the results have to be interpreted with caution. They concluded that neuropsychological findings indicative of subsequent cognitive injuries are difficult to find in groups of otherwise healthy dental personnel with previous occupational exposure to mercury.

Hilt *et al.,*(2009) examined if Norwegian dentists have an increased prevalence of symptoms consistent with neurological and/or cognitive malfunction. The study group consisted of 406 dentists from central Norway and 217 controls from the general population, all under the age of 70. They had responded to a standardised postal questionnaire (Euroquest) inquiring about seven symptoms in regard to neurology, psychosomatics, memory, concentration, mood, sleep disturbances, and fatigue. A score was calculated for each symptom based on 4 to 15 single questions scored on a scale from 1 (seldom or never) to 4 (very often).

The dentists and controls had a participation rate of 57.2 % and 42.9 % respectively. The dentists reported no more cognitive symptoms than the controls, with low average symptom scores from 1.16 for neurological symptoms in males to 1.73 for fatigue in females. Corresponding figures for the controls were 1.22 and 1.77. There were a total of 1.2 % of the dentists and 1.8 % of the controls who reported having three or more of the seven symptoms "often" or more frequently.

In conclusion, the Norwegian dentists did not report more cognitive and neurological symptoms than controls from the general population.

3.3.7. Genetic predisposition of individuals and subpopulations

As with many exogenous substances, genetic factors may also contribute to the individual susceptibility to mercury toxicity based on mercury toxicokinetics (Julvez and Grandjean, 2013). However, there is limited knowledge about genes that specifically influence mercury toxicokinetics and toxicity. GSH-related genes have broad substrate specificities.

Glutathione (GSH) related enzymes play a role in mercury toxicokinetics, and several studies have addressed the impact of polymorphisms in glutathione-related genes (Clarkson *et al.,* 2007). An association between GSTM1 and GSTT1 null genotypes and the GST polymorphisms may be associated with methyl mercury detoxification (Mazzaron Barcelos *et al.,* 2012). In

dental professionals from Michigan (US), the glutathione S-transferase GSTT1 deletion was associated with decreased urine mercury concentrations (Goodrich *et al.,* 2011).

The metabolism of mercury is also likely to be influenced by binding to certain ligands, such as selenoproteins and metallothioneins. In the same dental professionals, adjusted urinary mercury excretion was higher in individuals with selenoprotein 1 (SEPP1) rs7579 CT+TT genotypes compared to those with CC (Goodrich *et al.,* 2011). This is a possible protection mechanism.

In a population from Northern Sweden the glutathione transferase (GST) P1-105 and -114 genotypes influenced the retention of methylmercury in individuals that consumed fish 2-3 times a week. The erythrocyte mercury was higher, depending on the phenotype (Schlawicke Engstrom *et al.,* 2008). However, no association with clinical symptoms was demonstrated.

In Ecuadorean gold miners and gold buyers highly exposed to mercury vapour, the glutamyl-cysteine ligase GCLM-588T allele (which is associated with lower glutathione production) was associated with increased blood, plasma and urine mercury levels (Custodio *et al.,* 2005). Subjects with the GCLM-588 CC genotype had half as high a urinary mercury excretion as expected from exposure data. In regard to adverse effects linked to mercury exposure, there was no evidence that the glutathione genotypes modified the relationship between exposure and neurotoxic effects due to gold mining in Ecuador (Harari *et al.,* 2012).

For metallothionein, the small number of subjects with MT1M A or MT2A CC genotypes had lower urinary mercury levels than did those with MT1M or MT2A GG genotypes. The study gave little evidence of effect modification of the single nucleotide polymorphisms (SNPs) on the relationship between mercury biomarkers and peripheral nerve function. Their study suggested that some metallothionein genetic polymorphisms may influence the biomarker concentration at levels of exposure relevant to the general population (Wang *et al.,* 2012).

Although less certain, the data suggest that additional factors beyond glutathione metabolism affect mercury toxicokinetics. Certain mercury transporter genes may also modify the urinary excretion of mercury. In populations from Indonesia, the Philippines, Tanzania and Zimbabwe exposed to mercury vapour from gold mining, SNPs in four transporter genes appeared to affect mercury concentrations in urine, such as solute-carrier family 22 members 6 and 8 (SLCA22A6/OAT1 and SLCA22A8/OAT3), solute-carrier family 7 member 5 (SLC7A5/LAT1), and ATP-binding cassette sub-family C member 2 (ABCC2/MRP2) (Engstrom *et al.,* 2013). As this study was done in populations from Southeast Asia and Africa, confirmatory data are needed for European populations.

These data suggest that mercury toxicokinetics may depend on genetic polymorphisms including enzymes involved in glutathione metabolism, glutathione transferases, and other ligands or transporters, although no relationship was reported with these variants and Hg-induced adverse effects.

The impact of genetic variants was considered in regard to neurobehavioral outcomes or effects on moods in male dentists and female dental assistants from Washington State. Genetic polymorphisms include the brain-derived neurotropic factor (BDNF)(Echeverria *et al.,* 2005; Heyer *et al.,* 2004), coproporphyrinogen oxidase gene (CPOX) (Echeverria *et al.,* 2006), catechol O-methyltransferase (COMT) (Heyer *et al.,*2009, Woods *et al.,*2014), and the serotonin transporter gene promoter region (5-HTTLPR) (Heyer *et al.,* 2008). The biological plausibility of these association is link to the function of the gene product: CPOX is involved in the haeme biosynthesis of crucial biochemical importance. As a result, the mercury-associated porphyrin profile in urine is changed (Woods *et al.,* 2005; Heyer *et al.,* 2006). COMT is involved in the metabolism of catecholamine neurotransmitters, while 5-HTTLPR affects another key transmitter substance in the brain. However, some of these studies (Heyer *et al.,* 2006) have been challenged due to methodological problems (Björkman 2007).

The papers of the research group of Woods *et al.,*(Woods *et al.,* 2005, Heyer *et al.,* 2004, 2008, 2009) involved children of the Casa Pia Clinical Trial. Results suggest an interaction between various gene-polymorphisms (CPOX4, COMT, 5-HTTLPR, BDNF) and mercury neurobehavioral induced effects.

Similarly, the presence of the metallothionein MT1M mutant or MT2A mutant (33 and 39% of frequency, respectively) in boys, but not the girls in the Casa Pia trial, was reported in a post-hoc analysis to be associated with significant mercury-dependent deficits in neurobehavioral function (Woods *et al.,* 2013).

In the most recent of the studies related to the Casa Pia Clinical Trial, the 330 enrolled subjects were genotyped for 27 variants of 13 genes that are reported to affect neurologic functions and/or Hg disposition in adults (Woods *et al.,* 2014). Urinary mercury concentrations, reflecting mercury exposure from any source, served as the mercury exposure index. Modelling strategies were employed to evaluate potential associations between allelic status for individual genes or combinations of genes, mercury exposure, and neurobehavioral test outcomes assessed at baseline and for 7 subsequent years during the clinical trial. A significant modification of mercury effects on neurobehavioral outcomes was observed with variant genotypes for 4 genes (CPOX, MT1M, MT2A, COMT). Modification of mercury effects on a more limited number of neurobehavioral outcomes, was also observed for other variants in boys, but the modification was limited in girls (Woods *et al.,* 2014). This gender differences, although not explained by the authors, can be likely attributed to kinetic differences, affecting mercury exposure.

Julvez *et al.,*(2013) report that in a study population as a whole, no adverse effect of methylmercury exposure on neuropsychological outcomes could be identified, and indication of some effects became apparent only when the genetic variants were included in the analysis. The common BDNF polymorphism is shown to affect the neurotoxicity of methyl mercury exposure, but polymorphisms in CPOX appear unrelated to cognitive development (Julvez *et al.,* 2013) in contrast with results obtained by the Wood group.

Recently a review was published reporting the possible genotype–mercury interactions influencing health outcomes, in relation to Hg kinetics, transport, and dynamics (Basu *et al.,* 2014). Quantitative knowledge on the weight of polymorphism should help in improving the assessment factors in carrying out the risk assessment. Whenever data are available they should be used to refine the default factor. The authors highlighted that while different groups investigated the kinetic factors, a large portion of studies to date involving the interaction of polymorphisms and Hg exposure on health outcomes stem from a single research team studying a cohort of male dentists and female dental assistants with occupational elemental (inorganic) Hg exposure and the Casa Pia Clinical Trial on children.

Although the considerations given by the authors refer to data on methyl mercury, their estimation advocates for a default factor of 10. Indeed, they estimated that hair mercury predictions for frequent fish consumers (equivalent of 6 cans of tuna per week) varied 8-fold depending on genotype' (Basu *et al.,* 2014). So, irrespective of the existence of vulnerable sub-groups, the available data seem to indicate that they are covered by the default uncertainty factor of 10 generally used in the risk assessment to account for genetic heterogeneity in the human population.

Accordingly, the European Food Safety Authority argued that for methylmercury a partial uncertainty factor of 2 would be sufficient when a benchmark dose level (BMDL) had been obtained from a birth cohort that would represent the most vulnerable population (European Food Safety Authority, 2012).

The studies presented above seem to indicate that genetic variations, relatively common to the general human population, may have an influence also on responses to mercury -induced toxicity but gaps in knowledge still exist. However, no prospective clinical studies clearly showing the influence of genetic variations on the occurrence of adverse effects due to mercury from dental amalgam are available. Even in the Casa Pia group of papers, urinary mercury reflected mercury exposure from any source, therefore it could not be ascribed to dental amalgam.

There is no accepted and validated method available for identifying such risk groups.This is important as genetic variants may also play a role for alternative dental restorative materials (see below). Therefore, especially in this area further research is needed before clinical conclusions can be drawn.

3.3.8. Experience with non mercury-based fillings/amalgams

There does not seem to be any new information or new products based on non-mercury-based metallic fillings/amalgams for direct restorations, since the former Opinion (2008).

3.3.9. General Observations on Amalgam Efficacy

The efficacy, longevity and general performance of amalgam restorations has been assessed on many occasions in the past, and it is not necessary to review these studies here. Whatever the material chosen, direct restorations may fail, primarily through secondary caries, fracture of the restoration or tooth, marginal deficiencies or wear. The rates at which these failures occur are difficult to compare since they will vary with clinical technique and patient characteristics, and since there have been improvements to the quality of all materials over time.

It remains the view, however, that from mechanical functionality and longevity perspectives and resistance to secondary caries, possibly through anti-bacterial activity, amalgam will outlast alternative materials in many instances (Mitchell *et al.,* 2007, Soncini *et al.,* 2007). In a review from DIMDI (German Institute for Medical Documentation and Information) it was stated that only two out of six systemic reviews conclude that the expected survival time of composite fillings can be comparable to amalgams. However, these conclusions are based on the results of short term studies for composite resins which usually overestimate the longevity of filling materials (Antony *et al.,* 2008). From such perspectives, dental amalgam may still be the material of choice with many dental practitioners e.g. for large restorations and the replacement of large restorations. In a recent Cochrane systematic review on the comparative longevitiy of resin based composites and amalgams it is stated that the parallel group trials indicated that resin restorations had a significantly higher risk of failure than amalgam restorations and increased risk of secondary caries. The results from the split-mouth trials were consistent with those of the parallel group trials. More data with higher levels of evidence are warranted (Rasines Alcaraz *et al.,* 2014).

A main driving force for using composite materials instead of amalgam is the tooth-coloured appearance of composite restorations. One study from the Netherlands and one from Sweden showed very good long-term clinical effectiveness for posterior resin composite restorations with equal and better longevity than for amalgam (Opdam *et al.,* 2007; van Dijken, 2013; Opdam *et al.,* 2012). However, even under optimal conditions large composite restorations in caries risk patients failed more often than amalgam fillings (Opdam *et al.,* 2010). It is with respect to their aesthetics and non-adhesive character, which means that larger cavities have to be prepared, often with excessive tooth tissue removal, that amalgams may be seen to be inferior to the alternatives, and it is this, and not overall longevity, that is driving a change to these alternatives.

3.3.10. Conclusions on Dental Amalgam

It is recognised that mercury, which is the major metallic element used in dental amalgam, does constitute a toxicological risk, with reasonably well-defined characteristics for the major forms of exposure. The reduction in use of mercury in human activity would be beneficial, both for the general decrease in human exposure and from environmental considerations.

However, with respect to the debate about the possibility of causal relationships between the use of mercury containing amalgam and a wide variety of adverse systemic health effects and taking into account many studies and investigations into this putative causal link, there is no unequivocal evidence to support this possibility. These studies have included assessments in children and in pregnant and lactating women. The existence of susceptible subpopulations due to genetic predisposition needs further research before conclusions can be drawn.

It is generally concluded that no increased risks on adverse systemic effects have been documented in the general population as a whole and it is considered that the current use of dental amalgam does not pose any risk of systemic disease. It is recognised that some local adverse effects are occasionally seen with dental amalgam fillings, but the incidence is low and normally readily managed. In addition, allergy against mercury can occur. It is also recognised that there have been reports of reactions to dental amalgam, which indicate that very occasionally an individual may have unexplained atypical physical or other reactions attributed to mercury. The reasons for such hypersusceptibility are poorly understood.

The mercury release during placement and removal will result in transient exposure to the patients and also to the dental personnel. There is no general justification for removing clinically satisfactory amalgam restorations as a precaution, except in those patients diagnosed as having allergic reactions to amalgam constituents.

The SCENIHR recognises that current evidence does not preclude the use of amalgam in dental restorative treatment in the general population. Dental restorative therapy during pregnancy, as for any other therapeutic treatment, should be limited as much as possible in order to reduce the exposure of the foetus. The choice of material should be based on patient characteristics such as primary or permanent teeth, pregnancy, the already existent number of dental amalgam fillings, presence of allergies to mercury or other components of the restorative materials, and presence of decreased renal clearance.

As far as dental personnel are concerned, it is recognised that they may be more exposed to mercury exposure than the general population, although the incidence and type of reported adverse effects are similar to what is observed in the general population. However, the same considerations for caution in regard to patient exposure also applies to dental personnel.

To reduce the use of mercury-added products in line with the intentions of the Minamata Convention (reduction of mercury in the environment) and under the above mentioned precautions, it can be recommended that for the first treatment for primary teeth in children and in pregnant patients, alternative materials to amalgam should be the first choice. This decision should be made after informed consent from the patient or the legal guardians.

3.4. Alternatives

3.4.1. Classification of alternatives according to chemical composition

Dental filling materials in general can be classified into those used for direct and those used for indirect restorations; some materials like resin-based composites can in certain cases be used for both. With the indirect filling technique, an impression from the intraoral situation of the patient (after cavity preparation) is taken and the actual restoration is constructed outside the oral cavity. Traditionally, an impression material is used and from the impression a cast is made on which the dental technician then fabricates the restoration. The latter is mainly either made from a dental alloy or from ceramics. Dental alloys can be gold-based, but contain many other metals to improve the mechanical and corrosion properties. These metals can be silver, copper, palladium, platinum and others. For crowns, nickel-based alloys are also described. Recently other metals, like titanium/titanium-alloys are used as well as cobalt-chromium alloys; e.g. for CNC milling or laser sintering.

Several thousand different alloys are on the market today. Alternatively, silicate-based and zirconium oxide ceramics can be used for partial and full crowns. In pediatric dentistry, prefabricated metal crowns are used as amalgam alternatives. With this technique, out of a large variety of prefabricated crowns, the one with the best fit is selected and trimmed to further improve the fit. These steel crowns contain considerable amounts of nickel.
Recently, optical impression techniques have been introduced into dental practice; here, the impression is taken by a specifically designed camera and the restoration is constructed on a

computer. Based on this data set, the actual restoration is then grinded from a ceramic (or metal) block in a 3-D-grinding machine.
Common to all indirect restorations is the fact that they must be luted to the tooth substance. For this purpose, different cements are being used, for ceramics mainly resin-based composites materials with low viscosity.
Due to the additional impression technique and the rather complicated manufacturing process, costs of such restorations are comparatively high. Technical properties of the dental alloys and ceramics are generally good. However, when health risks of these restorations are to be evaluated, one must consider not only the composition of the alloys/ceramics but also the composition of other materials used like impression materials or luting substances.

Due to the high costs of indirect restorations, direct techniques are often preferred. Currently, most attention is focused in this context on materials, such as resin-based composites, glass ionomer cement, compomers, giomers and sealants.

A composite is generally defined as a material composed of two or more distinct phases (O'Brien 2002). Dental resin composites consist of a polymerisable resin base containing a ceramic filler. They may be classified in a number of ways, the normal method being based on the size, distribution, and volume percentage of the ceramic particles. With respect to their size, this classification yields the so-called macrofill, midifill, minifill, microfill and nanofill composites. Macrofill composites contain ceramic particles ranging in size from 10-100 µm, midifill in the range from 1-10 µm, minifill in the range from 0.1-1 µm, microfill in the range from 0.01-0.1 µm (10-100 nm) and nanofill in the range from 0.005-0.01 µm (5-10 nm). Recently the European Commission has published a recommendation of the definition of nanomaterial which mentions for nanomaterials a size range of 1-100 nm (Commission Recommendation 2011/696/EU, EC 2011). Hybrid composites contain a mix of two particle-size fractions of fillers, e.g. midi-hybrids consist of mix of microfillers and midifillers, mini-hybrids or micro-hybrids consist of a mix of microfillers and minifillers and nanohybrids consist of a mix of nanofillers and minifillers.

Filler loading varies significantly between the different resin composite materials. For example in a macrofill and hybrid composite, the filler material occupies 50-80% of the composite by weight, while in a microfill composite the filler loading is limited to about 35-50% by weight.

Silorane monomers replaced the methacrylates (e.g. Bis-GMA, UDMA, TEGDMA) in the resin matrix of a recently marketed posterior resin composite material. The ring-opening chemistry of the monomers reduces shrinkage of the resin composite below 1% (Weinmann *et al.,* 2005). Recently, other resin formulations have been marketed claiming reduced shrinkage/shrinkage stress (Roggendorf *et al.,* 2011). Clinical experience with these materials is very limited.

Currently, almost all resin composites are supplied as a pre-packed single-paste system, the curing of the resins occurring by light activation. Different types of commercially available curing units have different light intensities and utilise different light sources. Light-curing units use halogen-based, light-emitting diode (LED), plasma-arc, or laser technology. The energy levels range from 300 to more than 3,000 milliwatts/cm^2.

Glass ionomer cements were introduced in 1972 by Wilson and Kent (1972) and may be considered as a combination of silicate and polyacrylate cement system. Glass ionomer cements bind chemically to dental hard tissues. Polyalkenoate chains enter the molecular surface of dental apatite, replacing phosphate ions, which leads to the development of an ionenriched layer of cement that is firmly attached to the tooth (Wilson *et al.,* 1983). More recently, so-called high-viscosity glass ionomer cements have been marketed with somewhat improved mechanical properties (Lohbauer *et al.,* 2011; Sidhu, 2011). In addition to the original concept of glass ionomer cement, certain resin modified glass ionomer cements are now used in order to improve functionality.

Compomers were introduced in the 1990's and combine some of the benefits of composites

and glass-ionomer cements. A Giomer resin composite was introduced in the early 21st century and featured the hybridisation of glass-ionomer and resin composite.

Sealants are flowable resins or glass ionomers that are applied to seal pits and fissures in permanent teeth in order to prevent the occurrence of caries. A non-resinous calcium aluminate based filling cement received CE marking 2000 as alternative material. The material particles are based on alumina (Al_2O_3) and calcium oxide (CaO), and small amounts of ZrO_2-, TiO_2-, Fe_2O_3- and SiO_2. Mixing the particles with water, which contain small amounts of Na, Li and Fe additives, results after a crystalline phase formation into a hardened cement. Reported poor mechanical properties and unacceptable clinical efficiency resulted in that the materials continued clinical use could not be justified (Sunnegårdh-Grönberg *et al.,* 2003; van Dijken & Sunnegårdh-Grönberg, 2003).

3.4.2. Chemical characterisation of alternative materials

3.4.2.1. Resin composites

Dental resin composites are composed of a wide variety of components with different chemical composition (O'Brien 2002, Powers and Wataha 2007, Roeters and de Kloet 1998). Chemicals described in the literature as possible constituents of resin-based composites are summarised in Annex 1. There is inadequate data on the composition and leachables of these materials, which is sometimes reflected in the Material Safety Data Sheets (MSDS) (Henriks-Eckerman and Kanerva, 1997; Fleisch *et al.,* 2010). According to information from the manufacturers, the dental business environment is highly competitive, and, therefore, data on product composition and chemical characterisation are presently treated as confidential business information and are not typically available to the public.

Filler material
The filler materials are of inorganic composition, such as silica glass (SiO_2), alumina glass (Al_2O_3), and combinations of glass and sodium fluoride. Silica glass is made of beach sand and ordinary glass, but also of crystalline quartz, pyrolytic silica and specially engineered aluminium silicates (e.g. barium, strontium or lithium aluminium silicate glass). Alumina glass is made of crystalline corundum, while sodium-calcium-aluminafluorosilicate glass is an example of a combination glass. A combination glass has to be considered as an engineered mixture of various glasses, which can serve as a source of fluoride ions. The radiopacity of resin composites is obtained by the addition of barium, strontium, lithium or ytterbium fluoride (YF_3) to the filler particles.

Matrix material
The matrix is of organic composition. A large group of different aromatic and diacrylate monomers and oligomers is used, such as bisphenol A-glycidylmethacrylate (Bis-GMA), ethoxylated bisphenol A-methacrylate (Bis-EMA), triethyleneglycoldimethacrylate (TEGDMA) and urethane dimethacrylate (UDMA). In the silorane resin composite, the monomer is a silorane derived from the chemicals siloxanes and oxiranes (Weinmann *et al.,* 2005). As was mentioned above, other resin formulations are recently marketed for which publicly available information, especially on the biological characteristics and the clinical experience, is scarce.

Ormocers
To overcome the polymerisation and biocompatibility problems of conventional methacrylate based resin composites, the first restorative material based on ormocer technology was marketed in 1998. Ormocer is an acronym for organically modified ceramic and the material was originally developed for electronic applications by the Fraunhofer Silicate Research Institute (Würzburg, Germany). Ormocers are synthesised through a solution and gelation processes from multifunctional urethane and thioether(meth)acrylate alkoxysilanes (Moszner *et al.,* 2008). Monomers are better embedded in the matrix, reducing the release of monomers.

After incorporation of filler particles, the ormocer can be handled like a hybrid resin composite. Improved wear resistance has been observed compared to conventional hybrid resin composites (Manhart *et al.,* 2000). Shrinkage was equal to that of conventional hybrid resin composites despite having less filler content (Cattani –Lorente *et al.,* 2001). Ormocer with higher filler content showed shrinkage equal to low shrinkage resin composites (Yap and Soh, 2004). Due to problems with handling properties, conventional methacrylates had to be added as diluents to the marketed ormocer monomer matrix (Ilie and Hickel, 2009). Clinical performance of an ormocer material together with its adhesive system, however, was not satisfying: With a failure rate of 9.6% after 1 year, this material system did not fulfill ADA acceptance criteria for restorative materials (Oberländer *et al.,* 2001). A more recent preparation of an ormocer-based resin composite showed a better performance after four years (van Dijken and Pallesen, 2011). Studies with longer observation times are not available.

Filler particle incorporation
Coating of the filler particles with silane coupling agents (such as trialkoxysilane) ensures covalent coupling between filler and resin matrix. The carbon-carbon bond on silane molecules binds to the filler particles as well as resin monomer during polymerisation of the resin composite.

Curing of resin composite
Chemical agents (self or auto-cure) or, most commonly, light energy (ultraviolet or visible light) ensures polymerisation of dental resin composites. Dual curing, i.e. a combination of chemical and light curing is also possible. For most resin composite systems in current use, visible light polymerisation at 470 ± 20 nm wavelength is used. Depending on the curing method, various polymerisation initiators and accelerators are required. Initiators for chemical curing are usually benzoyl peroxide and benzene sulphinic acid which initiate polymerisation in the presence of an aromatic tertiary amine. For light curing systems, camphorquinone is normally used in conjunction with an aliphatic tertiary amine as accelerator. Due to the yellow color of camphorquinone, other initiators like trimethylbenzoyl-diphenyl-phosphine oxide (TPO) have been proposed as an alternative (Schneider *et al.,* 2012). In this context biphasic light curing units are now marketed with one peak at around 470 nm and one at around 420 nm.

Additional components
Resin composites contain a number of further additives, like stabilisers and inorganic oxides, and organic compounds are pigments that are added to create a range of various composite shades.

Bonding to enamel and dentine
Bonding of the resin composite, compomer and giomermaterials to hard tooth tissues is achieved by use of a bonding system that incorporates etchants, primers and bonding resins (van Landuyt *et al.,* 2007). Chemical etching using agents such as phosphoric acid, or acidic monomers are used to demineralise the tooth surface and increase the surface area. In etch-and-rinse systems, after rinsing and drying, a primer solution, composed of solvent and low viscosity resins such as HEMA, Phenyl-P, MDP, PENTA, is applied to obtain optimal wetting of the surface for the following bonding agent. Solvents used are water, acetone, ethanol and buthanol or a combination of these. The third step which bonds to the hydrophobic resin composite is achieved by the application of a very thin resin bonding layer. Classical bonding agents are composed of unfilled or with nano-filler filled resins of similar composition as the resin matrix of the composite material. Newer simplified etch-and-rinse bonding systems are composed of only two steps, combining in the second step the primer and bonding. In so-called self-etching adhesives (SEA), the phosphoric acid etching is replaced by etching of the tooth substance with acidic monomers which are included in the primer step. The applied acidic primer is not rinsed away as is the case for the phosphoric acid in the etch-and-rinse systems, but is included as a part of the hybrid layer. In the 2-step SEA, the primer application is followed by a separate low viscous bonding step. In the 1-step SEA adhesives, etching, priming and bonding are all combined in one application step.

Glass ionomer cements

In the original form, the powder component of these cements is a sodium-calciumalumino-fluoro-silicate glass. The liquid component is composed of polyacrylic acid and tartaric acid. When the powder and liquid are mixed together, a three phase acid-base reaction occurs, involving calcium and aluminium ions leaching as the acid attacks the glass particles, hydrogel formation as the polyacrylic acid molecules crosslink, and polyalkenoate salt gelation as the polyalkenoate salt captures un-reacted glass. More recently, high-viscosity glass-ionomer cements or those in combination with a surface varnish have been marketed with somewhat improved mechanical properties (Lohbauer *et al.,* 2011; Sidhu, 2011). Glass ionomer cements have also been used with the ART technique. They can be used to restore single-surface cavities both in primary and in permanent posterior teeth, but their quality in restoring multiple surfaces in primary posterior teeth cavities need to be improved. Insufficient information is available regarding the quality of ART restorations in multiple surfaces in permanent anterior and posterior teeth (Frencken *et al.,* 2012). Other authors claim better clinical performance of high viscosity glass ionomer materials in primary teeth, but data are comparatively scarce (Mickenautsch *et al.,* 2010).

In the resin-modified cements, methacrylate monomers like HEMA have been added to improve functionality with respect to higher strength and water resistance. The materials have been further modified by the addition of photo initiators so that light-curing can occur, but they maintain their ability to set by an acid-base reaction. The setting of resin modified glass ionomer cement is identical to the polymerisation of composite resin. During this process, free radical species are generated.

3.4.2.2. Compomers

The main components of compomers are polymerisable dimethacrylate resins, such as urethane dimethacrylate and TCB, which is a reaction product of butane tetracarboxylic acid and hydroxyethylmethacrylate, and ion-leachable glass filler particles such as strontium fluorosilicate glass. The glass particles are partially silanised to achieve bonding with the resin matrix. The setting reaction is based on free radical polymerisation using photoinitiators. During the setting reaction, HEMA is released while fluoride release occurs after setting.

3.4.2.3. Giomers

Giomers are based on the technology of a reaction between fluoride containing glass and a liquid polyacid. The prereacted glass particles are mixed with resins such as urethane dimethacrylate and hydroxyethylmethacrylate, and a catalyst to initiate polymerisation. Bonding of the material is achieved through the use of self-etching primers including methacrylate resins like 2-HEMA, 4-AETA , UDMA, and TEGDMA and pre-reacted glass-ionomer filler. The bonding agent releases fluoride. In a recent 6-year clinical evaluation, posterior restorations of giomer showed a rather high failure rate (van Dijken, 2013).

3.4.3. Toxicology of components of alternative materials

The alternative restorative materials are chemically complex, with many different components, setting reaction mechanisms and opportunities to interact with tissues of the individuals in whom they are placed. However, characteristics of exposure are very difficult to determine, bearing in mind that volumes of the materials used are very small, the residence time within the body of chemicals that take part in setting reactions is usually very short and the chemical and toxicological profiles of the set material are usually very different to those of the starting materials. In evaluating the possibilities for adverse effects arising from the clinical use of these materials, it is necessary to consider the evidence about the inherent toxicity of the chemicals used and the performance and behavior of the restorations over time. Of interest to

most investigations here have been the monomers used in polymerisation reactions, which may remain unreacted and therefore present in the set material, the acids used in various phases of the setting and etching processes and ions released from glasses.

3.4.3.1. Release of substances from alternative materials

Unbound monomers and/or additives are eluted within the first hours of placement in the tooth cavity. The very nature of the polymerisation processes, involving the absorption of light energy by the material that will vary with depth within the restoration, and the subsequent conversion of monomer molecules into cross-linked macromolecules, inevitably means that some monomer molecules do not have the opportunity to take part because of diffusion limitations. The completeness of the polymerisation process is reflected by the degree of conversion. Between 15 and 50% of the methacrylate groups may remain un-reacted according to Ferracane (1994). However, this may be enough to contribute to major cytotoxic effects *in vitro* (Stanislawski *et al.,* 1999). Improvements in the material formulations have resulted in increasingly superior degrees of conversion in recent years. The effects may also be dependent on dentine permeability and residual dentine thickness (Bouillaguet *et al.,* 1998, Galler *et al.,* 2005) since dentine may absorb unbound monomers and therefore contributes to decrease the cytotoxicity of the material. This is not directly under the control of the dental surgeon although the formation of reactionary dentine may be stimulated by preparative steps. Dentine permeability may also be modified by calcium phosphate precipitation in the lumen of the tubules leading to sclerotic dentine formation. It has also been shown that the surface of composite resins exposed to oxygen during curing produces a non-polymerised surface layer rich in formaldehyde, which by itself is an additional factor of cell toxicity (Schmalz, 1998).

Monomers have been identified in dental resin composites eluates by gas and liquid chromatography/mass spectrometry. A considerable concentration of the co-monomer triethyleneglycoldimethacrylate and minor concentrations of the basic monomers Bis-GMA and UDMA as well as the co-monomer HDDMA have been detected with these methods (Geurtsen 1998; Spahl *et al.,* 1998). Kopperud *et al.,*(2010) found no substances to leach from Silorane resin composite in water, whereas silorane monomers and an initiator component were eluted from the material into an ethanol solution.

Formaldehyde is released from resin-based composites into an aqueous environment especially from the superficial oxygen-inhibited surface layer after curing but also over a prolonged period of time (Oysaed and Ruyter, 1988). This also applies to resin modified glass ionomer cements (Ruyter, 1995). Formaldehyde is very likely generated by an oxidation of unsaturated methacrylate groups (Oysaed and Ruyter, 1988).

BPA is released into an aqueous environment from resin composites which contain Bis-DMA, because Bis-DMA itself is eluted, and it is then hydrolytically and enzymatically cleaved into BPA and methacrylic acid. This release mainly takes place during the first 24 hours after placement (Schmalz *et al.,* 1999; Myers and Hutz, 2011; Fleisch *et al.,* 2010). BPA is released in small amounts from some brands of Bis-GMA based resin composites continuously, because it is a residue from the production process of Bis-GMA, in which BPA is used (Imai, 2000; Imai and Komabayashi, 2000). Earlier data on larger amounts of BPA released from Bis-GMA resins (Olea *et al.,* 1996) could not be confirmed (Schmalz *et al.,* 1999; Myers and Hutz, 2011; Imai, 2000; Geurtsen *et al.,* 1999; Hamid and Hume, 1997; Moon *et al.,* 2000; Wada *et al.,* 2004). A recent study from NIH showed that BPA and related compounds could be found in saliva and urine after restoration with resin composites (Kingman *et al.,* 2012). In saliva, most compounds returned to prerestoration levels within 8 hours, while concentrations of the study compounds in urine returned to prerestoration levels nine to 30 hours after restoration placement with the exception of a 43 percent increase in BPA. In a recent study, the release of BPA after long-term storage was reported (Sevkusic *et al.,* 2014).
The SCENIHR Opinion "The safety of the use of bisphenol A in medical devices" (2015) concluded that release of BPA from some dental materials was associated with only negligible health risks.

Dental alloys continuously release metals into the oral environment depending e.g. on the metal content, the phase distribution within the alloy, thermal treatment and the corrosion conditions. Metals like Au, Cu, Ag, Pd are released and also Ni, Zn, Co, Ti, Cr and many others (Schmalz and Arenholdt-Bindslev, 2009).

Release of substances from and degradation of glass ionomer cements are generally regarded higher than for resin-based composites. These materials mainly release fluorides (Forsten, 1990) but also calcium, sodium, silicon, strontium, and aluminium. Some release silver or zinc (Guertsen, 1998; Hantsen *et al.,*1994). Ceramic releases – depending on the composition – substances like silicon, boron, sodium, potassium, and aluminium, some brands lithium in small amounts (Anusavice and Zhang, 1997).

3.4.3.2. Leachable substances generated by erosion and degradation

Leachable components are released due to degradation or erosion over time, the leaching process being determined not only by the degradation process itself but also diffusivity through the material. Chemical degradation is caused by hydrolysis or enzymatic catalysis. Non-specific esterases, human saliva derived esterase and pseudocholinesterase may catalyse the biodegradation of resin composite (Geurtsen 2000; Jaffer *et al.,*2002; Finer *et al.,*2004). Incubated *in vitro* with cholesterol esterase, the composites may release 2,2-bis [4(2,3-hydroxypropoxy)-phenyl]propane (bis-HPPP) and TEGDMA for up to 32 days, the amount depending on the matrix/filler ratio (Shajii and Santerre, 1999).

These esterases have been shown to hydrolyse Bis-GMA to bis-(2,3-dihydroxypropyl) ether (BADPE-4OH) by the loss of two molecules of methacrylic acid. The same enzyme converted TEGDMA into triethylene glycole and methacrylic acid and HEMA hydrolyses under acidic conditions into thylene glycole and methacrylic acid (Schmalz and Arenholt-Bindslev, 2009). During cell metabolism of TEGDMA and HEMA epoxy-intermediate 2,3-epoxymethacrylic acid is formed which is considered to be mutagenic (Durner *et al.,* 2010). The hydrolytic degradation of Bis-DMA to BPA has already been mentioned above.

It is also assumed that bonds in the pendant side chains of the macromolecule are attacked through the effect of thermal, mechanical and photochemical factors.

Water or other solvents may diffuse into the polymer, facilitating the release of degradation products, including oligomers and monomers. The leaching process is influenced by size and polarity and by hydrophilic and lipophilic characteristics of the released components (Geurtsen 1998). Softening of the Bis-GMA matrix allows the solvents to penetrate more easily and expand the polymer network, a process that facilitates the long-term diffusion of unbound monomers (Finer and Santerre 2004).

3.4.3.3. Release of ions

Ions are released from both metallic and non metallic alternative materials. Ions from dental alloys comprise a large variety like gold, palladium, platinum, silver, copper, zinc, tin, nickel, cobalt, chromium and others (Schmalz and Arenholt-Bindslev, 2009). But also non metallic alternative dental restorative materials release ions, such as fluoride, strontium and aluminium ions. The fluoride is expected to be beneficial and reduce the development of secondary caries. Presumably, the fluoride content of toothpastes and nutriments reload the material so that the resins or resin modified glass ionomer cements do not become porous. Other ions are implicated in the colour of the restorative material, and these metal elements may interfere with the biocompatibility of the resin because they are implicated in the Fenton reaction producing reactive oxygen species that are cytotoxic. The concentration of fluoride and strontium is considered to be too low to produce cytotoxicity. In contrast, however, copper,

aluminium and iron may be present in toxic concentrations. The cytotoxic cascade has been shown to be enhanced by metals such as aluminium and iron present in various amounts in some of these materials (Stanislawski *et al.,*1999; Stanislawski *et al.,* 2000; Stanislawski *et al.,* 2003).

3.4.3.4 Toxicity of resin composite monomers

Toxicity evaluation of resin composite materials is very complex, because a large variety of different substances are contained in these materials, which vary from one manufacturer to another. Furthermore, other substances may be produced during the polymerisation process, like formaldehyde. Also, different biological endpoints need to be critically discussed. This all would go well beyond the scope and the range of this report. Therefore, only key elements are mentioned here and more detailed information can be obtained from the literature (e.g. Schmalz and Arenholt-Bindslev, 2009).

The first ormocer that was markeded initially showed low cytotoxicity and mutagenicity, which further decreased after prolonged aging (Wataha *et al.,* 1999; Bouillaguet *et al.,* 2002, Schweikl *et al.,* 2005). On the other hand, Al-Hiyasat *et al.,*(2005) showed a higher cytotoxicity for another commercial ormocer in comparison with two other resin composites.
Its flowable material showed lower cytotoxicity than the restorative material. Furthermore, estrogenic effects have been described with an ormocer material, although the clinical relevance is yet unclear (Wataha *et al.,* 1999). Polydorou *et al.,*(2009) showed that an ormocer released significantly less monomers such as Bis-GMA, TEGDMA or UDMA compared to either a nanohybrid composite or a self-curing composite.

Monomers caused cytotoxicity in cultured cells with ED50 in the low millimolar to submillimolar concentrations (Kleinsasser *et al.,* 2006; Schweikl *et al.,* 2005; Schweikl and Schmalz, 1996a; Schweikl and Schmalz 1997; Schweikl *et al.,* 1998a; Schweikl *et al.,* 1996b; Schweikl *et al.,* 1998b; Schweikl *et al.,*2006). In an *in vitro* embryotoxicity screening study, BisGMA induced effects at low, non-cytotoxic concentrations suggesting a potential for embryotoxicity or teratogenicity (Schwengberg *et al.,* 2005). Siloranes showed reduced cytotoxicity (Brackett *et al.,* 2007). They also showed low genotoxic potential and can be suitable components for development of biomaterials (Schweikl *et al.,* 2004; Krifka *et al.,* 2012).

TEGDMA and the photostabiliser 2-hydro-4-methoxybenzophenone (HMBP) are cytotoxic and inhibit cell growth (Geurtsen and Leyhausen 2001). The intracellular glutathione level may be decreased by 85% by TEGDMA (Stanislawski *et al.,* 1999; Stanislawski *et al.,* 2000; Stanislawski *et al.,* 2003; Engelmann *et al.,* 2001; Engelmann *et al.,* 2002).

An *in vitro* evaluation of the cytotoxicity of 35 dental resin composite monomers and additives indicated moderate to severe cytotoxic effects (Geurtsen *et al.,* 1998). The effects vary according to the material tested, but also they strongly dependon the cells used for testing. For example, human periodontal ligament and pulp fibroblasts are more sensitive than 3T3 and gingival fibroblasts (Geurtsen *et al.,* 1998). With the exception of a very few reports, there is a general consensus that resin-containing restorative materials are cytotoxic (Geurtsen *et al.,* 1998; Geurtsen, 2000; Schmalz, 1998), greater effects generally been seen at early intervals after preparation.

At clinically relevant concentrations and for different cell lines, TEGDMA and HEMA have been shown to increase the intracellular concentration of reactive oxygen species (ROS) (Stanislawski *et al.,* 2003; Schweikl *et al.,* 2006). Monomer-induced oxidative stress is associated with the depletion of the non-enzymatic antioxidant glutathione and modified expression of enzymatic antioxidants (Volk *et al.,* 2006; Schweikl *et al.,* 2006; Krifka *et al.,* 2012). The presence of these resin monomers also leads to DNA damage (genotoxic effect) in vitro probably due to oxidation processes (Schweikl *et al.,* 2007), DNA strand breaks (Kleinsasser *et al.,* 2006; Durner *et al.,* 2011), a cell cycle delay (Schweikl *et al.,* 2006;

Schweikl *et al.,* 2007) and to apoptosis (Janke *et al.,* 2003; Krifka *et al.,* 2012). In p53 deficient culture systems (V79 cells), mutation can be observed after exposure to TEGDMA or HEMA (Schweikl *et al.,* 1998; Schweikl *et al.,* 2001). Furthermore, the ability of dental human pulp cells for biominerlisation (here: formation of new dentin) is blocked by TEGDMA (Galler *et al.,* 2011) as well as the bacterial defense system of macrophages (Schmalz *et al.,* 2011).

Only limited toxicity data for the monomers used in dental resin composite systems are available. Major differences in the degrees of cytotoxicity of various resin composite materials have been found (Schedle *et al.,* 1998; Franz *et al.,* 2003; Franz *et al.,* 2007). Most tested materials showed only mild cytotoxicity comparable to amalgam or less than amalgam but there were a few exceptions. Most of the available toxicity data have been generated in in-vitro systems that focus on genetic toxicity of the compounds in standard test systems such as the Ames-test, and on cytotoxicity in gingival fibroblasts. TEGDMA, UDMA and HEMA have all been shown to be positive in the COMET assay indicating induction of DNA-damage in mammalian cells. HEMA, BisGMA and TEGDMA also induced gene mutations in mammalian cells by a clastogenic mechanism.

The limited data on these monomers in experimental animals include studies on absorption, distribution, metabolism and elimination (ADME) on HEMA, TEGDMA and Bis-GMA after oral application of radiolabelled compounds. A rapid absorption of these compounds from the gastrointestinal tract and a rapid catabolism by physiological pathways to carbon dioxide, which is exhaled, has been described, although important details are still unknown (Reichl *et al.,* 2001a; Reichl *et al.,* 2002a; Reichl *et al.,* 2002b; Reichl *et al.,* 2001b; Reichl *et al.,* 2002c; Reichl *et al.,* 2008; Durner *et al.,* 2009). During this process, highly mutagenic epoxy compounds (2,3-epoxymethacrylic acid) are produced (Durner *et al.,* 2010).
No direct data on toxic effects of resin monomers in animals are available from publicly accessible sources. However, since the materials used as a basis for resin generation are derivatives of methacrylic acids and glycidyl ethers, the well-studied toxicology of methacrylate and its esters may be used as a basis for structure activity relationships to predict major toxicities.

Methylmethacrylate, as a relevant resin monomer, is rapidly absorbed after oral administration in experimental animals and is rapidly catabolised by physiological pathways to carbon dioxide. The major toxic effects of methylmethacrylate in animals are skin irritation and dermal sensitisation. In repeated dose-inhalation studies, local effects on respiratory tissue were noted after methylmethacrylate inhalation. Neurotoxicity and liver toxicity were observed as systemic effects after inhalation of methylmethacrylate in rats and in mice to concentrations above 3000 ppm for 14 weeks. For developmental toxicity of methylmethacrylate a NOAEC > 2000 ppm was observed. Methylmethacrylate is also clastogenic at toxic concentrations (EU-RAR 2002).

A detailed overview of the toxicity of glycidyl ether compounds is available (Gardiner *et al.,* 1992), although it is based mainly on unpublished study reports. Skin irritation and sensitisation were the major toxicities observed. In addition, positive effects in genetic toxicity testing were seen with many glycidyl ethers at comparatively high concentrations.

For BPA release from dental materials acute exposure was reported (Joskow *et al.,* 2006) to be in total 110 µg for six fissure sealants placed at one time with Bis-DMA containing material and 5.5 µg for sealants free of Bis-DMA. For chronic exposure, data are scarce. It is known from the elution behavior of resin-based materials that most of all eluable substances are eluted during the first 24 hours (Ferracane *et al.,* 1994; Ferracane *et al.,* 1995). No further degradation of Bis-GMA or related products to BPA was observed so far. However, recently it was reported that BPA was released only after storage of several months (Sevkusic *et al.,* 2014).

EFSA (2015) established a temporary (t)-TDI of 4 µg/kg b.w./day for oral exposure to BPA based on kidney alterations as the critical effect. The latter dose would mean for a 25kg child a tolerable daily intake would be 100 µg, which is higher than the amount of BPA acutely

released immediately after placement of a Bis-DMA-containing fissure sealant material on 6 teeth. Therefore, no acute or chronic estrogenic effect is to be expected from the use of Bis-GMA (and Bis-DMA free) resin-based composites/sealants. Even for the Bis-DMA containing resin composites/sealants the risk cannot be regarded as unacceptable under the given assumptions.

Saliva had been collected from 8 male volunteers; 4 had received 38 $\pm$ 3 mg of a Bis-DMA containing sealer and one which was Bis-DMA free. The saliva samples had been collected before and immediately after placement as well as 1 hour and 24 hours later (Arenholt-Bindslev *et al.,* 1999). The results show an estrogenic activity elicited by those saliva samples from patients with the Bis-DMA contains fissure sealant, but not from patients with a Bis-DMA free Bis-GMA based sealant (Arenholt-Bindslev *et al.,* 1999). The estrogenic activity could only be observed immediately after placement. After one or 24 hours no estrogenic effect could be observed. Other authors have reported similar results (Tarumi *et al.,* 2000; Fung *et al.,* 2000; Kingman *et al.,* 2012). Other components than BPA of composite resin eluates like a photostabiliser [HMBP], a photointiator [DMPA], an inhibitor [BHT] or a phthalate compound [BBP] were in vitro estrogenic, but the amounts of these substances released were very small and the risk possibly negligible in the clinical situation (Wada *et al.,* 2004).

In ovariectomised mice, a high dose of bis-GMA via subcutaneous route had no effect on DNA, RNA and DNA/RNA ratio compared to the control group were observed but a modest increase of uterus weight (Mariotti *et al.,* 1998). This was apparently due to an unspecific increase in collagen but not due to an increase of the cell number, and the dose in this experiment was far higher than any expected exposure in humans (Mariotti *et al.,* 1998); thus no unacceptable risk for the patient was concluded.

In conclusion, resin-based composite materials are today for many clinical situations recognised tooth-coloured materials to restore lesions; e.g. due to caries, erosion or trauma or to prevent caries (fissure sealants). According to present knowledge, for Bis-GMA-based materials with no Bis-DMA, additional exposure evaluation shows no risk for BPA-related acute or chronic effects, because no or very little BPA is released from dental materials (SCENIHR, 2015). However, BPA present as impurity/residue from the manufacturing process may be released.

For Bis-DMA containing materials, BPA release was consistently shown. The amount was so low that according to present knowledge, no adverse effect is expected (SCENIHR, 2015). However, if for personal considerations and wishes of a patient, any BPA exposure shall be minimised, products containing Bis-DMA should not be used. To better inform the user (dentist and patient), the content of dental materials should be declared.
No adverse effects were noted in reproductive toxicity studies of BisGMA (Moilanen *et al.,*2014) or TEGDMA (Moilanen *et al.,*2013) conducted in mice at doses at least 100-fold higher than estimated clinical exposure from use of composite restoratives.

3.4.3.5 Toxicity of other alternative materials

Under this heading dental alloys, glass ionomer cement including those with resin ingredients and ceramics are summarised. Metals released from dental alloys are – depending on the element and its oxidation stage – cytotoxic (Schedle *et al.,*1995; Schmalz *et al.,* 1997). Cytotoxicity of alloys depends on the corrosion rate, which with high gold alloys is generally smaller than with less noble alloys. Some Ni-containing alloys and Pd-Cu alloys but also Cu containing gold alloys are clearly cytotoxic (Wataha and Schmalz, 2001). Some metals are mutagenic, but the clinical relevance is not yet clarified for the use in dentistry (IARC, 1996). Alloys used for ceramic metal restorations may cause inflammation of the surrounding gingiva due to the release of metals (Schmalz and Arneholdt-Bindslev, 2009). Certain metals released from dental alloys like Ni, Cr, Co and Pd are well known to elicit as haptens allergic reactions. Also, Au has been described as an allergen (Møller, 2002). Cross reaction between Ni and Pd

have been reported (Garhammer *et al.,* 2001; Hindsen *et al.,* 2005). Oral lichenoid reactions could be associated with an Au or Pd allergy (Raap *et al.,* 2009). Like for amalgam, patient groups claimed systemic reactions caused by dental alloys, but these claims could not be substantiated except for allergies (Schmalz and Arneholdt-Bindslev, 2009).

Glass ionomer cements are only cytotoxic, when not fully set (Ersev *et al.,* 1999). Neither mutagenicity nor allergic reactions have been reported, but in direct contact with the dental pulp, severe tissue damage occurs (Schmalz *et al.,* 1994). Resin modified glass ionomer cements and compomers have biologic characteristics similar to resin composites. One resin modified glass ionomer cement was strongly cytotoxic and mutagenic (Heil *et al.,* 1996; Ribeiro *et al.,* 2006).

Ceramic materials are – with very few exceptions – not cytotoxic, mutagenic and do not cause allergic reactions. Radioactivity was measured, but the doses were considered low (Schmalz and Arneholdt-Bindslev, 2009). Many ceramic materials have to be luted to the dental hard tissues using resin-based materials and therefore biological problems associated with resin materials (see above) have to be considered.

3.4.4. Exposure

As noted earlier there are very limited data on exposure levels to the components of alternative dental restorative materials. Unlike the situation with amalgam, there are no obvious markers for exposure. Moreover, there are significant limitations to the determination of these exposure levels. The molecules used in any setting reaction, whether that is a polymerisation or an acid – base reaction, are by definition chemically reactive with a potential to exert toxic effects in humans. However, the reaction involves a small amount of material and usually takes place very quickly, following which many of these molecules have been irreversibly changed into far less reactive species or trapped within a solid mass with very limited capacity to diffuse and leach out. It is therefore expected that there will be a low but detectable level of exposure to many of these molecules during placement of the restoration. This is followed by a considerably reduced level, during the lifetime of the restoration.

The monomers used in dental resin-based materials are volatile and it is usually possible to smell them in dental clinics. The exposure of dental personnel to airborne methacrylates was studied during the placing of resin composite restorations in six dental clinics in Finland by Henriks-Eckermann *et al.,*(2001). Both area and personal sampling were performed, and special attention was paid to measurement of short-term emissions from the patient's mouth. The median concentration of HEMA was 0.004 mg/m^3 close to the dental nurse's work-desk and with a maximum concentration of 0.003 mg/m^3 in the breathing zone of the nurse with a maximum concentration of 0.033 mg/m^3. Above the patient's mouth the concentration of 2-HEMA was about 0.01 mg/m^3 during both working stages, i.e., during application of adhesive and resin composites and during finishing and polishing of the fillings. Maximum concentrations of 3-5 times higher than median concentrations were also measured.

TEGDMA was released into the air during the removal of old resin composite restorations (0.05 mg/m^3) but only to a minor extent during finishing and polishing procedures. The results showed that, except for short-term emissions from the patient's mouth, the exposure of dental personnel to methacrylates is very low. Measures to reduce exposure were discussed, as the airborne concentrations of methacrylates should be kept as low as possible in order to reduce the risk of hypersensitivity. In a study from Germany similar concentrations for HEMA and TEGDMA have been measured (Marquardt *et al.,* 2009). Other than those papers, there seems to be limited information about the actual level of exposure to volatile monomers in a clinical situation.

Polymerised resin-based materials contain various amounts of residual monomers and polymerisation additives that may leach from restorations. The release may remain on a high level for some days (Polydorou *et al.,* 2007). In addition, as noted above, chemical,

microbiological and wear impacts are observed over time, and occlusal or approximal degradation of resin composite restorations occurs (Groger *et al.,* 2006; Söderholm, 2003).

Most information on the release of material components is based on laboratory models with solvents such as ethanol, water, saline, artificial saliva or culture media. Gas chromatography and mass spectrometry of the solutes from resin composites, compomers and resin modified glass-ionomers have demonstrated the presence of a number of organic leachables such as monomers, co-monomers, initiators, stabilizers, decomposition products and contaminants. Some of them have been identified as the low viscosity monomers EDGMA, TEGDMA and HEMA together with initiator and co-initiators such as hydroquinone, camphorquinone, and DMABEE and an ultraviolet absorber, Tinuvin P (Lygre *et al.,* 1999; Michelsen *et al.,* 2003). Attempts at quantification have shown that elution from different materials differs significantly (Michelsen *et al.,* 2006) and the data are contradictory. Bis-GMA, Bis-EMA, UDMA and various additives have been shown to leach (Rogalewicz *et al.,* 2006), although others have failed to demonstrate BisGMA and UDMA in aqueous extracts, even though TEGDMA-based composites released high amounts of monomers (Moharamzadeh *et al.,* 2007). Under simulated in vitro chewing conditions TEGDMA release from a resin composite was analysed; with or without chewing most TEGDMA was released in the first 26 hours, then the amount declined. Around 2.6% of the included ^{14}C labeled TEGDMA was released after 86 hours (Durner *et al.,* 2010).

It is reasonable to assume that similar leaching reactions take place in patients, depending on the composition of the material, the effectiveness of the polymerisation process and the chemical impact of the oral environment, although limited information is available on the concentration of components from amalgam alternatives in patient saliva or other body fluids. There are some exceptions, such as acrylic monomers from soft liners and phthalates from denture base materials (Lygre *et al.,* 1993; Lygre 2002).

Bisphenol-A (BPA) can be released from resin-based materials (Olea *et al.,* 1996; Pulgar *et al.,* 2000) with more BPA being eluted in the polymerised state than in the unpolymerised. However, from unpolymerised samples fewer substances are released than from polymerised ones, which is in contradiction to studies reported elsewhere in the literature (Schmalz and Arenholt-Bindslev, 2009). Furthermore, a large number of other authors who studied BPA release using a large variety of test methods and materials could not detect BPA with the exception of a Bis-DMA containing sealant. Because of the contradictory results, the analytical methods used by Olea *et al.,*(1996) and Pulgar *et al.,*(2000) were heavily questioned (Imai, 2000; Imai and Komabayashi, 2000; Schmalz and Arenholt-Bindslev, 2009; Fleisch *et al.,* 2010; Myers and Hutz, 2011).

It was shown that materials containing Bis-DMA released BPA immediately after application into the patient's saliva. After 24 hours the BPA concentrations in saliva returned to pretreatment level (Schmalz *et al.,* 1999; Arenholt-Bindslev *et al.,* 1999). In the same study, a Bis-GMA-based pit and fissure sealant that contained no primary BPA contamination was not found to release BPA into saliva. BPA release from Bis-DMA containing sealants have been reported by other authors (e.g. Joskow *et al.,* 2006). BPA could not be detected in the blood samples and urine content of BPA was most elevated in patients after Bis-DMA material application one hour after placement and then decreased after 24 hours.

Bis-DMA was cleaved hydrolytically under alkaline conditions, using porcine esterases and human saliva. BPA could be detected, but this was not the case with Bis-GMA (Schmalz *et al.,* 1999). It can be concluded that Bis-DMA is initially eluted from Bis-DMA-containing pit and fissure sealants, which is then degraded to BPA in saliva. BPA degradation from Bis-GMA could not be demonstrated under the given analytical conditions (Schmalz *et al.,* 1999).

As BPA is used during the production process of Bis-GMA, residues of BPA may be present. These have been estimated by Imai (2000) to be at maximum 10µg/g unpolymerised resin. Experimental addition of 100 µg/g of BPA to a resin composite resulted in a BPA release being lower than for TEGDMA, and over ten years 12% (water) or 53% (methanol) BPA from the original BPA content of the resin was calculated to be released. From 1 g of this resin during 10 years patients may be exposed to minute amounts of 4 ng/day (water) or 16ng/day (methanol) (Imai and Komabayashi, 2000).

A study performed by the American Dental Association (2014) shows that bis-GMA-based dental restorative materials have the potential to release BPA at a detectable level. Furthermore, bis-DMA and bis-EDMA also demonstrated a high potential to release BPA. All sources of raw bis-GMA had detectable levels of BPA. However, all of the tested dental restorative composites released BPA at levels that are far below the daily exposure limits set by the U.S. Environmental Protection Agency and the European Food Safety Authority.

Summarising the data, it can be stated that patients may only be exposed to minute amounts of BPA from Bis-GMA resins due to possible impurities. From materials containing Bis-DMA, BPA exposure of patients could consistently be found, but mainly during the first 24 hours after placement. In addition the risk assessment due to BPA release from dental amalgam has been carried out and described in the recent opinion by SCENIHR (2015), showing that no concern is associated with these dental material.

Nano-particles

Recently, attention was drawn to another exposure source for patients and dental personnel with a possible toxicological relevance: the formation of nanoparticles during the placement or the removal of resin composite fillings (van Landuyt *et al.,* 2012). From a large group of contemporary resin composite materials, blocks were formed, ground as is done in a dental practice and the dust was analysed. Small respirable dust particles were found and the ratio of dust particles < 1 µm to those >1 µm ranged between 3:1 to 9:1.

This was confirmed in a recent study by Bogdan *et al.,*(2014), showing that nanoparticles were generated during shaping of materials independent of the amount and size of the filler particles.

Exposure measurements of dust in a dental clinic revealed high peak concentrations of nanoparticles in the breathing zone of both dentist and patient, especially during aesthetic treatments or treatments of worn teeth with composite build-ups (Van Landuyt *et al.,* 2014). Analysis of the particles generated by abrasive procedures confirmed that all tested composites, including both conventional and nano-composites, released airborne nanoscale particles.

3.4.5. Potential adverse effects in patients

On the basis of the above comments on the composition of the alternatives to amalgam, the possible exposure levels associated with their components and known *in vitro* data on their toxicity, a general assessment of potential adverse effects in patients may be made.

3.4.5.1. General

The components released from dental restorative materials comprise a long list of xenobiotic organic substances and metallic elements (Schmalz 2005; Wataha and Schmalz 2005). The components are subject to oral mucosal, pulpal and gastrointestinal absorption, and, for aerosols, pulmonary absorption, the passive diffusion through cell membranes being guided by factors such as the concentration gradient, molecular size, polarity, lipophilicity, and hydrophilicity.

Toxic effects after inadvertent contact with chemicals associated with restorative dentistry may appear as acute soft tissue injuries among dental patients. Local chronic reactions of irritation, or of combined irritation and hypersensitivity, appear as lichenoid reactions of the gingiva or mucosa. It is generally accepted that the amount of potentially toxic substances absorbed from alternatives to amalgam is too small to cause systemic reactions by dose-dependent mechanisms in target organs. However, this statement does not deny that adverse reactions may occur, elicited by minute quantities of released substances, including allergies and genotoxicity. Of these, only allergy has been confirmed among dental patients.

The cytotoxicity and genotoxicity of substances leached from resin-based materials and metallic elements have been the subject of extensive studies using cell culture techniques and a bacterial mutation test (Ames test). Substances such as TEGDMA and HEMA cause gene mutations *in vitro*. Studies on the intracellular biochemical mechanisms have clarified various effects such as cell membrane damage, inhibition of enzyme activities, protein or nucleic acid synthesis etc. (Schweikl *et al.,* 2006). At present, the clinical relevance of these *in vitro* studies is uncertain.

The release of Bisphenol A from Bis-GMA based materials such as fissure sealants and composites into saliva has been of special interest because of its potential estrogenic effect (Joskow *et al.,* 2006). The concentration of released Bis-GMA from certain types of sealants has been reported to be within the range at which estrogen receptor-mediated effects were seen in rodents (Schmalz *et al.,* 1999). However, the release from resin-based restoratives is much lower. The conversion of Bis-GMA to Bis-MA is minimal in resin-based materials if pure base monomers are used (Arenholt-Bindslev and Kanerva, 2005). The minute concentration in resin-based amalgam alternatives is not considered to be a problem.

It must be noted that there are other alternatives to amalgams in addition to these resin- and cement-based materials. These primarily include a variety of different alloys and ceramics used for indirect restorations. These, however, do not represent clinically relevant options for the treatment of the vast majority of teeth and are only used when direct restorations are contra-indicated. Although idiosyncratic responses may be encountered with most materials (Ahlgren *et al.,* 2002), and there may be exposure even to gold from such restorations (Ahlgren *et al.,* 2007), there are very few indications that such materials have the potential for adverse effects with the exception of allergies towards metals like nickel, cobalt, palladium and even gold (Schmalz and Arenholt-Bindlev, 2009).

3.4.5.2. Allergy/Immune system

Potential allergens among amalgam alternatives

There is limited possibility to predict the allergenic potential for a foreign substance on the basis of chemical composition using Quantitative Structure-Activity Relationship (QSAR) analysis. However, experimental testing such as the Guinea Pig Maximisation Tests or the Murine Local Lymph Node Assay, and empirical results after years of testing substances causing allergies have given some leads: the strongest allergens are often low molecular weight, aromatic, lipid soluble substances, or otherwise chemically active substances that react with proteins. Metal and metal salts are also high ranking haptens. On this basis, monomers, cross-linking agents, chemicals associated with the polymerisation process, and degradation products, all associated with resin-based materials, are important candidates for allergic responses among users of these alternatives, including dental patients and professionals. A short list of allergens relevant to resin-based amalgam alternatives is presented in Table 4.

Although an allergic reaction may be provoked by haptens derived from dental materials, the sensitisation process may be caused by substances unrelated to dentistry. Plastics are met with in everyday life and in occupations such as construction work and printing. For anatomical reasons both the allergic sensitisation and the allergic response are more easily obtained on skin than in the oral tissues. Epidermal tests are therefore adequate also for observations of intraoral adverse effects. A positive patch test is an indication of a causal relationship between the substance and the suspected allergic reaction, but does not provide definitive evidence without other criteria of causality, which often cannot be performed for practical and ethical reasons.

Table 4: Some allergens in resin-based amalgam alternatives (primers, bonding agents, resin composites, glass ionomers, resin modified glass-ionomers, compomers etc.).

Methacrylate monomers
2-hydroxy ethyl methacrylate
Triethylene glycol dimethacrylate
Pyromelilitic acid dimethylmethacrylate
Bisphenol-A glycidyl methacrylate
Urethane dimethacrylate
Bis-phenol-A polyethylene glycol diether dimethacrylate
Ethylene glycol dimethacrylate (EGMDA)
Other substances
Benzoyl peroxide, camphorquinone (initiators)
Tertiary aromatic amine (activator)
Methylhydroquinone (inhibitor)
2-hydroxy-4-methoxy benzophenones, (UV absorber)
2-(2-hydroxy-5 methylphenyl) benzotriazole (UV absorber)

3.4.5.3. The role of bacteria

The presence of bacteria located at the interface between composite materials and dental tissues may be important (Hansel *et al.,* 1998). EGDMA and TEGDMA promote the proliferation of cariogenic microorganisms such as *Lactobacillus acidophilus* and *Streptococcus sobrinus*; TEGDMA stimulates the growth of *S mutans* and *S salivarius* in a pH-dependent manner (Khalichi *et al.,* 2004). This provides one explanation for caries that develop beneath restorations of resin-containing materials. In addition, bacterial exotoxins have harmful effects on pulp cells after diffusion throughout dentine tubules.

It is also important to note that effects on dental pulp associated with restorations may be caused by bacterial contamination rather than the materials themselves (Bergenholtz *et al.,*1982; Bergenholtz 2000). This is still a matter of controversy and a few reports still consider that the pulp reaction to adhesive systems is generally minimal (Murray *et al.,* 2002; Murray *et al.,* 2003). Improvements of resin-containing materials and bonding agents and techniques have reduced the significance of shrinkage and gaps at the interface, which may be less than 1 µm (Hashimoto *et al.,* 2004). However this is still a large gap for many microorganisms such as lactobacilli that are less than 0.1 µm in diameter, and therefore the microbial parameter cannot be ignored.

Clinical studies in high risk caries groups report more secondary caries when composites restorations are used compared to amalgam (Opdam *et al.,* 2010), and recurrent caries is the primary reason for composite replacement (Burke *et al.,* 2001). Recurrent caries is primarily located at the gingival margin of the restoration (Mjor, 1998). The vitality of the biofilm formed on composites is higher compared to amalgam (Auschill *et al.,* 2002).

Biofilm grown on dental composites in vitro have been shown to lead to chemical degradation of the composite and to increase the surface roughness of the composite material (Beyth *et al.,*2008; Gregson *et al.,* 2012). However, a 30-day old *S. mutans* biofilm did not have a negative impact on surface roughness or hardness of a composite, but surface degradation was evident. (Fucio *et al.,* 2008).

Resin composites are vulnerable to hydrolytic degradation of polymerised methacrylates (Gopferich, 1996), and the dentin-resin interface have been shown to be degraded by water

sorption, possibly by two degradation patterns, disorganisation of the collagen fibrils and loss of resin in interfibrillar spaces (Hashimoto *et al.,* 2003). In addition, degradation of resin composite materials by bacterial and salivary esterases have been shown to occur (Shokati *et al.,* 2010; Bourbia *et al.,* 2013). These findings show that bacteria may have an active role in breakdown of adhesives and composites, and that degradation at the dentin-resin interface may increase bacterial microleakage (Kermanshahi *et al.,* 2010). In addition, it has been shown that the presence of a multi-species biofilm may lead to degradation at the dentin-composite interface, and that the degree of degradation varies between different composite restorative systems (Li *et al.,* 2014).

Interestingly, both unlined and bonded amalgam restorations show reduced marginal leakage when compared to composites (Ozer *et al.,* 2002; Alptekin *et al.,* 2010).

Monomers used in dental composites have been described to promote proliferation of oral microorganisms such as *Lactobacillus acidophilus* and *Streptococcus sobrinus* by measuring an increase in absorbance during growth (Hansel *et al.,* 1998). However, the effect of monomers is controversial, as others have shown that the actual bacterial number, colony forming units, of *S. sobrinus* and *S. sanguinis* did not increase when exposed to TEGDMA or DEGDMA. The increase in absorbance was described to be caused by vesicular formation around bacteria when exposed to ethylene-glycol monomers, causing an increase in particle size and not increase in the actual number of bacteria (Takahashi *et al.,* 2004). However biodegradation products of the monomers have been shown to affect bacterial growth. The hydrolysed end products of TEGDMA, metacrylic acid and triethylene glycol, have been shown to exert opposite effects on bacterial growth. Metacrylic acid may inhibit growth of *S. mutans* and *S. salivarius*, whereas triethylene glycol accelerated the growth of *S. mutans* at low pH (Khalichi *et al.,* 2004). Triethylene glycol has also been shown to affect gene expression of glycosyltransferase B, a known virulence factor involved in production of extracellular polysaccharides of *S. mutans*. This finding shows that low concentrations of monomers and their degradation products may affect virulence gene expression of bacteria (Khalichi *et al.,* 2009).

3.4.6. Epidemiological and clinical evidence concerning adverse effects of alternatives in patients

Studies published by Maserejian *et al.,*(2012 a, 2012 b; 2014) concerning possible adverse effects related to exposure to bisGMA-based dental composite restorations have contrasting results. A post-hoc analysis of the Casa Pia Study showed that exposure to bisGMA-based dental composite restorations was associated with impaired psychosocial function in children in comparison to amalgam (Maserejian *et al.,* 2012a). The same authors published another study related to neuropsychological development, finding insignificant associations (Maserejian *et al.,* 2012b). A more recent analysis showed that use of sealants (containing BPA) or preventive resin restorations were not associated with behavioural, neuropsychological, or physical development in children (Maserejian *et al.,* 2014).

3.4.6.1. Case reports

Several cases and series of cases confirming allergic reactions caused by tooth-coloured restorative materials have been published. For example, an early case report described a female patient who developed a rash and hives on her chest, arms and legs after treatment with a composite (Nathanson and Lockhart, 1979). Patch-testing indicated that Bis-GMA was the provoking agent, whereas the sensitisation might have taken place by contact with a cross-reacting epoxy product. Patch tests also indicated Bis-GMA in a case of peri-oral erythema and crusting of cheeks following the application of a bonding agent for resin composite and glass ionomer fillings (Carmichael *et al.,* 1997). Moreover, stomatitis and peri-oral dermatitis was attributed to Bis-GMA in a filling material (Kanerva and Alanko 1998). Even

immediate type allergic reactions have been described after contact with a Bis-GMA resin composite used for fissure sealing (Hallström, 1993; Schmalz and Arenholt-Bindslev, 2009). Other relevant molecules were reported to be TEGDMA and HEMA, which are used in materials for bonding resin composites to the tooth structures (Aalto-Korte *et al.,* 2007; Drucker and Pratt, 2011; Schmalz and Arenholt-Bindslev, 2009). In general, clinical symptoms comprise intraoral, perioral and extraoral reactions (Tillberg *et al.,* 2009). Local lichenoid reactions similar to those described for amalgam, have also been attributed to composite fillings. In one case patch testing indicated EGDMA as the allergen (Auzeerie *et al.,* 2002), whereas other cases indicated formaldehyde derived from the resin (Lind, 1988). Ulcerating gingivitis localised to resin composite fillings was explained as a delayed reaction to the UV-absorber Tinuvin P (Björkner and Niklasson, 1979).

Metals and alloys are another group of materials which can be used as alternatives to amalgam. While cases of allergic reactions to nickel are well known (Schmalz and Arenholt-Bindslev, 2009), reactions towards palladium (Garhammer *et al.,* 2001) have also been reported and a cross reactivity between nickel and palladium was proposed (Garhammer *et al.,* 2001). Also, cases of contact allergy to gold and the relationship with OLL have been reported (Ahlgren *et al.,*2012).

Reactions to cobolt-chromium metal-ceramic fixed partial dentures and crowns have also been reported (Sélden *et al.,* 1995; Wang *et al.,* 1996). Alloys must be processed by dental technicians to produce crowns, partial crown or inlays. This is traditionally done after taking an impression of the patient's mouth and then making a cast.. Cases of allergic reactions towards impression materials have been described (Mittermüller *et al.,* 2012).

For deciduous teeth, steel crowns are advocated as amalgam replacement. A case of delayed hypersensitivity with perioral skin eruptions after insertion of such a crown in a 13-year-old girl was reported (Yilmaz *et al.,* 2012).

The multitude of case reports with the various alternatives used indicate a concern for adverse reactions of these alternatives. However, currently no general conclusions can be made based on the available information.

3.4.6.2. Reports from adverse reaction registry units

In the years 1999-2002 the Norwegian Dental Biomaterials Adverse Reaction Unit received an increasing number of reports of adverse reactions associated with composite materials, although these were still outnumbered by reactions to amalgam and other alloys (Lygre *et al.,* 2003; Vamnes *et al.,* 2004). Swedish data showed a similar tendency. Patch-testing of referred patients demonstrated positive reactions to methacrylates and additives relevant to resin- based materials, although the most frequent allergens were nickel, gold, cobalt, palladium, mercury and chromium. A survey by the UK registry indicated that the number of adverse reactions caused by resin-based materials, amalgam alternatives included, was about 14 % of the total number of patient reactions (Scott *et al.,* 2004). The UK Registry and the Swedish Registry have been discontinued since the former version of this Opinion was published.

The discussions concerning potential adverse reactions related to the use of dental amalgam have also focused on potential side-effects from other materials, such as polymer-based filling materials and associated products, e.g. bonding agents, and cast gold alloys. There are no harmonised criteria for what can be classified as an adverse reaction related to dental materials. Under-reporting was a recognised problem and lack of awareness and lack of clarity as to what constitutes an adverse reaction may be contributory factors.

The Dental Biomaterials Adverse Reaction Unit is a permanent activity funded by the Norwegian Government and located at the Department of Clinical Dentistry, University of Bergen.The Dental Biomaterials Adverse Reaction Unit has three main purposes:

1) Recording of adverse reactions

Dentists, dental assistants and physicians report to the Adverse Reaction Unit when any kind of side effect related to dental materials is observed. Both subjective and objective reactions can be recorded. The information is evaluated, coded and collected in a database at the Unit.

2) Clinical examination of referred patients

At the Adverse Reaction Unit, patients who exhibit reactions that are suspected to be associated with dental biomaterials can be referred from the patient's primary dentist or physician for additional examination. No dental treatment is given at the Adverse Reaction Unit. The aim is to collect clinical data on the various aspects of adverse reactions, particularly those which are not directly related to local reactions. The referral routines are designed so that a co-operation is required between the patient's primary physician and dentist.

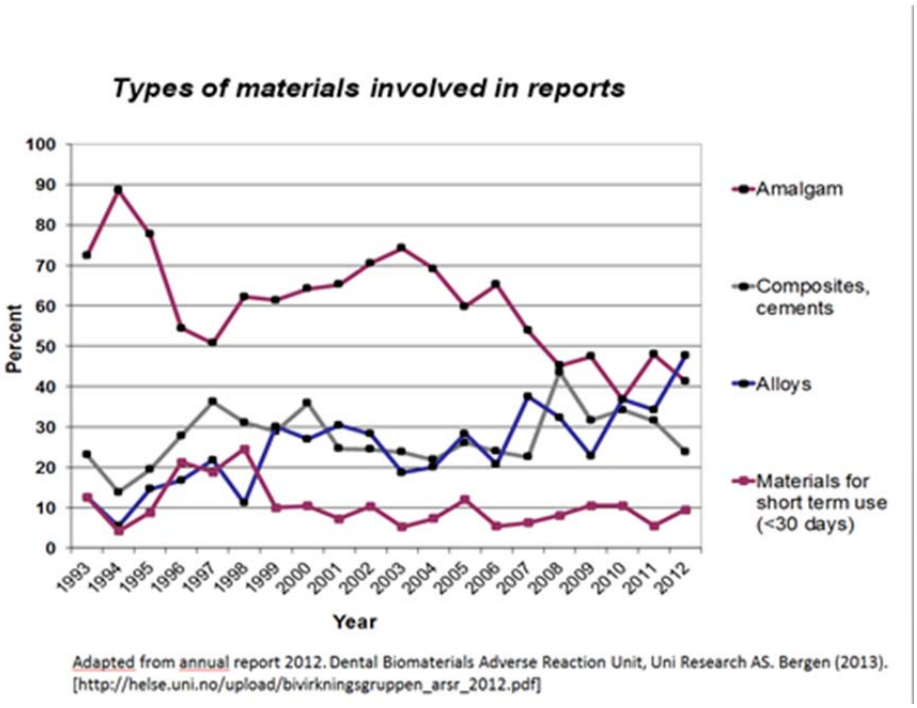
3) Information activities

The Unit gathers informational material pertaining to dental materials and their potential risks for both health personnel and the public.

Unfortunately, the Unit only publishes its Annual Report in Norwegian. However, the following graph indicates the types of materials involved in reports from 1993 to 2012.

Figure 2: Types of dental materials involved in adverse reaction reports

Adapted and translated from Annual Report Dental Biomaterials Reaction Unit 2012, courtesy of Professor Lars Björkman.



Adapted from annual report 2012. Dental Biomaterials Adverse Reaction Unit, Uni Research AS. Bergen (2013). [http://helse.uni.no/upload/bivirkningsgruppen_arsr_2012.pdf]

Since all dental materials pose a potential risk to patients and members of the dental team, the post-market monitoring of adverse reactions caused by dental materials should be considered essential.

The Directive concerning medical devices (93/42/EEC) requires the manufacturers to have postmarketing surveillance data which are reviewed by the Notified Bodies on audits. The Competent Authorities have a vigilance system for adverse events with medical devices. However, this information is not publically accessible.

The US Food and Drug Administration has active reporting systems for adverse events concerning all types of medical devices, including dental materials. Their MAUDE database houses medical device reports submitted to the FDA by mandatory reporters (manufacturers, importers and device user facilities) and voluntary reporters such as health care professionals, patients and consumers.

3.4.6.3. Reports from dermatological units

A Finnish multicentre study based on dental screening allergens on 4000 patients concluded that methacrylates, particularly HEMA, were responsible for 2.8 % of reactions, which were otherwise dominated by metal salts (Kanerva *et al.,* 2001). A Swedish investigation showed positive patch tests to methacrylate allergens in 2.3 % of the patients (Goon *et al.,* 2006). The most common of these allergens was HEMA, followed by EDGMA, TEGDMA, and MMA. Simultaneous positive reactions were frequent. Only one patient reacted to Bis-GMA, whereas reactions to HEMA alone were seen in most patients. Data from Israel after testing of patients with oral manifestations such as cheilitis, burning mouth, lichenoids, and orofacial granulomatosis also ranked HEMA as the most frequent dental allergen after the metal salts (Khamaysi *et al.,* 2006).

1632 subjects had been patch tested to either the dental patient series or dental personnel series at the department of Occupational and Environmental Dermatology, Malmö, Sweden. Positive patch tests to (meth)acrylate allergens were seen in 2.3% (30/1322) of the dental patients and 5.8% (18/310) of the dental personnel. The most common allergen for both groups was HEMA, followed by EGDMA, TEGDMA, and methyl methacrylate (Goon *et al.,* 2006). The prevalence of acrylate/methacrylate allergy was in Singapore – slightly lower compared to Malmö (Goon *et al.,* 2008).

In a series of 121 patch-tested patients suffering from several intra-, peri- and extraoral symptoms, the most common allergens detected included goldsodiumthiosulphate (14.0%), nickel sulfate (13.2%), mercury (9.9%), palladium chloride (7.4%), cobalt chloride (5.0%), and 2-hydroxyethyl methacrylate (5.8%) (Khamaysi *et al.,* 2006). Twenty-eight of 206 patients had positive patch-test reactions to metals used in dentistry. The number of positive patch-test reactions was highest for gold sodium thiosulfate, palladium chloride, and nickel sulfate (Raap *et al.,* 2009).

3.4.6.4. Questionnaire studies

A few attempts have been made to estimate the incidence of adverse effects of dental materials among dental patients. However, no studies have focused specifically on alternatives to amalgam. After about 10 000 dental treatments, one fifth of which were resin composite restorations, 22 adverse reactions were observed, none of them being related to tooth coloured restorative materials. Thirty-one dentists, representing a collective practice time of 387 years, recollected 70 cases of adverse effects, of which two were attributed to temporary resin-based and denture base materials, and 5 to copper cement, but none to alternatives to amalgam (Kallus and Mjør, 1991).

Other questionnaire studies have aimed at obtaining incidence rates of material related side effects in dental specialty practices such as paedodontics, orthodontics, and prosthodontics. Data from paedodontics indicated one reaction in 2400 patients, but only a minimal part was attributed to alternatives to amalgam (Jacobsen *et al.,* 1991). Orthodontics and prosthodontics do not regularly include the placement of restorative amalgam alternatives, but resin-based materials of similar composition are used. In orthodontics, only one of 41 000 patients showed an intra-oral reaction to an orthodontic composite, but nine others reacted to resin-based removable appliances, retention appliances, activators, and polymeric brackets (Jacobsen and Hensten-Pettersen 2003). However, some of these appliances are often made by chemically

polymerised methacrylates, containing relatively higher concentration of potentially allergenic residual monomers as compared to well-cured restorative composites. Questionnaire data from prosthodontics could be interpreted to indicate a reaction rate of one per 600 patients for resin-based prosthodontic materials (Hensten-Pettersen and Jacobsen 1991).

More recently, New Zealand dentists were asked about their experience with (non-amalgam) dental alloy allergies. As many as one in six general practising dentists have encountered allergic reactions to metal alloys in their patients (Zhou *et al.,* 2010).

3.4.6.5. General Comments

Case reports and reports from dermatological units highlight the possibility of adverse effects related to identified dental materials. Information from these sources is helpful in a field where these events are infrequent. The adverse reaction registry units in some countries contribute data on the relative frequency of the different adverse reactions, including those to amalgam alternatives. However, since participation by dental personnel is voluntary, the amount of under-reporting of patient reactions is unknown. The existing epidemiological studies offer an impression of the different material related adverse effects as perceived by dental personnel. However, none of these studies are well suited as a basis for estimation of the prevalence of reactions caused by specific allergens associated with amalgam alternatives or other materials.

In spite of these drawbacks, an attempt to rationalise the risk of material-related adverse effects in dentistry on the basis of published reports has appeared (Schedle *et al.,* 2007). Large variations were found, ranging between 1:10 000 and 1:100 for dental patients. A FDI-report also points to the fact that the vast majority of patients have encountered no adverse reactions, but dentists were advised to be aware of the possibility of reactions to resin-based materials (Fan and Meyer, 2007). The importance of satisfactory curing of these materials was specifically underlined. It is assumed that the most frequent potential allergens associated with resin-based amalgam alternatives are found in Table 5.

Furthermore, non-amalgam dental alloy-based alternatives for dental amalgam used in inlays, partial or full crowns contain metals such as nickel, palladium or gold for which allergic reactions are repeatedly being reported with partially higher frequencies than for dental amalgam (Schmalz and Arenholt-Bindslev, 2009).

3.4.7. Epidemiological and clinical evidence concerning adverse effects of alternatives in dental personnel

The potential for adverse effects due to alternative restorative materials amongst dental personnel is widely recognised (Hume and Gerzina, 1996). Most of the evidence of adverse effects takes the form of case reports, findings from surveys (Örtengren, 2000) and reports from national reporting systems (van Noort *et al.,* 2004) as well as from dermatological units (Goon *et al.,* 2006).

The study from Sweden shows a 2-3 times higher sensitisation rate for dental personnel as compared to patients. Given the extent of the use of alternative restorative materials, hundreds of millions of restorations annually, and the possibility that <7% of dental personnel may report skin symptoms when working (Örtengren, 2000), it is surprising that the reported incidence of adverse effects due to alternative restorative materials is low (van Noort *et al.,* 2004). The prevalence of verified allergic contact dermatitis amongst dental personnel (<1%) is much lower than the prevalence of self-reported skin symptoms (<7%) (Örtengren, 2000).

Most of the adverse reactions reported take the form of contact dermatitis, which in severe cases may be associated with paraesthesia of the finger tips (Kanerva *et al.,*1998). Reactions around the eyes, generalised skin itching and bronchial problems have been reported, but these are rare (Hume and Gerzina 1996).

HEMA appears to be a common sensitiser, although a small minority of dental personnel may have positive patch-tests to BisGMA and/or TEGDMA (Kanerva *et al.,* 2001). It is relevant that relatively low molecular weight resin monomers, including HEMA and TEGDMA take only a few minutes to diffuse through latex gloves of the type worn by dental personnel, while higher molecular weight monomers, such as BisGMA, take a little longer to pass through the relatively thin latex of treatment gloves (Jensen *et al.,* 1991; Munksgaard, 1992). These findings emphasise the importance of a "no-touch" technique when handling resin-based restorative materials, even when wearing gloves. This approach to the handling of resin-based restorative materials is highlighted in manufacturers' directions for use.

Regarding the lower incidence of allergic responses to resin-containing alternative restorative materials in patients relative to dental personnel, Kallus and Mjör (1991) and Hensten-Pettersen and Jacobsen (1991) suggest that this may be related to the fact that the principal exposure of dental personnel is to methacrylates as monomers during the handling of uncured materials. Adverse effects of alternative restorative materials in dental personnel may, as a consequence, be minimised by the avoidance of contact with, in particular, low molecular weight monomers during the handling and placement of uncured materials. The effects may be further reduced by the use of effective face protection, water cooling and suction, as appropriate, in all operative procedures involving both cured and uncured resin-based materials and associated systems. On the other side, it was reported that in a room where resin composites are used, monomer concentration in the air is elevated which means that a further source of exposure exists (Marquardt *et al.,* 2009). However, the concentrations were very low.

Between 1995 and 1998, 174 dental personnel were referred as patients to the Department of Occupational and Environmental Dermatology, Stockholm (Wrangsjö *et al..* 2001). After clinical examination, 131 were patch tested with the Swedish standard series and 109 with a dental screening series. Furthermore, 137 were tested for IgE-mediated allergy to natural rubber latex. Hand eczema was diagnosed in 109/174 (63%), 73 (67%) being classified as irritant contact dermatitis and 36 (33%) as allergic. Further diagnoses included other eczemas, urticaria, rosacea, psoriasis, tinea pedis, bullous pemphigoid or no skin disease. 77/131 (59%) had positive reactions to substances in the standard series and 44/109 (40%) to substances exclusive to the dental series. 24/109 (22%) patients had positive reactions to (meth)acrylates, the majority with reactions to several test preparations. Reactions to HEMA, EGDMA and MMA were most frequent. Nine of the 24 were positive only to (meth)acrylates, the remaining 15 also had reactions to allergens in the standard series. Irritant hand dermatitis was the dominant diagnosis. Contact allergy to (meth)acrylate was seen in 22% of the patch-tested patients, with reactions to three predominant test substances. In one third of these cases the (meth)acrylate allergy was seen together with atopy and/or further contact allergies.

Also, less severe allergic skin reactions among dental personnel have been diagnosed as caused by methacrylates, secondary in frequency only to chemicals related to natural rubber latex (Alanko *et al..* 2004). Hand dermatoses, together with eye-, nose-, and airway reactions are consistent findings among dental personnel, although the role played by amalgam alternatives is undecided (Sinclair and Thomson 2004; Andreasson *et al..* 2001).

The Finnish Register of Occupational Diseases diagnosed 24 cases of occupational asthma or rhinitis caused by methacrylates during the years 1990-98 .The incidence rate of occupational respiratory disease was considered greater than in the whole population (Piirilä *et al.,*2002).

Preventive actions such as change in hygiene factors, use of no-touch techniques when working with methacrylates, less use of latex and awareness of risk factors seems to keep the prevalence of skin and respiratory symptoms low among dental personnel (Schedle *et al.,* 2007).

3.4.8. Potential adverse effects of ancillary items and equipment

3.4.8.1. Photopolymerisation energy sources

Light sources are used to activate chemical photoinitiators, by absorption of photons, in order to initiate polymerisation in many restorative materials (Small, 2001). The applied light dose (radiant exposure; [J/m^2]) depends on the radiation power emitted per unit area (irradiance; [W/cm^2]) multiplied by time [in seconds]. Each photoinitiator has its unique radiation absorption spectrum, i.e. photons of specific wavelengths (energies) only are absorbed and to different degrees. The most common photoinitiator is camphorquinone which absorbs visible light between ~400-500 nm with an absorption peak at 468 nm. The main advantages of light-cured resin composites compared to chemically cured products are based on the fact that mixing of components in the clinic is not required, resulting principally in less porosity, better curing control, less curing time and ease of placement (Krämer *et al.,* 2008).

Types of light curing units

Dental curing systems use light sources such as light-emitting diodes (LEDs), quartz-tungsten-halogen lamps (QTH), xenon-plasma arcs (PAC) and lasers of which LEDs are the most widely used. A small percentage of the lamp source emission is visible light: 15%; 5%; 1% for LEDs; QTH and PAC, respectively. The remaining emission is heat (all lamps) and infrared radiation (IR) (not from visible light LEDs). LED dental curing lamps, based on solid-state semiconductor technology emit radiation in the visible and IR part of the electromagnetic spectrum within relatively narrow wavelength bands. Typical bandwidth for dental LEDs are 30-50 nm, and since bands exist that match the absorption spectra of commonly used photoinitiators, both around 400 nm and around 470 nm filters are not required. The irradiance of 13 lamp products measured in the 400 to 515 nm range varied from ~600 - 2000 mW/cm^2 (Bruzell and Wellendorf 2008). Some LEDs marketed in 2008 claim irradiance values up to 5000 mW/cm^2. The lifetime is longer and irradiance more stable for LEDs than for halogen lamps.

QTH lamps with halogen inside quartz bulbs generate light through the heating of a tungsten filament to high temperatures. A drawback of halogen bulbs is that the generation of heat causes a degradation of the components of the curing unit over time. The irradiance declines consecutively, which compromises the curing ability of the unit. The IR and some UV radiation is filtered to emit wavelengths in the violet-blue range only (~380-515 nm). The irradiance of halogen lamps tested between 2002-2007 varied from ~400 to ~3400 mW/cm^2.

Plasma-arc lights are made up of two electrodes in a gaseous, e.g. xenon-filled bulb. The plasma is heated to several thousand degrees Celsius and emits UV, visible and IR radiation which is filtered to allow mainly blue light (390-500 nm). Typical irradiance is ~3000 mW/cm^2.

Lasers can emit optical radiation at single (monochromatic) wavelengths as a result of the excitation of atoms of suitable gases/liquids/solids to specific energy levels. Argon lasers suitable for photopolymerisation emit at 488 nm and may have a power output up to 5000 mW, but the operating power is usually around 250 mW.

Dental curing lamps are classified as medical electrical equipment and should comply with a specific standard to indicate the potential risk of adverse health effects (International Electrotechnical Commission (IEC) 60601-2-57:2011). According to the rules in this standard, several dental curing lamps will be classified in the second highest group, indicating that the risk is moderate, but that the aversion response of the eye cannot be relied on completely.

Light-curing of resin composites

The dental curing lights initiate polymerisation of resin-based dental restorative materials by emission of radiation to be absorbed by photoinitiators in the material. The surface of the light delivery device should, ideally, be positioned a few mm from the material surface. Increasing the distance will normally decrease the irradiation, depending on the area of the emission relative to the area to be cured. The radiant exposure required for optimal curing, i.e. achieving adequate depth of resin composite layer without sacrificing mechanical properties while minimizing heat generation, is material dependent and is of the magnitude 10 000-50

000 mJ/cm^2. Recommended irradiances and curing times may vary from 300 mW/cm^2 to > 2000 mW/cm^2 and ~5-100 s respectively, to obtain a 1.5-4 mm thick layer of resin composite polymer, depending on the material colour, degree of opacity/translucency, particle size and -volume and chemical composition. So-called bulk-fill materials have increased translucency that increase the layer thickness (Musanje & Darvell, 2003; Bruzell and Wellendorf, 2008; Ilie *et al.,*2013).

Risk issues

Exposure of the eyes

The eyes of the lamp operators and assistants are at risk from acute and cumulative effects, mainly due to back-reflection of the blue light. Some LEDs emit shorter wavelengths (close to UV, approx. 400 nm) in addition to the blue, and this radiation is potentially damaging to anterior parts of the eye, such as the cornea and lens. Exposure to intense visible light radiation sources in a dental clinic necessitates the use of eye protective filters to avoid blue-light photochemical retinal damage. Normally, the light from a curing lamp does not reach the patient's eyes. However, if the risk is increased, eye protection should be used by patients as well. Increased risk includes for e.g. light curing of the front teeth and treating patients with ocular disease or intraocular lens implants (due to e.g. cataract surgery). Such lenses offer various degrees of UV- and blue light protection, but they offer less protection from wavelengths emitted from an LED lamp than the middle-aged eye does (Mainster, 2006). Bruzell *et al.,*(2007) measured the visible light transmittance of eye protective filters of which half the number were unsuitable for use with light curing.

Exposure of skin and oral tissues

Both materials and radiation intended for curing can be exposed to patients' oral tissue or dentists' finger skin. The two agents combined can cause photosensitisation effects, which is typical of UVA (320 -400 nm) and visible radiation (400-800 nm). UV can also induce direct effects. Although in vitro studies have shown that blue light of doses relevant for dental light curing can induce small cytotoxic effects (Bruzell Roll *et al.,*2004; Opländer *et al.,* 2011), these lamps do not appear to cause damage to healthy skin under normal use. However, thermal effects can occur with irradiances above ~100 mW/cm2 after a few minutes depending on local tissue factors such as blood circulation. There are reports of accidental oral soft tissue burns with the use of LEDs (Spranley *et al.,* 2012). Quartz-halogen lamps and a few LEDs emit some radiation in the UV-and short wavelength visible band (380-410 nm). Chadwick *et al.,*(1994) assessed the level of UVA (340- 400 nm) emitted from three previously used halogen sources and the level of protection afforded by six brands of surgical gloves. It was concluded that the risk of initiating adverse dermatological consequences such as photosensitisation as a result of exposure to relatively low irradiance of UVA, is minimal in normal usage. Furthermore, glove material absorption of UVA has been reported to be up to a third lower than reported in the Chadwick-study (Lehtinen *et al.,* 1990). However, some LED lamps on the market today emit up to 1100 times higher irradiance in the UVA, which implies that the risk for photosensitisation of skin, due to the combined effect of curing lamp emission and chemicals, has increased during the last 10 years. Nevertheless, it should be kept in mind that UV (bandwidth 100–400 nm, encompassing UVC, UVB and UVA) is classified as carcinogenic to humans (International Agency for Research on Cancer (IARC)).

Dental light curing units with emission mainly in the visible spectrum, but also with a fraction of UVA (380-580 nm; unknown irradiance) have been shown to cause the disappearance of Langerhans cells (antigen presenting cells of the skin) 3 days after exposure in a model of human skin heterotransplanted into nude mice (Bonding *et al.,* 1987). Several studies have shown that UV radiation on skin has immunosuppressive effects, in particular wavelengths shorter than about 320 nm (reviewed by Schwartz, 2008). The suppression is primarily affecting the adaptive immune response due to an impairment of antigen presenting cells and an emergence of T regulatory cells (Duthie *et al.,* 1999). The innate immune response may in contrast be enhanced, explaining why solar exposure does not favour bacterial infections in general (Liu *et al.,* 2006).

There does not seem to be any scientific studies on the possibility of adverse reactions other than the thermal mentioned above in the oral mucosa after exposure to high intensity visible blue light.

Light as a cofactor in photobiological reactions

Most manufacturers state in the instructions for use that dental curing lights should not be used in patients with light sensitivity diseases such as urticaria solaris or porphyrias – or who are currently on photosensitising medication. Examples of such drugs are found in the groups of NSAIDS, antidepressants, antipsoriatics and antibiotics (tetracyclines) (Kleinman *et al.,* 2010; deLeo 2000). Some photosensitising drugs can accumulate in skin, nails, teeth and ocular tissue. Photosensitising reactions, i.e. phototoxic and photoallergic reactions due to the absorption of UV or light by absorbing molecules, chromophores, with subsequent production of reactive oxygen species (ROS), radicals and other toxic photoproducts constitute a potential risk with the use of light sources in dentistry. Exogenous chromophores are, for example, the above-mentioned drugs, edibles and dental material components. Endogenous chromophores are for example DNA, porphyrins, flavins, haemoglobin and bilirubin. An example of combined chemical substance and light effect (no phototsensitisation) was shown *in vitro*: the depletion of glutathione (GSH) by methacrylates led to increased cytotoxicity following UVA/blue light irradiation with the formation of ROS (Christensen and Bruzell, 2010). Although the dose from the high intensity lamps are in the same range of what is used for dermatological skin testing of photobiological reactions, phototoxic or photoallergic reactions have not been documented in the context of oral medicine. This may partly be explained by the fact that the diagnoses of photoallergic/-toxic reactions are difficult to distinguish from other allergic reactions as the manifestations are similar. Furthermore, tissue reactions experienced by a patient after a dental treatment will easily be associated with any material used. The EU-directive (2006) on safety regarding occupational exposure to artificial optical sources includes photosensitising reactions as a risk factor, and the dental curing lamp is encompassed by this directive. The possibility of photo-related reactions should be taken into account in the evaluation of dermatological conditions in dental personnel.

Exposure of teeth

The curing lamps with high irradiance may cause local heating. Laboratory studies show temperature rises, at 3 mm distance from the light source, from 4.1°C to 12.9°C (~300 mW/cm^2), and from 17.4°C to 46.4°C (~11000 mW/cm^2) for LED and halogen lamps, respectively (Yap and Soh, 2003). Furthermore, a LED with irradiance of 1100 mW/cm^2 caused a pulpal temperature increase of 6 °C after 10 s (Durey *et al.,* 2008). In vitro studies with thermocouples placed in pulp chambers of extracted teeth showed a moderate rise in pulpal temperature. In a vital tooth this does not seem to be a problem, possibly due to the heat convection effect of the blood circulation. In subjects with impaired blood circulation and with many restorations or carious teeth, temperature increases may be higher. The recent introduction of LEDs with irradiance of more than ca. 1500 mW/cm^2 might increase the risk of thermal damage to the pulp.

Temperature rise

For high irradiance light curing units (e.g. > 3000 mW/cm^2 as presented in Rueggeberg, 2010) temperature raise in the pulp chamber with 0.75 to 1 mm residual dentin thickness was over the critical value of 5 to 6 °C (Rueggeberg, 2010). As dentin is known to be a good thermal isolator, generally heat damage to the pulp in shallow and medium depth cavities is not expected. However, heat damage on the pulp in cavities closer to the pulp or with pulp exposure is to be expected. In addition, this may also occur with lower irradiance but prolonged curing times.

Furthermore, if the treatment is performed under local anaesthesia with vasoconstrictors, blood circulation is reduced and heat removal from the pulp is impaired (Jandt and Mills, 2013). If inadvertently, the light source is directed to the soft tissues, like the lips, severe burning has been described with rubber dam offering no protection (Spranley *et al.,* 2012). On

the other side, insufficient curing, which is observed in daily practice (Price, 2013), may increase the release of substances and increase its toxicity (Sigusch *et al.,* 2009).

Electromagnetic compatibility

Although a report exists of headache associated with curing light exposure in a Parkinson's patient with implanted brain stimulator electrodes (Vangstein, 2003), two studies of possible electrical or electromagnetical interference of implants with dental curing lamps concluded that no significant effects on the equipment were found (Miller *et al.,* 1998; Roberts, 2002). However, a battery-operated LED curing lamp was found to interfere with the sensing and pacing activity of pacemakers and implantable cardioverter-defibrillator devices (Roedig *et al.,* 2010).

Ineffective treatment/inferior quality of restoration

Inferior curing caused by e.g. cracks or material build-up on the light guide will increase the amount of monomers and may lead to increased risk of toxicity. Incorrect positioning of the lamp, such as too large a distance between the light and the material to be cured, may cause less than optimal curing and overexposure of oral tissues (Price *et al.,* 2014). Many dental curing lights have an integrated photometer to check that the irradiance is sufficient for the intended use. Alternatively, a separate photometer or a more advanced spectrophotometer or radiometer can be used. When performing irradiation measurements it is important that the equipment used is intended to measure the wavelength range and the irradiance emitted from the lamp in question. Equipment used to measure halogen lamps 15 years ago is not necessarily intended for today's LEDs. It is also recommended to check that the depth of cure for the various composites is sufficient. The latter method checks both the quality of the light source and the quality of the composite material. This is an important aspect, since the resin-based materials have a limited shelf life. Some polymer composite materials contain photoinitiators with absorption peaks in the range 390-410 nm, and thus require radiation of lower wavelengths than does camphorquinone for polymerisation to take place.

Overall risk assessment of light curing units

There are inherent problems in the assessment of adverse effects of light exposure from dental curing lamps. Spectral characteristics vary among the different products, tissues treat radiation differently and the repair mechanisms for photo-induced damage may be insufficiently developed in oral mucosa.

The dental curing lights, when used according to the manufacturer's instructions and with proper eye protection, seem to be safe for use in most patients and users. However, the potential for adverse reactions to occur are definitely present and the manufacturer's cautionary statements about not using them in specific situations should be heeded (Bruzell Roll *et al.,*2004).

3.4.8.2. Glove use

The wearing of gloves, often of latex, but increasingly of non-latex alternatives, has become routine in the everyday dental practice. Although not advised, should alternative resin-based filling materials be handled during use, low molecular weight components may quickly pass through the glove (Jensen *et al.,* 1991; Munksgaard, 1992) and will remain in contact with the moist skin of the clinician until the gloves are removed and the hands washed at the end of the treatment. With practitioners who are sensitive to such constituents, or in the presence of skin conditions, cuts or abrasions, an adverse reaction may occur. Such reactions may be avoided by strict adherence to the no-touch techniques recommended by manufacturers of alternative restorative materials.

3.4.9. General Observations on Efficacy of Alternatives

The general observations on the efficacy of amalgam restorations (Sections 3.3.9 and 3.3.10) may be reinforced here. Alternatives to amalgam have been in clinical use for well over 30 years. They have not only addressed the issues on the aesthetics of amalgams but have facilitated a radical change in the concepts of restorative dentistry through the introduction of more minimally invasive techniques and the associated retention of more tooth substance when treating caries. This has been achieved through the use of tooth coloured materials that are themselves adhesive to tooth substances or that can achieve adhesion through the use of intermediary agents. It is recognised that their use is technique sensitive and that the procedures for their placement take longer and therefore be more expensive. It is also true that they may be more susceptible to secondary caries and, in some situations, have less longevity than amalgams (for references see sections 3.3.9 and 3.3.10). In general therefore, these tooth-coloured alternatives offer an effective modality for the treatment of dental caries in many situations.

Non-amalgam alloys and – more recently – ceramics have also been used as amalgam alternatives, although the costs involved are considerably higher because the restoration must be separately fabricated and then luted to the tooth (indirect restoration). Survival rates of such restorations are high (Felden *et al.,* 1998; Krämer *et al.,* 2008; Federlin *et al.,* 2010); however, due to the specific requirements of the technique, more sound tooth tissue has to be removed to fit such a restoration than is the case with a direct restoration using amalgam or resin-based composites.

Used as inlay/onlay more tooth substance is replaced but in the case of overlays of partial crowns the preparation should be minimal and the longevity is rather good with AFR of 2% (van Dijken and Hasselrot, 2010).

3.4.10. Conclusions on Alternatives

Alternatives to amalgam comprise a large variety of materials based on mainly acrylic resin technology, cements, ceramics or dental alloys. The materials used as alternatives to dental amalgam for direct restorations (so-called resin-based composites or resin composites) are usually chemically very complex, with certain clinical limitations or may present some toxicological risks. They frequently contain a variety of organic substances, for which toxicological data are scarce or even missing and they may undergo chemical reactions within the tooth cavity and adjacent soft tissues during placement releasing newly formed substances like formaldehyde. Therefore, it should not be assumed that non-mercury containing alternatives are free from any concerns about adverse effects (Goldberg, 2007).

The amount of the released substances from resin composites and related materials depend on the degree of conversion. During application, the low viscous dental adhesives in non-polymerised state will in many cases be in direct contact with the oral tissues which makes penetration of the tissues possible and has potential biological risks.

With respect to those materials that incorporate polymerisable resins, it is known that some of the monomers involved in their intra-oral placement and polymerisation are highly cytotoxic to pulp and gingival cells *in vitro* and there is also evidence that some of them are mutagenic, although it is far from clear whether this has any clinical significance. Some of these substances are irritants when used by themselves in various situations and the occupational risks associated with their use are similar to those found in the printing and automotive industries. Allergies to these substances have been reported, both in patients and in dental personnel. We note that the full chemical specification of these alternative restorative materials is not always divulged and it may be difficult to ascertain exactly what they contain. In the absence of data, it may not be possible to provide a scientifically sound statement on the safety of individual products. It is also noted, however, that there are very limited scientific data available concerning exposure of patients and dental personnel to these substances.

Nevertheless, these alternative materials have now been in clinical use for well over thirty years, and this use has revealed little evidence of clinically significant adverse events. The commercially available materials have either changed substantially or been improved considerably during this time, with reduced bioavailability of harmful components through improved polymerisation processes. It is recognised that many of the new forms of these alternative materials lack long-term clinical data and as such, need to be monitored for possible risks to patients and dental personnel.

As a separate issue, it should be borne in mind that these photo-polymerisable systems require activation and that the powerful light sources now used for this purpose may constitute an additional risk for adverse effects, both to patients and dental personnel. Eye protection is extremely important.

As for amalgam, genetic predisposition may play a role for the occurrence of adverse reactions towards alternative materials. It is known that the catalase system is necessary for compensating the increase of cellular reactive oxygen species, which takes place after dental methacrylate monomer exposure (Krifka *et al.,* 2013 and 2012). Several common mutations of the catalase gene (CAT) are known (see above); however, as for amalgam, the clinical impact for alternative materials is unclear. Furthermore, it was reported that glutathione (GSH) plays an important role in the detoxification of dental methacrylate monomers: toxicity of these monomers can be increased by GSH inhibition and decreased by the addition of N-acetyl-cysteine (NAC), a precursor of GSH (Krifka *et al.,* 2012; Stanislawski *et al.,* 2003). Vulnerable individuals and subpopulations with a genetic predisposition, e.g. of a glutamyl-cysteine ligase GCLM-588T allele with a reduced glutathione production (Goodrich *et al.,* 2011; Custodio *et al.,* 2005), may also exist for dental methacrylate monomers. The influence of different variants of glutathione transferase on the cellular reactions towards resin monomers was shown (Lefeuvre *et al.,* 2004). However, clinical data are missing and more research is warranted.

Indirect restorations used as amalgam alternatives have a good survival rate, but the involved costs are considerably higher than with direct restorations and more sound tooth tissue has to be removed in order to place such a restoration in a tooth. Furthermore, metals used in these alloys are not without biological risk and ceramic restorations have to be luted in many cases with resin-based composite materials and thus the same biological problems occur as with such direct fillings.

In a recent Cochrane systematic review on the comparative longevitiy of resin-based composites and amalgams it is stated that the parallel group trials indicated that resin restorations had a significantly higher risk of failure than amalgam restorations and increased risk of secondary caries. The results from the split-mouth trials were consistent with those of the parallel group trials. More data with higher levels of evidence are warranted.

3.4.11. Comments on costs

Generally, costs for restorative treatment are based on the costs of the materials and the time needed to perform the work within the given environment. Furthermore, the longevity of a restoration influences the costs by higher replacement rates. There is general agreement in the literature that the treatment costs for amalgam fillings are lower than for resin composite restorations. The latter were rated 1.7 to 3.5 times more expensive than amalgam for a one tooth year restoration (Chadwick *et al.,* 1999). Other estimates amounted to initial costs for resin composite fillings to be 25% higher, cost per year of function to be 2.5 times higher than for amalgam (Sjögren & Halling, 2002). In a recently published report from Norway (Skjelvik and Schou Grytli, 2012) a price increase for a resin composite filling compared to an amalgam filling in the range of € 48 to 72€ was reported, which means an increase of 33 and 50 percent. However, for amalgam fillings additional costs should be considered; e.g. for amalgam waste/separator management and for cremation. In the above-mentioned Norwegian report, such costs have been estimated to be about 1 to 2 € per amalgam filling for waste/separator costs. However, such costs are varying, e.g. according to the price of the

recycled metals; presently, e.g. in Germany, recycling companies even pay (a small amount) for amalgam waste. Another problem is related to cremation, by which mercury from amalgam fillings is released into the environment. Installation of additional filters for mercury and maintaining them may add up to 18€ per cremation with an assumed 5 fillings per cremation (Skjelvik and Schou Grytli, 2012). It can be concluded that even taking the more indirect costs for amalgam into consideration, the costs for treatment of cavities with resin composites will increase the costs compared to amalgam fillings.

4. OPINION

The cited scientific evidence constitutes an update of the 2008 scientific Opinion concerning the safety of dental amalgam and alternative dental restorative materials. It evaluates new information and also some scientific articles that were not included in that version. The Opinion provides answers to the questions posed in the mandate.

4.1. The scientific and clinical evidence

The SCENIHR recognises that dental amalgam, for the general population, is an effective restorative material. From the perspectives of longevity, the mechanical performance and economics, it has long been considered and still is a material of choice, especially for certain types of restorations in posterior teeth, including replacement therapy for existing amalgam fillings. However, because dental amalgam is neither tooth-coloured nor adhesive to remaining tooth tissues, its use has been decreasing in recent years and the alternative tooth-coloured filling materials have become increasingly more popular. This is consistent with the trend towards minimal interventional, adhesive, techniques in dentistry. At the same time the quality and durability of these materials have improved. This trend towards non-amalgam restorations is emphasised by the significant reduction of training in the placement of dental amalgam restorations, and the corresponding increase in training in the use of amalgam alternatives in many dental schools in European countries.

Mercury is the metallic element of concern used in dental amalgam. Mercury is a well-recognised toxicological risk, with reasonably well-defined characteristics for the major forms of exposure such as ingestion of organic and inorganic mercury compounds and inhalation, of elemental mercury vapour. Respiratory air concentrations, blood levels and urinary excretion of mercury in individuals with amalgam fillings indicate that the levels of exposure encountered are 5 to 30 times lower than those permitted for occupational exposure. Tolerable limits for dietary exposures to mercury are relevant to amalgam safety considerations, as inhaled elemental mercury may add to the body burden of inorganic mercury. Dietary mercury exposure in the general population in Europe does not exceed the TWI for methyl mercury and inorganic mercury, except in heavy fish-consumers. EFSA (2012) reported that the tolerable weekly intake for inorganic mercury might be exceeded due to the additional inhalation exposure in people with a high number of amalgam fillings. However, evidence is weak as the data are mainly derived from model-based calculations. Studies on large patient collectives did not show any correlation of health effects with the number of amalgam restorations.

Local adverse effects in the oral cavity are occasionally seen with dental amalgam fillings, including allergic reactions and an association with clinical features characteristic of lichen planus, but the incidence is low (<0.3% for dental materials in general) and usually readily managed. Regarding systemic effects, elemental mercury is a well-documented neurotoxicant, especially during early brain development, and inorganic mercury also constitutes a hazard to kidney function. The presence of dental amalgam has been suggested to be associated with a variety of systemic conditions, particularly neurological and psychological/psychiatric diagnoses, including Alzheimer's disease, Parkinson's disease, and multiple sclerosis as well as kidney disease. These possible risks are not substantiated. However, recent studies suggest that the genetic make-up may be the cause of a higher mercury internal dose for some individuals, possibly making them more vulnerable to mercury toxicity than the average.

Mercury concentration in the adult brain is associated with the number of amalgam fillings. In the foetus mercury concentration in the foetal kidney but not the brain showed a trend associated with the mothers'number of amalgam fillings. Because the elimination half-life for inorganic mercury in the brain estimated by means of a PB-PK model exceeds 10 years, mercury is likely to accumulate in the central nervous system.

The accumulated concentrations in brain tissue may reach values that are similar to those inducing neurochemical changes in *in vitro* experimental models. Such effects have not been

convincingly demonstrated in humans and so far, studies in children of school age did not convincly demonstrate amalgam-associated neuropsychological deficits. However, recent studies suggest that genetic polymorphisms concerning mercury kinetics may influence the degree of individual susceptibility with regard to mercury internal exposure and consequently toxicity. This may raise some concern for possible effects on the brain of mercury originating from dental amalgam. However, so far such effects have not been documented in humans, although some evidence on alteration of mercury dynamics have been reported. The transient mercury release during placement and removal will result in transient exposure to the patients (resulting in a transient increase in plasma mercury levels) and also to the dental personnel. Therefore there is no general justification for removing clinically satisfactory amalgam restorations, except in those patients diagnosed of having allergic reactions to one of the amalgam constituents. Recent studies do not indicate that dental personnel in general, despite somewhat higher exposures than patients, suffer from adverse effects that could be attributed to mercury exposure due to dental amalgam. Exposure of both patients and dental personnel can be minimised by the use of appropriate clinical techniques.

The alternative materials also have certain clinical limitations and toxicological risks. They contain a variety of organic substances and may undergo chemical reactions within the tooth cavity and adjacent soft tissues during placement. Therefore, it should not be assumed that non-mercury containing alternatives are free from any concerns about adverse effects. With respect to dental composite restorative materials and hybrid systems that incorporate polymerisable resins, it is known that some of the monomers used are highly cytotoxic to pulp and gingival cells in vitro. There is also evidence that some of these are mutagenic *in vitro* although it is far from clear whether this has any clinical significance. Allergies to some of these substances have been reported, both in patients and in dental personnel.

It is noted that there are very limited scientific data available concerning exposure of patients and dental personnel to the substances that are used in alternative restorative materials. It is recognised that such data are very difficult to obtain. Further toxicological research on the various components of these alternative dental materials is warranted.

Alternative materials have now been in clinical use for more than thirty years, initially in anterior teeth and later also for restorations in posterior teeth. This clinical use has revealed little evidence of clinically significant adverse events. However, there is an increase in patients' claims with increasing use of these materials. It is also important to note that the commercially available materials have changed substantially and improved considerably over this time, especially concerning their physical and mechanical properties and their adhesion to dental hard tissues.

Resin-based composites contain a large variety of organic substances, for which toxicological data are scarce or missing and available information on the composition and on leachables of these materials is inadequate. Leaching occurs directly after curing from remaining un-reacted groups in the body of the restoration and in the non-polymerised surface layer of the restoration exposed to oxygen during curing. Leachable components may also be released due to degradation or erosion over time, the leaching process being determined not only by the degradation process itself but also by diffusivity through the material. Chemical degradation is caused by hydrolysis or enzymatic catalysis. Other degradation factors are thermal, mechanical and photochemical. Unreacted monomers, catalysts, formaldehyde and – in some cases – bisphenol A are released. Dental alloys continuously release metals into the oral environment depending e.g. on the metal content, the phase distribution within the alloy and the corrosion conditions. Metals like gold, copper, silver and palladium are released but also nickel, zinc, cobalt and chromium and many others. Glass ionomer cements release fluorides and calcium, sodium, silicon, strontium, and aluminium. Ceramics release substances like silicon, boron, sodium, potassium, and aluminium, some brands also release lithium in small amounts.

The cytotoxicity and genotoxicity of substances leached from resin-based materials, of metallic elements from alloys and of glass ionomer cements have been the subject of extensive studies using cell culture techniques and bacterial mutation tests. Some of the released substances, especially from resin-based composites and from alloys, are highly cytotoxic to pulp and gingival cells *in vitro* and there is also evidence that some of the released monomers are

mutagenic, although it is unclear whether this has clinical significance. Studies on the intracellular biochemical mechanisms have clarified various effects such as cell membrane damage, inhibition of enzyme activities, protein or nucleic acid synthesis, increase of radical oxygen species concentration, etc. The risk associated with the release of Bisphenol A from resin dental materials was recently evaluated and considered to be negligible. (SCENIHR 2015). Substances from resin materials such as TEGDMA and HEMA, but also metals from alloys like nickel, cobalt and palladium, cause allergies in patients and dental personnel. Recently, increased attention has been directed to the possibility of photo-related reactions and to the effect of high energy light curing units. Specific safety precautions are necessary to prevent eye damage of patients and dental personnel (by proper eye protection) and heat related effects (burning of the gingiva or the dental pulp). Photo-related reactions should be taken into account in evaluation of dermatological conditions in patients and dental personnel.

The SCENIHR notes that the full chemical specification of these alternative restorative materials is not always divulged, and it may be difficult to know exactly what they contain. As a result, there is limited toxicological data publicly available for these materials. Dental restorative materials are defined as medical devices according to EU-Directive 93/42/EEC and belong to class IIa. Consequently, the certification process does not include review of the design dossier and, therefore, the chemical specification does not have to be revealed to the third party. Although manufacturers are obliged to assess biocompatibility and the risk from unintended side effects, accessible information on the toxicity of the constituents of the materials as well as relevant exposure data is lacking. Therefore, the SCENIHR notes that it is not possible to provide a scientifically sound statement on the generic safety of these materials.

It is noted that there are very limited scientific data available concerning exposure of patients and dental personnel to substances that are used in alternative restorative materials. Many of the monomers and other organic solvents used in them are volatile and need to be better identified and quantified.

More publically available research data are also needed to have a broader basis for risk evaluation. In view of the controversial nature of this subject, it would also be beneficial for the community in general to be better informed of the recognised benefits and risks.

In light of the above comments we conclude that dental amalgam already in place is not considered a health risk for the general population. Thus, pre-existing amalgam restorations should not be removed, as this intervention would result in a greater exposure to mercury. As with any other medical or pharmaceutical intervention, caution should be exercised when considering the (re-)placement of any dental restorative material in pregnant women. There is no evidence that infants or children are at risk of adverse effects arising from the use of alternatives to dental amalgam. As far as dental personnel are concerned, it is recognised that they may be at greater risk with respect to mercury exposure than the general population, although the incidence of reported adverse effects is very low.

Far less information is available concerning exposure, toxicity and clinical outcomes for alternative materials. There is some evidence that certain low molecular weight substances used in their preparation are associated with local allergic reactions, although the incidence is very low. There is no evidence that there is any association between these materials, as used clinically, and any neurological disorders or any other health disorders. We do emphasise, however, that data is sparse and the continuing evolution of these materials suggests that caution should be exercised before new variations are introduced into the market. As far as dental personnel are concerned, again there is evidence of limited numbers of cases of allergies to these materials. The pervasiveness of some of the volatile low molecular weight species throughout dental clinics should be noted.

The SCENIHR concludes that dental health can be adequately ensured by alternative types of restorative material. Furthermore, the use of resin-based alternatives allows the use of minimally interventional adhesive techniques. The longevity of restorations of resin-based alternative materials in posterior teeth has improved with the continuing development of these materials and the practitioner's familiarity with effective replacement techniques. However, in certain clinical situations (e.g. large cavities and high caries rates), the alternative materials

are still inferior to amalgam. The clinical trend towards the use of adhesive alternatives implies that a sustained reduction in the use of dental amalgams in clinical practice will continue across the European Union.

As a separate issue, it should be borne in mind that these photo-polymerisable systems require activation and that the powerful light sources now used for this purpose may constitute an additional risk for adverse effects, both to patients and dental personnel. Eye protection is extremely important.

The SCENIHR noted that indirect restorative techniques, involving the use of variety of different alloys and ceramics may also be used when direct restorations are contra-indicated. Their use, which is both time-consuming and expensive, has remained at a comparatively low level in recent years. This use is not seen as a health concern with the exeption of allergies to some metals. As a general principle, the relative risks and benefits of using dental amalgam or the various alternatives should be explained to patients to assist them to make informed decisions. This has implications concerning the provision of improved product information from the manufacturers.

The SCENIHR concludes that dental restorative treatment can be adequately ensured by amalgam and alternative types of restorative material. The longevity of restorations of alternative materials in posterior teeth has improved with the continuing development of these materials and the practitioner's familiarity with effective placement techniques, but is in certain clinical situations (e.g. large cavities and high caries rates) still inferior to amalgam.

The choice of material should be based on patient characteristics such as primary or permanent teeth, pregnancy, presence of allergies to mercury or other components of the restorative materials, and presence of decreased renal clearance. The clinical trend towards the use of adhesive alternatives is considered advantageous as it implies that a sustained reduction in the use of dental amalgam in clinical practice will continue across the European Union.

The SCENIHR recognises a lack of knowledge and a need for further research, in particular in regard to genetic susceptibility related to mercury effects and to the constituents of alternative restorative materials. Furthermore, there is a need for the development of new alternative materials with a high degree of biocompatibility.

4.2. Answers to Terms of reference

In particular, the SCENIHR is asked the following questions.

4.2.1. Question 1

Is there any new scientific evidence that justify reasons for concern from the health point of view in the use of dental amalgam as dental restoration material?

A variety of systemic adverse effects, particularly developmental neurotoxicity as well as neurological and psychological or psychiatric diseases, have been suggested to be associated with the presence of dental amalgam. The causality evidence for such effects due to dental amalgam is weak, also considering other source of mercury exposure. A recent study (Sherman *et al.,*2013) indicates that demethylation of methyl mercury from seafood gave a major contribution to the mercuric mercury in the urine with fewer than 10 amalgam fillings.

The most recent *in vitro* evidence provides new insight into the effects of mercury on developing neural brain cells at concentrations similar to those found accumulated in human brain in post-mortem specimen.

Neurological effects associated to dental amalgam have not been convincingly demonstrated in humans as caused by dental amalgam. The effects of genetic polymorphism concerning mercury kinetics may influence the degree of individual susceptibility in regard to mercury internal exposure and consequently toxicity. Some evidence on alteration of Hg dynamics have been also reported. They may raise some concern, although so far such effects have not been clearly demonstrated in humans.

4.2.2. Question 2

In view of the above, is the use of dental amalgam safe for patients and users, i.e. dental health professionals? Are certain populations particularly at risk, e.g. pregnant women or children? Is it possible to recommend certain practices to minimize patient's and user's exposure to dental amalgam?

The current evidence does not preclude the use of dental amalgam in restorative treatment in the general population. The SCENIHR recognises that dental amalgam is an effective restorative material for the general population, with low risk of adverse health effects.

The choice of material should be based on patient characteristics. The use of amalgam restorations is not indicated in primary teeth, in patients with mercury allergies, and persons with chronic kidney diseases with decreased renal clearance. As with any other medical or pharmaceutical intervention, caution should be taken when considering the placement of any dental restorative material in pregnant women. A decision to perform dental treatment during pregnancy should take into account the dental therapeutic needs of the patient and balance any potential risks (including the use of anaesthetics, along with all dental materials) against therapeutic benefits to the patient. Generally, extensive dental treatment during pregnancy is discouraged.

Placement and removal results in short-time exposure to the patients compared to leaving the amalgam intact. Therefore there is no general justification for unnecessarily removing clinically satisfactory amalgam restorations, except in those patients diagnosed as having allergic reactions to one of the amalgam constituents.

Recent studies do not indicate that dental personnel, despite somewhat higher exposures than general population as mercury in the urine, suffer from adverse effects that could be attributed to mercury exposure due to dental amalgam. In a recent study in Canada, it was observed that mercury vapour exposure during dental training on amalgam removal remained below occupational exposure limits (Warwick *et al.,* 2013).

The mercury release during placement and removal results in exposure of dental personnel. Exposure of both patients and dental personnel could be minimised by the use of appropriate clinical techniques.

Genetic polymorphisms involved in alteration of mercury kinetics and dynamics may raise some concern for vulnerable groups, although so far such effects have not been clearly demonstrated in humans.

To reduce the use of mercury-added products in line with the intentions of the Minamata Convention (reduction of mercury in the environment) and under the above mentioned precautions, it can be recommended that for the first treatment of primary teeth in children and for pregnant patients, alternative materials to amalgam should be the first choice. This decision should be made after informed consent from the patient or the legal guardians.

4.2.3. Question 3

Is there new scientific evidence on the safety and performance of alternative materials?

Alternatives to amalgam comprise a large variety of materials based mainly on acrylic resin technology, cements, ceramics or dental alloys. Except for certain metals such as gold, there are no relevant markers for assessing patient- or user exposure to the alternative materials.

Ceramics have to be luted to the dental hard tissues usually using acrylic technology products. Resin materials have to be cured mainly using light curing units. Resin-based materials achieve adhesion to tooth substances through the use of intermediary agents containing highly reactive chemicals. Their use is still technique sensitive and the procedures for their placement takes more time than for amalgam.

The data base required for safety evaluation of alternative materials is still inadequate and less complete than for amalgam. Many of the new alternative materials lack long-term clinical data. There are very limited scientific data available concerning identification and quantification of the exposure of patients and dental personnel to released substances from these materials. Further toxicological research on the various components of these alternative dental materials is warranted.

The SCENIHR notes that alternative materials are chemically very complex and also have clinical limitations and represent toxicological risks. They contain a variety of substances including organic solvents and undergo chemical reactions within the tooth cavity and adjacent soft tissues during placement. . The SCENIHR Opinion "The safety of the use of bisphenol A in medical devices" (2015) concluded that release of BPA from some dental materials was associated with negligible health risks. Non-mercury containing alternatives are not free from concerns about adverse effects. With respect to resin composite restorative materials and hybrid systems that incorporate polymerisable resins, there is *in vitro* evidence that some of the monomers used are highly cytotoxic to pulp and gingival cells. There is also *in vitro* evidence that some monomers are mutagenic although it not known whether this has any clinical significance. Allergic reactions to some of these substances have been reported, both in patients and in dental personnel. Similar to treatment with dental amalgam, the use of these materials in pregnant women is discouraged.

Studies comparing amalgam with resin-based materials showed generally better longevity for amalgam. Alternative restorations fail, primarily through secondary caries and fracture of the restoration and tooth. However, some recent studies from the Netherlands, Sweden and Denmark showed very good long-term clinical effectiveness for posterior resin composite restorations with equal and better longevity than for amalgam. But even under optimal conditions, large composite restorations in caries risk patients failed more often than amalgam fillings.

In one study, exposure to bisGMA-based dental composite restorations was associated with impaired psychosocial function in children, whereas no adverse psychosocial outcomes were observed with either urethane dimethacrylate–based compomer or amalgam treatment levels.

The indirect restorations have a good survival rate, but require removal of some additional healthy tooth tissue. The involved costs are considerably higher than with direct restorations.

Due to reported mediocre mechanical properties and clinical failures, glass ionomer cements can only be used in small, one-surface cavities. Recently, resin-based materials with reduced cytotoxicity, e.g. the methacrylate-free siloranes, have been introduced, showing good short term clinical performance. They also show low genotoxic potential and may be suitable components for development of new biomaterials.

In conclusion, amalgam alternatives have certain clinical limitations and toxicological risks. More experimental, clinical and epidemiological research is required to ensure patient safety in the future. The development of better amalgam alternatives is still the prime aim.

4.2.4. Question 4

Is it possible to recommend alternative materials and certain practices related to these materials to reduce potential risks for patients and users?

The current evidence does not preclude the use of alternative materials in dental restorative treatment in the general population.

The choice of the restorative material for treating dental cavities depends on a large number of variables, e.g. the size of the defect, the technical circumstances for restoration placement, and individual health problems like allergies, material properties, or the available funds. Therefore, the final decision on which material should be used in the individual case can only be made in the single situation between the dentist and the patient, based on informed consent. Based on current information, dental composites do not pose unacceptable risks to pregnant patients. However, the data base is scarce. A decision to perform dental treatment during pregnancy should take into account the dental therapeutic needs of the patient and balance any potential risks (including the use of anesthetics, along with all dental materials) against therapeutic benefits to the patient. Generally, extensive dental treatment during pregnancy is discouraged.

Alternative materials may also represent some health risks, so no general recommendations on the use of alternative materials can be given. One exception is for patients with a proven allergy to one of their components, which requires more information about their constituents.

4.2.5. Question 5

In case there is not enough scientific data to answer these questions, the SCENIHR is asked to formulate recommendations for research that could help to provide the necessary data.

The SCENIHR recognises a lack of knowledge and a need of further research, in particular in regard to genetic polymorphism related both to mercury and to the constituents of alternative restorative materials. Furthermore, there is a need for the development of new alternative materials with a high degree of biocompatibility.

The ideal new material meant as a true amalgam alternative should have a similar gradient in properties from cavity floor to surface, as in a natural tooth, and be cost effective and non-toxic to human health and the environment (safe and efficacious). It would seal the interface between the tooth and the restoration against the penetration of bacteria and common ions from saliva and food, be adhesive to the tooth with little to no shrinkage, interact favourably with carious dentin and enamel (preferably with healing/demineralising properties), be clinically easy to use in a variety of settings, and be fracture- and wear-resistant and repairable.

The present report has clearly identified that in some areas there are not enough scientific data to provide firm answers to the questions formulated by the EU Commission. Therefore, the future research agenda should first of all address improvement of knowledge on toxicological profile of alternative material and the development of new materials, both organic and inorganic. Improved tools for their evaluation are also needed and both points are specified below. In addition further research on the individual susceptibility of the mercury from amalgam and on the constituents of alternatives currently in use is necessary.

However, equal or more research emphasis should be placed on the further development and implementation of new caries management concepts like early intervention and of new tools for caries prevention in risk groups. It is generally accepted that restorations do not only fail due to insufficient mechanical and biological properties, but also due to a high caries activity in some patients.

<u>Improving information for materials in use.</u>

- Studies in clinical and community practice settings for materials in use should be further supported with study designs and study reports that follow internationally recognised guidelines.
- More human and environmental safety studies including mechanistic approaches, especially for chemicals from alternative materials or for nanoparticles from restorative materials, are needed.
- Risk groups for the exposure to chemicals including genetic approaches are to be identified.

Developing new materials

- While advances in polymer sciences are being made, there is a concern that we may need to move away from Bis-GMA polymer based materials for human safety and environmental reasons.
- New organic non-acrylic materials (like the siloranes) should be refined or new materials, both organic and inorganic, should be developed.
- Biomimetic material approaches should be followed to develop materials with the ability to remineralise dental hard tissues with the aim to further increase and support the minimal invasive approach to treat carious lesions.
- New materials – as true amalgam alternatives – must aim to be easily used in a variety of clinical and community settings on primary and permanent teeth and on low and high caries risk patients.
- New materials must be tested in randomized clinical trials. In addition to patient and user safety aspects, environmental safety has to be addressed.

Developing new research tools to improve knowledge for existing and for new materials

- Laboratory tests must be developed which reliably predict clinical material performance over the lifetime of the materials and, ultimately integrated into specifications for acceptance of new materials/products.
- New clinical testing schemes should be developed, by which the long term clinical behaviour of new materials can be predicted from short-term testing.
- International networks for Centres advising patients who claim health problems from dental materials should be established.
- Close collaboration with medical disciplines (e.g. allergology) and human genetics should be further developed.
- Tools should be developed, by which the process of pre-market certification can be accelerated.

5. CALL FOR INFORMATION

A call for information was issued by the Commission on 8 August 2012 with a deadline of 10 October 2012.

In total, 68 responses were received of which 35 were from organisations, 20 from individuals and 13 concerning 1 case report. Of the organisations, 15 were non-governmental, 7 public authorities and 13 other institutions, including dental associations.

In evaluating the responses from the call, submitted material has only been considered for the update of the Opinion if

1. it is directly referring to the content of the report and relating to the issues that the report addresses,
2. it contains specific comments and suggestions on the scientific basis of the Opinion,
3. it refers to peer-reviewed literature published in English, the working language of the SCENIHR and the working group,
4. it has the potential to add to the preliminary Opinion of the SCENIHR.

Each submission which met these criteria has been carefully considered by the Working Group. Overall, many of the comments were of good quality. The scientific rationale of the report has been revised to take account of relevant comments. The literature has been updated with relevant publications.

As indicated in the Opinion, the information on adverse effects of alternatives is limited. During the call for information, some additional information became available regarding the alternative restorative materials, especially concerning the release of BPA from dental resin-based materials.

6. CONSIDERATION OF THE RESPONSES RECEIVED DURING THE CONSULTATION PROCESS

A public consultation on this Opinion was opened on the website of the Scientific Committees from 09 September to 16 November 2014. Information about the public consultation was broadly communicated to national authorities, international organisations and other stakeholders.

25 organisations and individuals participated in the public consultation providing 102 comments to different chapters and sections of the Opinion. Each submission was carefully considered by the SCENIHR and the scientific Opinion has been revised to take account of relevant comments. The literature has been accordingly updated with relevant publications. The scientific rationale and the Opinion section were clarified and strengthened.

The text of the comments received and the response provided by the SCENIHR is available here:
http://ec.europa.eu/health/scientific_committees/consultations/public_consultations/scenihr_consultation_24_en.htm

7. MINORITY OPINION

None

8. LIST OF ABBREVIATIONS

4-AETA	4-Methacryloxyethyl trimellitic anhydride
ADA	American Dental Association
ADME	Absorption, distribution, metabolism and elimination
Al2O3	Alumina glass
ALS	Amyotrophic Lateral Sclerosis
ATSDR	Agency for Toxic Substances Disease Registry
BAT	Biologischer Arbeitsplatz Toleranzwert (biological tolerance value at the workplace)
BBP	n-butyl benzyl phthalate
BHT	Butylhydroxytoluene
BDNF	Brain derived neurotrophic factor
Bis-EMA	Ethoxylated bisphenol A-methacrylate
Bis-GMA	Bisphenol A – glycidylmethacrylate
Bis-HPPP	2,2-bis[4(2,3-hydroxypropoxy)-phenyl]propane
BPA	Bisphenol A
CAT	Catalase gene
CPOX	Coproporphyrinogen oxidase
COMT	Catechol O-methyltransferase
COMET	The Single Cell Gel Electrophoresis assay
DMABEE	4-N,N-Dimethyl amino benzoic acid ethylester
DPMS	Dimercaptopropane sulfonate
EDS	Energy-dispersive X-rays spectroscopy
EFSA	European Food Safety Authority
EGDMA	Ethyleneglycoldimethacrylate
EPA	Environmental Protection Agency
DIMDI	German Institute for Medical Documentation and Information
GCLM-588T	Glutamyl-cysteine ligase allele
GSTs	Glutathione S-transferases
GSH	Glutathione
HDDMA	Hexanediol dimethacrylate
HEMA	Hydroxyethylmethacrylate
Hg	Mercury
HMBP	2-Hydroxy-4-methoxybenzophenone
IARC	International Agency for Research on Cancer
ICD	International Classification of Diseases
IEC	International Electrotechnical Commission

IRIS	Integrated Risk Information System
IR	Infrared
ISO	International Standards Organisation
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
LED	Light-emitting diode
MAK	Maximale Arbeitsplatz Konzentration (maximum concentration at the workplace)
MAF	Minor allele frequency
MBRN	Medical Birth Registry of Norway
MeHg	Methylmercury
MSDS	Material safety data sheets
MT	Metallothioneins
MT1M	Metallothionein mutant
α1-MG	Alpha 1 microglobulin
MMA	Methylmethacrylate
MRL	Minimal Risk Level
MS	Multiple Sclerosis
NAC	N-acetylcysteine
NAG	N-acetyl-β-D-glucosaminidase
NIOSH	National Institute for Occupational Safety and Health
NOAEL	No Observable Adverse Effect Level
NOAEC	No Observed Adverse Effect Concentration
NSAIDS	Nonsteroidal anti-inflammatory drugs
OES	Occupational Exposure Standard
8-OHdG	8-hydroxy-2-deoxyguanosine
OLP	Oral Lichen Planus
PAC	Xenon-plasma arcs
PBPK	Physiologically based pharmacokinetic modeling
PTWI	Provisional Tolerable Weekly Intake
RNA	Ribonucleic acid
ROS	Reactive oxygen species
QTH	Quartz – tungsten – halogen
QSAR	Quantitative Structure-Activity Relationship
SCENIHR	Scientific Committee on Emerging and Newly Identified Health Risks
SCHER	Scientific Committee on Health and Environmental Risks
SEA	Self-etching adhesives
SiO_2	Silica glass
SNPs	Single nucleotide polymorphisms
5-HTTLPR	Serotonin transporter gene promoter region

TCB	Reaction product of butane tetracarboxylic acid and hydroxyethylmethacrylate,
TEGDMA	Triethyleneglycoldimethacrylate
TPO	Trimethylbenzoyl-diphenyl-phosphine oxide
TWI	Tolerable weekly intake
UBA	Umweltbundesamt (German Federal Environment Agency)
UDMA	Urethane dimethacrylate
UNEP	United Nations Environment Programme
UV	Ultraviolet
WHO	World Health Organisation
YF_3	Ytterbium fluoride

9. REFERENCES

Aalto-Korte K, Alanko K, Kuuliala O, Jolanki R, Methacrylate and acrylate allergy in dental personnel. Contact Dermatitis. 2007 Nov;57(5):324-30.

Ahlgren C, Ahnlide I, Björkner B, Bruze M, Liedholm R, Möller H, Nilner K., Contact allergy to gold – correlation with dental gold, Acta Dermat Venerol 2002; 82:41-4.

Ahlgren C, Axéll T, Möller H, Isaksson M, Liedholm R, Bruze M. Contact allergies to potential allergens in patients with oral lichen lesions. Clin Oral Investig. 2014;18(1):227-37.

Ahlgren C, Bruze M, Moller H, Gruvberger B, Axell T, Liedholm R, Nilner K., Contact allergy to gold in patients with oral lichen lesions. Acta Derm Venereol 2012; 92(2):138–143.

Ahlgren C, Molin M, Lundh T, Nilner K. Levels of gold in plasma after dental gold insertion. Acta Odont Scand 2007; 65(6):331-4.

Ahlqwist M, Bengtsson C, Furunes B, Hollender L, Lapidus L. , Number of amalgam tooth fillings in relation to subjectively experienced symptoms in a study of swedish women. Community Dent Oral Epidemiol 1988; 16:227-231.

Ahlqwist M, Bengtsson C, Lapidus L., Number of amalgam fillings in relation to cardiovascular disease, diabetes, cancer and early death in swedish women. Community Dent Oral Epidemiol 1993; 21:40-44.

Alanko K, Susitaival P, Jolanki R, Kanerva L. Occupational skin diseases among dental nurses. Contact Dermatitis 2004; 50:77-82.

Al-Hiyasat AS, Darmani H, Milhem MM. Cytotoxicity evaluation of dental resin composites and their flowable derivatives. Clinical Oral Inv 2005; 9:21-25.

Alptekin T, Ozer F, Unlu N, Cobanoglu N, Blatz MB., In vivo and in vitro evaluations of microleakage around Class I amalgam and composite restorations. Oper Dent 2010; 35(6): 641-648.

Al-Saleh I, Al-Sedairi AA, Elkhatib R. Effect of mercury (Hg) dental amalgam fillings on renal and oxidative stress biomarkers in children. Sci Total Environ. 2012 Aug 1;431:188-96

Al-Saleh I, Al-Sedairi AA. Mercury (Hg) burden in children: the impact of dental amalgam. Sci Total Environ. 2011 Jul 15;409(16):3003-15.

Altmann L, Sveinsson K, Krämer U, Weishoff-Houben M, Turfeld M, Winneke G, Wiegand H. Visual functions in 6-year-old children in relation to lead and mercury levels. Neurotoxicol Teratol. 1998; 20(1):9-17.

American Dental Association., Determination of Bisphenol A Released from Resin-Based Dental Composite Restoratives. ADA Professional Product Review 2014; 9(3).

American Dental Association Council on Scientific Affairs (DA). Dental mercury hygiene recommendations. J Am Dent Assoc 2003; 134:1498-9.

Aminzadeh KK, Etminan M. Dental amalgam and multiple sclerosis: a systematic review and meta-analysis. J Publ Health Dent 2007; 67(1):64-66.

Andreasson H, Örtengren U, Barregård L, Karlsson S. Work-related skin and airway symptoms among Swedish dentists rarely cause sick leave or change of professional career. Acta Odontol Scand 2001; 59: 267-72.

Antony K, Genser D, Hiebinger C, Windisch F., Longevity of dental amalgam in comparison to composite materials. GMS Health Technol Assess. 2008 Nov 13;4:Doc12.

Anusavice, K.J., Zhang, N.Z.: Chemical durability of Dicor and lithia-based glass-ceramics. Dent Mater 1997, 13, 13–19 .

Arenholt-Bindslev D, Kanerva L. Die Diagnose von Nebenwirkungen. In: Schmalz G, Arenholt-Bindslev D, editors. Biokompatibilität zahnärztlicher Werkstoffe. München: Elsevier GmbH; 2005. p. 337-68.

Arenholt-Bindslev D., Breinholt V., Preiss A., Schmalz G. Time-related bisphenol-A content and estrogenic activity in saliva samples collected in relation to placement of fissure sealants. Clin Oral Investig 1999; 3, 120–125.

ATSDR (Agency for Toxic Substances Disease Registry). Toxicological profile for mercury. Update. Atlanta-GA: 1999. http://www.atsdr.cdc.gov/toxprofiles/tp46.html (accessed 11 January 2008).

Auschill TM, Arweiler NB, Brecx M, Reich E, Sculean A, Netuschil L. The effect of dental restorative materials on dental biofilm. Eur J Oral Sci 2002; 110(1): 48-53.

Auzeerie V, Mahé, Marck Y, Auffret N, Descamps V, Crickx B. Oral lichenoid eruption due to methacrylate allergy. Contact Dermatitis 2002; 45:241.

Baccaglini L, Thongprasom K, Carrozzo M, Bigby M., Urban legends series: lichen planus. Oral Dis. 2012; Jun 6.

Ballatori N, Clarkson TW. Biliary secretion of glutathione and of glutathione-metal complexes. Fundam Appl Toxicol. 1985; 5(5): 816-31.

Barcelos GR, Grotto D, de Marco KC, Valentini J, Lengert A, de Oliveira AA, Garciac SC, Leite Bragaa GU, Engströmd KSh, de Syllos Cólusb IM, Brobergd K, Barbosa Jr. F., Polymorphisms in glutathione-related genes modify mercury concentrations and antioxidant status in subjects environmentally exposed to methylmercury. Sci Total Environ. 2013; 463-464: 319-25.

Barregård L, Sällsten G, Järvholm B. People with high mercury uptake from their own dental amalgam fillings. Occup Environ Med 1995; 52:124-128.

Barregård L. Mercury from dental amalgam: Looking beyond the average. Occup Environ Med 2005; 62:352-353.

Barregard L, Fabricius-Lagging E, Lundh T, Mölne J, Wallin M, Olausson M, Modigh C, Sallsten G.. Cadmium, mercury, and lead in kidney cortex of living kidney donors: Impact of different exposure sources. Environ Res. 2010; 110(1): 47-54.

Barregard L, Trachtenberg F, McKinlay S. Renal effects of dental amalgam in children: the New England children's amalgam trial. Environ Health Perspect. 2008 Mar; 116(3):394-9.

Basu, N., Goodrich, J. M. & Head, Ecogenetics Of Mercury: From Genetic Polymorphisms And Epigenetics To Risk Assessment And Decision-Making J. Environ. Toxicol. Chem. 2014; 33, 1248–58.

Bates MN, Fawcett J, Garrett N, Curtess T, Kjeilstrom T. Health effects of dental amalgam exposure: a retrospective cohort study. Int J Epidemiol 2004; 33:894-902

Bates MN. Mercury amalgam dental fillings: an epidemiological assessment. Int J Hyg Environ Health 2006; 209(4):309-316.

Basu, Goodrich, & Head, Ecogenetics of mercury: from genetic polymorphisms and epigenetics to risk assessment and decision-making. Environ Toxicol Chem. 2014 Jun;33(6):1248-58.. Epub2014 Apr 25.

Bellinger DC, Trachtenberg F, Barregard L, Tavares M, Cernichiari E, Daniel D. Neuropsychological and renal effects of dental amalgam in children. A randomized clinical trial JAMA 2006; 295:1775-1783.

Bellinger DC, Trachtenberg F, Daniel D, Zhang A, Tavares MA, McKinlay S. A dose-effect analysis of children's exposure to dental amalgam and neuropsychological function. J Amer Dent Assoc 2007; 138:1210-6.

Bellinger DC, Trachtenberg F, Zhang A, Tavares M, Daniel D, McKinlay S. Dental amalgam and psychosocial status: the New England Children's Amalgam Trial. J Dent Res. 2008 May;87(5):470-4

Bergdahl M, Habib R, Bergdahl J, Nyberg L, Nilsson Lg. Natural teeth and cognitive function in humans, Scand J Psychol, 2007, 48, 557–565

Bergenholtz G, Cox CF, Loesche WJ. Bacterial leakage around dental restorations and bacterial growth in cavities. J Oral Pathol 1982; 11:439-50.

Bergenholtz G. Evidence for bacterial causation of adverse pulpal responses in resin-based dental restorations. Crit Rev Oral Biol Med 2000; 11:467-80.

Berglund A., Molin M. Mercury levels in plasma and urine after removal of all amalgam restorations: The effect of using rubber dams, Dent Mater September, 1997; 13:297-304.

Berlin M, Jerksell LG, von Ubisch H. Uptake and retention of mercury in the mouse brain. A comparison of exposure to mercury vapor and intravenous injection of mercuric salt. Arch Environ Health. 1969; 12(1): 33-42.

Bernardo M, Luis H, Martin MD, Leroux BG, Rue T, Leitão J, DeRouen TA., Survival and reasons for failure of amalgam versus composite posterior restorations placed in a randomized clinical trial. J Am Dent Assoc. 2007 Jun; 138(6):775-83.

Beyth N, Bahir R, Matalon S, Domb AJ, Weiss EI. Streptococcus mutans biofilm changes surface-topography of resin composites. Dent Mater 2008; 24(6): 732-736.

Björkman L, Brokstad KA, Moen K, Jonsson R. Minor changes in serum levels of cytokines after removal of amalgam restorations. Toxicol Lett. 2012 Jun 1; 211(2):120-5

Björkman L, Lundekvam BF, Laegreid T, Bertelsen BI, Morild I, Lilleng P, Lind B, Palm B, Vahter M. Mercury in human brain, blood, muscle and toenails in relation to exposure: an autopsy study. Environ Health. 2007 Oct 11; 6:30.

Björkman L, Pedersen NL, Lichtenstein P. Physical and mental health related to dental amalgam fillings in Swedish twins. Community Dent Oral Epidemiol 1996; 24:260-267.

Björkner B, Niklasson B. Contact Allergy to the UV Absorber Tinuvin P in a dental restorative Material. Am J Contact Derm 1979; 8:6-7.

Bjornberg KA, Vahter M, Berglund B, Niklasson B, Blennow M, Sandborgh-Englund G. Transport of methylmercury and inorganic mercury to the fetus and breast-fed infant. Environ Health Perspect 2005; 113:1381-5.

Bogdan A, Buckett MI, Japuntich DA. Nano-Sized Aerosol Classification, Collection and Analysis - Method Development Using Dental Composite Materials. J Occup Environ Hyg. 2014.

Bonding N, Graem N, Rygaard J, Dabelsteen E. Effects of irradiation with dental light curing units on Langerhans cells in human stratified epithelium in heterotransplanted skin. Scan J Dent Res 1987; 95:463-6.

Bouillaguet S, Shaw L, Gonzalez L. Wataha JC, Krejci I. Long-term cytotoxicity of resin-based dental restorative materials. Journal of Oral Rehabilitation 2002; 29:7-13.

Bouilliaguet S, Virgillito M, Wataha J, Ciucchi B, Holz J. The influence of dentine permeability on cytotoxicity of four dentine bonding systems, *in vitro*. J Oral Rehab 1998; 25:45-51. http://www.edwardtufte.com/tufte/hill

Bourbia M, Ma D, Cvitkovitch DG, Santerre JP, Finer Y. Cariogenic bacteria degrade dental resin composites and adhesives. J Dent Res 2013; 92(11): 989-994.

Brackett M. G, Bouillaguet S., Lockwood P. E., Rotenberg S., Lewis J. B., Messer R. L. W., Wataha J. C. In vitro cytotoxicity of dental composites based on new and traditional polymerisation chemistries. J Biomed Mater Res Part B: Appl Biomater, 2007; 2:397–402.

Bratel J, Haraldson T, Ottosson JO. Potential side effects of dental amalgam restorations. (II). No relation between mercury levels in the body and mental disorders. Eur J Oral Sci 1997b; 105(3):244-50.

Bratel J, Haraldsson T, Meding B, Yontchev E, Ohman SC, Ottosson JO. Potential side effects of dental amalgam restorations. (I). An oral and medical investigation. Eur J Oral Sci 1997a; 105(3):234-43.

Broberg K, Engström K, Ameer S. Mercury, In Handbook of Toxicology of metals. Nordberg GF, Fowler BA, Nordberg M (eds.). Academic Press 7. 2014; Aug. 2014, ISBN 01239733. Vol II Specific metals 255-258.

Bruzell E, Johnsen B, Aalerud TN, Christensen T. Evaluation of eye protection filters for use with dental curing- and bleaching lamps. J Occup Environ Hyg 2007; 4: 432-9.

Bruzell E, Wellendorf H. LED (Light Emitting Diodes) – lampor för ljushärdning av dentala material. Kunskapsdokument från KDM. Kunskapscenter för Dentala Material. Socialstyrelsen, Stockholm, 2008. In Swedish. http://www.niom.no/content/tested-depth-cure-and-curing-lamps.

Bruzell Roll EM, Jacobsen N, Hensten-Pettersen A. Health hazards associated with curing light in the dental clinic. Clin Oral Invest 2004; 8:113-7.

Burke FJ, Wilson NH, Cheung SW, Mjör IA. Influence of patient factors on age of restorations at failure and reasons for their placement and replacement. J Dent 2001; 29(5): 317-324.

Callaghan B, Feldman D, Gruis K, Feldman E. The association of exposure to lead, mercury, and selenium and the development of amyotrophic lateral sclerosis and the epigenetic implications. Neurodegener Dis. 2011; 8(1-2):1-8.

Carmichael AJ, Gibson JJ, Walls WG. Allergic contact dermatitis to bisphenol-A-glycidylmethacrylate (BIS-GMA) dental resin associated with sensitivity to epoxy resin. Br Dent J 1997; 183:297-8.

Castoldi AF, Onishchenko N, Johansson C, Coccini T, Roda E, Vahter M, Ceccatelli S, Manzo L. Neurodevelopmental toxicity of methylmercury: Laboratory animal data and their contribution to human risk assessment. Regul Toxicol Pharmacol. 2008; 51(2):215-229.

Cattani –Lorente M, Bouillaguet S,Godin CH, Meyer JM, Polymerisation shrinkage of ormocer based dental restorative composites. Eur Cell Mater 2001; 1:25-26.

Chadwick BL, Dummer PM, Dunstan FD, Gilmour AS, Jones RJ, Phillips CJ, Rees J, Richmond S, Stevens J, Treasure ET., What type of filling? Best practice in dental restorations. Qual Health Care. 1999 Sep; 8(3):202-7.

Chadwick RG, Traynor N, Moseley H, Gibbs N. Blue light curing units – a dermatological hazard. Brit Dent J 1994; 176:17-31.

Christensen T and Bruzell EM. Methacrylate monomers lower the level of reduced glutathione and increase the in vitro sensitivity of cells to optical radiation. Photochem. Photobiol. Sci., 2010; 9:1597-1600.

Clarkson TW, Vyas JB, Ballatori N. Mechanisms of mercury disposition in the body. Am J Industr Med 2007; 50: 757-764.

Costa MF, Tomaz S, de Souza JM, Silveira LC, Ventura DF. Electrophysiological evidence for impairment of contrast sensitivity in mercury vapor occupational intoxication. Environ Res. 2008; 107(1):132-8.

Costa L, Giordano G. Methylmercury neurotoxicity: A sinopsis of in vitro effects. In: Methylmercury and Neurotoxicity (S. Ceccatelli & M. Aschner Eds). Current Topics in Neurotoxicity 2012; 2: 219-227.

Counter SA, Buchanan LH, Ortega F. Acoustic stapedius muscle reflex in mercury-exposed Andean children and adults. Acta Otolaryngol. 2012 Jan;132(1):51-63.

Custodio HM, Harari R, Gerhardsson L, Skerfving S, Broberg K. Genetic influences on the retention of inorganic mercury. Arch Environ Occup Health 2005; 60: 17-23.

Da Costa SL, Malm O, Dórea JG., Breast-milk mercury concentrations and amalgam surface in mothers from Brasília, Brazil. Biol Trace Elem Res. 2005; Aug;106(2):145-51.

Dahl JE, Sundby J, Hensten-Pettersen A, Jacobsen N. Dental workplace exposure and fertility. Scand J environ Health, 1999, 25,285-90.

DeLeo V Occupational Phototoxicity and Photoallergy. In: Kanerva L, Elsner P, Wahlberg JE, Maibach HI (eds) Handbook of Occupational Dermatology. Springer, Berlin, Heidelberg, New York, 2000 pp 314-324.

DeRouen TA, Martin MD, Leroux BG, Townes BD, Woods JS, Leitão J, Castro-Caldas A, Luis H, Bernardo M, Rosenbaum G, Martins IP. Neurobehavioral effects of dental amalgam in children-A randomized clinical trial. JAMA 2006; 295:1784-92.

DeRouen, T, Woods J, Leroux B, Martin M. Letter to the Editor. Critique of reanalysis of Casa Pia data on associations of porphyrins and glutathione-S-transferases with dental amalgam exposure. Hum Exp Toxicol, 2014 (pii: 0960327114542885.

DeRouen T, Woods J, Leroux B, Martin M. Critique of reanalysis of Casa Pia data on associations of porphyrins and glutathione-S-transferases with dental amalgam exposure.Hum Exp Toxicol. 2014 Jul 8.

Drexler H., Schaller K. H., The mercury concentration in breast milk resulting from amalgam fillings and dietary habits, Environ. Res. 1998; 77, 124–129.

Directive 2006/25/EC of the European Parliament and of the Council of 5 April 2006 on the minimum health and safety requirements regarding the exposure of workers to risks arising from physical agents (artificial optical radiation). http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:32006L0025:EN:NOT.

Doméjean-Orliaguet S, Tubert-Jeannin S, Riordan PJ, Espelid I, Tveit AB: French dentists' restorative treatment decisions. Oral Health PrevDent 2004; 2:125-131.

Drasch G., Aigner S., Roider G., Staiger F., Lipowsky G., Mercury in human colostrum and early breast milk. Its dependence on dental amalgam and other factors, J. Trace Elements Med. Biol. 1998; 12, 23–27.

Drasch G, Schupp I, Höfl H, Reinke R, Roider G.Mercury burden of human fetal and infant tissues. Eur J Pediatr. 1994; 153, 607-610.

Drucker AM, Pratt MD, Acrylate contact allergy: patient characteristics and evaluation of screening allergens. Dermatitis. 2011 Mar-Apr;22(2):98-101

Duplinsky TG, Cicchetti DV., The health status of dentists exposed to mercury from silver amalgam tooth restorations. Int J of Statistics in Med Res 2012; 1, 1-15.

Durey K, Santini A, Miletic V. Pulp chamber temperature rise during curing of resin-based composites with different light-curing units. Prim Dent Care 2008; 15:33-38.

Durner J, Dębiak M, Bürkle A, Hickel R, Reichl FX. Induction of DNA strand breaks by dental composite components compared to X-ray exposure in human gingival fibroblasts. Arch Toxicol. 2011 Feb; 85(2):143-8.

Durner J, Glasl B, Zaspel J, Kunzelmann KH, Hickel R, Reichl FX., Release of TEGDMA from composite during the chewing situation. Dent Mater. 2010 Jul; 26(7):e197-204.

Durner J, Kreppel H, Zaspel J, Schweikl H, Hickel R, Reichl FX., The toxicokinetics and distribution of 2-hydroxyethyl methacrylate in mice. Biomaterials. 2009 Apr; 30(11):2066-71.

Durner J, Walther UI, Zaspel J, Hickel R, Reichl FX., Metabolism of TEGDMA and HEMA in human cells. Biomaterials. 2010 Feb; 31(5):818-23.

Duthie MS, Kimber I, Norval M. The effects of ultraviolet radiation on the human immune system. Br J Dermatol. 1999 Jun; 140(6):995-1009.

Dutton DJ, Fyie K, Faris P, Brunel L, Emery JH. The association between amalgam dental surfaces and urinary mercury levels in a sample of Albertans, a prevalence study. J Occup Med Toxicol. 2013 Aug 29; 8(1):22. doi: 10.1186/1745-6673-8-22.

Dye BA, Schober SE, Dillon CF, Jones RL, Fryar C, McDowell M, Sinks TH. Urinary mercury concentrations associated with dental restorations in adult women aged 16-49 years. Occ Environ Med 2005; 62:368-75.

Echeverria D, Woods JS, Heyer NJ, Martin MD, Rohlman DS, Farin FM, Li T., The association between serotonin transporter gene promotor polymorphism (5-HTTLPR) and elemental mercury exposure on mood and behavior in humans. J Toxicol Environ Health A. 2010; 73(15):1003-20.

Echeverria D, Woods JS, Heyer NJ, Rohlman D, Farin FM, Li T, Garabedian CE. The association between a genetic polymorphism of coproporphyrinogen oxidase, dental mercury exposure and neurobehavioral response in humans. Neurotoxicol Teratol 2006; 28(1): 39-48.

Echeverria D, Woods JS, Heyer NJ, Rohlman DS, Farin FM, Bittner AC Jr, Li T, Garabedian C., Chronic low-level mercury exposure, BDNF polymorphism, and associations with cognitive and motor function. Neurotox Teratol 2005; 27:781-96.

Edwards T, McBride BC. Biosynthesis and degradation of methylmercury in human faeces. Nature. 1975; 253(5491):463-4.

EFSA (European Food Safety Authority). Opinion of the Scientific Panel on Contaminants in the Food Chain on a request from the Commission related to mercury and methylmercury in food. The EFSA Journal 2004; 34:1-14.

EFSA (European Food Safety Authority). Opinion of the Scientific Panel on contaminants in the food chain on a request from the European Parliament related to the safety assessment of wild and farmed fish. The EFSA Journal 2005; 236:1-118.

EFSA CONTAM Panel (2012). Scientific Opinion on the risk for public health related to the presence of mercury and methylmercury in food. EFSA Journal 2012; 10(12):2985 [241 pp.doi:10.2903/j.efsa.2012.2985

Ekstrand J, Nielsen JB, Havarinasab S, Zalups RK, Soderkvist P, Hultman P. Mercury toxicokinetics--dependency on strain and gender. Toxicol Appl Pharmacol. 2010; 243(3): 283-91.

Engelmann J, Leyhausen G, Leibfritz D, Geurtsen W. Effects of TEGDMA on the intracellular glutathione concentration of human gingival fibroblasts. J Biomed Mater Res 2002; 63:746-51.

Engelmann J, Leyhausen G, Leibfritz D, Geurtsen W. Metabolic effects of dental resin components *in vitro* detected by NMR spectroscopy. J Dent Res 2001; 80:869-75.

Engström K1, Ameer S, Bernaudat L, Drasch G, Baeuml J, Skerfving S, Bose-O'Reilly S, Broberg K. Polymorphisms in genes encoding potential mercury transporters and urine mercury concentrations in populations exposed to mercury vapor from gold mining. Environ Health Perspect 2013; 121(1): 85-91.

Engström, KS, Strömberg U, Broberg K. Genetic Variation in Glutathione-Related Genes and Body Burden of Methylmercury. Environ Health Perspect 2008: 116, 734-739.

EPA (Environmental Protection Agency, US). Water quality criterion for the protection of human health Report EPA-823-R-01-001. Washington DC, USA: Environmental Protection Agency; January 2001.

Ersev, H., Schmalz, G., Bayirli, G., Schweikl, H.: Cytotoxic and mutagenic potencies of various root canal filling materials in eukaryotic and prokaryotic cells in vitro. J Endod 1999; 25, 359–363.

Ethier AA, Muckle G, Bastien C, Dewailly É, Ayotte P, Arfken C, Jacobson SW, Jacobson JL, Saint-Amour D. Effects of environmental contaminant exposure on visual brain development: a prospective electrophysiological study in school-aged children. Neurotoxicology. 2012 Oct; 33(5):1075-85.

EU-RAR (European Union Risk Assessment Report). Methyl methacrylate, CAS No: 80-62-6, EINECS-No. 201-297-1. Institute for Health and Consumer Protection, European Chemicals Bureau, European Commission Joint Research Centre, 1st Priority List, Luxembourg: Office for Official Publications of the European Communities; 2002.

European Commission Recommendation 2011/696/EU, EC 2011.

European Food Safety Authority. Scientific Opinion on the risk for public health related to the presence of mercury and methylmercury in food. EFSA Journal. 2012; 10(12): 2985.

Eyeson J, House I, Yang YH, Warnakulasuriya KA. Relationship between mercury levels in blood and urine and complaints of chronic mercury toxicity from amalgam restorations. Br Dent J. 2010 Feb 27;208(4):E7; discussion 162-3.

Fan PL, Meyer DM. FDI report on adverse reactions to resin based materials. Int Dent J 2007; 57:9-12.

Federlin M, Hiller KA, Schmalz G., Controlled, prospective clinical split-mouth study of cast gold vs. ceramic partial crowns: 5.5 year results. Am J Dent. 2010 Jun; 23(3):161-7.

Feitosa-Santana C, Barboni MT, Oiwa NN, Paramei GV, Simões AL, Da Costa MF, Silveira LC, Ventura DF. Irreversible color vision losses in patients with chronic mercury vapor intoxication. Vis Neurosci. 2008; 25(3):487-91.

Feitosa-Santana C, Bimler DL, Paramei GV, Oiwa NN, Barboni MT, Costa MF, Silveira LC, Ventura DF. Color-space distortions following long-term occupational exposure to mercury vapor. Ophthalmic Physiol Opt. 2010; 30(5):724-30.

Felden AA., G. Schmalz, K.-A. Hiller Retrospective clinical study and survival analysis on partial ceramic crowns: results up to 7 years. Clin Oral Invest 1998; 2: 161–167.

Ferracane JL. Elution of leachable components from composites. J Oral Rehabil 1994; 21:441-52.

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

Finer Y, Santerre JP. The influence of resin chemistry on a dental composite's biodegradation. J Biomed Mater Res 2004; 69A:233-46.

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

Fonfria E, Vilaro MT, Babot Z, Rodriguez-Farre E, Sunol C. Mercury compounds disrupt neuronal glutamate transport in cultured mouse cerebellar granule cells. Journal of neuroscience research. 2005; 79(4): 545-53.

Forsten, L.: Short- and long-term fluoride release from glass ionomers and other fluoride-containing filling materials in vitro. Scand J Dent Res 1990; 98, 179–185.

Franz A, König F, Anglmayer M, Rausch-Fan X, Gille G, Rausch WD, Lucas T, Sperr W, Schedle A. , Cytotoxic effects of packable and nonpackable dental composites. Dental Mat 2003; 19:382–392.

Franz A, König F, Skolka A, Sperr W, Bauer P, Lucas T, Watts DC, Schedle A., Cytotoxicity of resin composites as a function of interface area. Dental Mat 2007; 23:1438–1446.

Frencken JE, Leal SC, Navarro MF.Twenty-five-year atraumatic restorative treatment (ART) approach: a comprehensive overview.Clin Oral Investig. 2012 Oct;16(5):1337-46.

FSA (2014) Draft Scientific Opinion on the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs. Endorsed For Public Consultation Draft Scientific Opinion. Efsa Panel On Efsa Panel On Food Contact Materials, Enzymes, Flavourings And Processing Aids (Cef) , European Food Safety Authority (Efsa), Parma, Italy. January 2014.

Fúcio SB, Carvalho FG, Sobrinho LC, Sinhoreti MA, Puppin-Rontani RM. The influence of 30-day-old Streptococcus mutans biofilm on the surface of esthetic restorative materials--an in vitro study. J Dent 2008; 36(10): 833-839.

Fung EY, Ewoldsen NO, St Germain HA Jr, Marx DB, Miaw CL, Siew C, Chou HN, Gruninger SE, Meyer DM. Pharmacokinetics of bisphenol A released from a dental sealant J Am Dent Assoc. 2000. 131(1):51-8.

Galler K, Hiller KA, Ettl T, Schmalz G. Selective influence of dentin thickness upon cytotoxicity of dentin contacting materials. J Endod. 2005 May; 31(5):396-9.

Galler KM, Schweikl H, Hiller KA, Cavender AC, Bolay C, D'Souza RN, Schmalz G. TEGDMA reduces mineralization in dental pulp cells. J Dent Res 2011; 90:257-62.

Gardiner TH, Waechter JM, Wiedow MA, Solomon WT. Glycidyloxy compounds used in epoxy resin systems: a toxicology review. Regul Toxicol Pharmacol 1992; 15:S1-77.

Gardner RM, Nyland JF, Silbergeld EK. Differential immunotoxic effects of inorganic and organic mercury species in vitro. Toxicol Lett. 2010 Oct5; 198(2):182-90.

Garhammer P, Schmalz G, Hiller KA, Reitinger T, Stolz W., Patients with local adverse effects from dental alloys: frequency, complaints, symptoms, allergy. Clin Oral Investig. 2001 Dec; 5(4):240-9.

Garner LA. Contact dermatitis to metals. Dermatol Ther 2004; 17:321-27.

Gassó S, Cristòfol RM, Selema G, Rosa R, Rodríguez-Farré E, Sanfeliu C. Antioxidant compounds and Ca(2+) pathway blockers differentially protects against methylmercury and mercuric chloride neurotoxicity. J Neurosci Res. 2001; 66(1):135-145.

Geier D, Carmody T, Kern J, King P, Geier M. A significant dose-dependent relationship between mercury exposure from dental amalgams and kidney integrity biomarkers: A further assessment of the Casa Pia children's dental amalgam trial. Hum Exp Toxicol. 2012 Aug 14. [Epub ahead of print].

Geier DA, Carmody T, Kern JK, King PG, Geier MR. A significant relationship between mercury exposure from dental amalgams and urinary porphyrins: a further assessment of the Casa Pia children's dental amalgam trial. Biometals. 2011 Apr; 24(2):215-24.

Geier DA, Kern JK, Geier MR. A prospective study of prenatal mercury exposure from maternal dental amalgams and autism severity. Acta Neurobiol Exp (Wars). 2009;69(2):189-97.

Geurtsen W, Leyhausen G. Chemical-biological interaction of the resin monomer triethyleneglycoldimethacrylate (TEGDMA). J Dent Res 2001; 80:2046-50.

Geurtsen W, Spahl W, Leyhausen G. Variability of cytotoxicity and leaching of substances from four light-curing pit and fissure sealants. J Biomed Mater Res 1999; Jan;44(1):73-7.

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

Geurtsen W. Biological Interactions of Non-Metallic Restorative Materials with Oral Tissues. Acad Dent Mater Trans 1999; 13:75-93.

Geurtsen W. Substances released from dental resins composites and glass ionomer cements. Eur J Oral Sci 1998; 106:687-95.

Ghasemi H, Murtomaa H, Torabzadeh H, Vehkalahti MM: Restorative treatment threshold reported by Iranian dentists. Community Dent Health 2008; 25:185-190.

Gibson GR, Macfarlane GT, Cummings JH. Sulphate reducing bacteria and hydrogen metabolism in the human large intestine. Gut. 1993; 34(4):437-9.

Gioda A, Hanke G, Elias-Boneta A, Jiménez-Velez B., A pilot study to determine mercury exposure through vapor and bound to PM10 in a dental school environment. Toxicol Ind Health; 2007; 23(2):103-13.

Goldberg M. In vitro and in vivo studies on the toxicity of dental resin components: a review. Clin Oral Invest 2007.

Gopferich, A. Mechanisms of polymer degradation and erosion. Biomaterials 1996; 17(2): 103-114.

Goodrich JM, Wang Y, Gillespie B, Werner R, Franzblau A, Basu N. Glutathione enzyme and selenoprotein polymorphisms associate with mercury biomarker levels in Michigan dental professionals. Toxicol Appl Pharmacol 2011; 257: 301-308.

Goon AT, Bruze M, Zimerson E, Goh CL, Soo-Quee Koh D, Isaksson M., Screening for acrylate/methacrylate allergy in the baseline series: our experience in Sweden and Singapore. Contact Dermatitis. 2008 Nov; 59(5):307-13.

Goon AT, Isaksson M, Zimerson E, Goh CL, Bruze M. Contact allergy to (meth)acrylates in the dental series in southern Sweden: simultaneous positive patch test reaction patterns and possible screening allergens. Contact Dermatitis 2006; 55:219-26.

Gordan VV, Bader JD, Garvan CW, Richman JS, Qvist V, Fellows JL, Rindal DB, Gilbert GH: Restorative treatment thresholds for occlusal primary caries among dentists in the dental practice-based research network. J Am Dent Assoc 2010; 141:171-184.

Grandjean P, Budtz-Jørgensen E, Weihe P. Cardiac autonomic activity in methylmercury neurotoxicity: 14-year follow-up of a Farose birth cohort. J Pediatr 2004; 144:169-76.

Grandjean P, Budtz-Jørgensen E. An ignored risk factor in toxicology: The total imprecision of exposure assessment. Pure Appl Chem 2010; 82: 383-391.

Grandjean P, Landrigan PJ. Neurobehavioural effects of developmental toxicity. Lancet Neurol 2014; 13: 330-8.

Grandjean P, Yorifuji T. Mercury (Chapter 8). In: Bingham E, Cohrssen B, eds. Patty's Toxicology, 6th ed. New York: Wiley 2012, Vol. 1, pp 213-27.

Grandjean P. Seven deadly sins of environmental epidemiology and the virtues of precaution. Epidemiology. 2008; 19(1): 158-62.

Gregson KS, Shih H, Gregory RL. The impact of three strains of oral bacteria on the surface and mechanical properties of a dental resin material. Clin Oral Investig 2012; 16(4): 1095-1103.

Groger G, Rosentritt M, Behr M, Schroder J, Handel G. Dental resin materials in vivo – TEM results after one year: a pilot study. J Mater Sci Mater Med 2006; 17:825-8.

Gundacker C, Scheinast M, Damjanovic L Fuchs C, Rosner M, Hengstschläger M. Proliferation potential of human amniotic fluid stem cells differently responds to mercury and lead exposure Amino Acids 2012; 43:937–949.

Guzzi G, Grandi M, Cattaneo C, Calza S, Minoia C, Ronchi A, Gatti A, Severi G.,Dental amalgam and mercury levels in autopsy tissues: food for thought. The American journal of forensic medicine and pathology. 2006; 27(1): 42-5.

Guzzi G, Pigatto PD. Urinary mercury levels in children with amalgam fillings. Environ Health Perspect. 2008 Jul; 116(7):A286-A287.

Halbach S., Welzlb U, G., Kremersc L., Willruthc H., Mehlc A., Wackd F.X., Hickelc R., Greim H., Steady-state transfer and depletion kinetics of mercury from amalgam fillings. The Science of the Total Environment 259 _2000. 1321.

Halbach S., Kremers L., Willruth H., Mehl A., Welzl G., Wack F. X., Hickel R., Greim H., Systemic Transfer of Mercury from Amalgam Fillings before and after Cessation of Emission Institute of Toxicology and àInstitute of Biomathematics and Biometry, GSFÐNational Research Center for Environment and Health, Neuherberg, D-85758-Oberschleissheim; Department of Restorative Dentistry and Periodontology, University of Munich,Goethestrasse 70, D-80336-Munich; and °Army Dentistry Unit 612/2, Fu¬ rst-Wrede-Kaserne, Ingolsta dter Strasse 240,D-80935-Munich, Germany, Received July 11, 1997.

Hall BM. Distribution of mercury resistance among Staphylococcus aureus isolated from a hospital community. J Hyg (Lond). 1970 Mar; 68(1):111-9.

Hallström, U: Adverse reaction to a fissure sealant. Report of a case. J Dent Child 1993; 60, 143–146.

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

Hanf V, Forstman A, Costea JE, Schieferstein G, Fischer I, Schweinsberg F. Mercury in urine and ejaculate in husbands of barren couples. Toxicol Lett 1996; 88:227-31.

Hansel C, Leyhausen G, Mai UE, Geurtsen W. Effects of various resin composite (co)monomers and extracts on two caries-associated micro-organisms *in vitro.* J Dent Res 1998; 77:60-7.

Hansson P, Sunnegårdh-Grönberg K, Bergdahl J, Bergdahl M, Nyberg L, Nilsson LG. Relationship between natural teeth and memory in a healthy elderly population. Eur J Oral Sci. 2013 Aug; 121(4):333-40.

Hantson P, Mahieu P, Gersdorff M, Sindic CJM, Lauwerys R. Encephalopathy with seizures after use of aluminum containing bone cement. Lancet 1994; 344, 1647.

Harari R1, Harari F, Gerhardsson L, Lundh T, Skerfving S, Strömberg U, Broberg K., Exposure and toxic effects of elemental mercury in gold-mining activities in Ecuador. Toxicology letters 2012; 213(1): 75-82.

Hashimoto M, Ito S, Tay FR, Svizero NR, Sano H, Kaga M, Pashley DH. Fluid movement across the resin-dentine interface during and after bonding. J Dent Res 2004; 83:843-48.

Hashimoto M, Ohno H, Sano H, Kaga M, Oguchi H. In vitro degradation of resin-dentin bonds analyzed by microtensile bond test, scanning and transmission electron microscopy. Biomaterials 2003; 24(21): 3795-3803.

Health Canada 1995: The Safety of Dental Amalgam. ©Minister of Supply and Services Canada, 1996. Cat. H49-105/1996E.ISBN 0-662-24873-2.

Heggland I, Irgens ÅI, Tollånes M, Romundstad P, Syversen T, Svendsen K, Melø I, Hilt B. Pregnancy outcomes among female dental personnel – a registry-based retrospective cohort study. Scand J Work Environ Health.2011; 37(6):539–546. doi:10.5271/sjweh.3175)

Heil, J., Reifferscheid, G., Waldmann, P., Leyhausen, G., Geurtsen, W.: Genotoxicity of dental materials. Mutat Res 1996; 368, 181–194.

Henriks-Eckerman ML, Kanerva L. Product analysis of acrylic resins compared to information given in material safety data sheets. Contact Dermatitis 1997; 36:164-5.

Henriks-Eckerman ML, Alanko K, Jolanki R, Kerusuo H, Kanerva L. Exposure to airborne methacrylates and natural rubber latex allergens in dental clinics. J Environ Monit 2001; 3:302-5.

Hensten-Pettersen A, Jacobsen N. Perceived side effects of biomaterials in prosthetic dentistry. J Prosthet Dent 1991; 65:138-44.

Herrstrom P, Hogstedt B. Clinical study of oral galvanism: no evidence of toxic mercury exposure but anxiety disorder an important background factor. Scand J Dent Res 1993; 101(4):232-237.

Hertz-Picciotto I, Green PG, Delwiche L, Hansen R, Walker C, Pessah IN. Blood mercury concentrations in CHARGE Study children with and without autism. Environ Health Perspect. 2010 Jan; 118(1):161-6.

Heyer NJ, Bittner AC, Jr., Echeverria D, Woods JS. A cascade analysis of the interaction of mercury and coproporphyrinogen oxidase (CPOX) polymorphism on the heme biosynthetic pathway and porphyrin production. Toxicol Lett 2006; 161(2): 159-166.

Heyer NJ, Echeverria D, Bittner AC, Jr., Farin FM, Garabedian CC, Woods JS. Chronic low-level mercury exposure, BDNF polymorphism, and associations with self-reported symptoms and mood. Toxicol Sci 2004; 81(2): 354-363.

Heyer NJ, Echeverria D, Farin FM, Woods JS. The association between serotonin transporter gene promoter polymorphism (5-HTTLPR), self-reported symptoms, and dental mercury exposure. J Toxicol Environ Health Part A 2008; 71(19): 1318-1326.

Heyer NJ, Echeverria D, Martin MD, Farin FM, Woods JS. Catechol O-methyltransferase (COMT) VAL158MET functional polymorphism, dental mercury exposure, and self-reported symptoms and mood. J Toxicol Environ Health Part A 2009; 72(9): 599-609.

Hilt B, Svendsen K, Syversen T, Aas O, Qvenild T, Sletvold H, Melø I., Occurrence of cognitive symptoms in dental assistants with previous occupational exposure to metallic mercury, Neurotoxicology. 2009 Nov; 30(6):1202-6

Hindsén M., Spiren A., Bruze M.: Cross reactivity between nickel and palladium demonstrated by systemic administration of nickel. Contact Dermatitis 2005; 53, 2–8.

Hock C, Drasch G, Golombowski S, Müller-Spahn F, Willershausen-Zönnchen B, Schwarz P, Hock U, Growdon JH, Nitsch RM. Increased blood mercury levels in patients with Alzheimer's disease. J Neural Transm 1998; 105: 59-68.

Hogberg HT1, Kinsner-Ovaskainen A, Coecke S, Hartung T, Bal-Price AK, mRNA expression is a relevant tool to identify developmental neurotoxicants using an in vitro approach, Toxicol Sci. 2010 Jan;113(1):95-115. doi: 10.1093/toxsci/kfp175.

Hörsted-Bindslev P. Amalgam toxicity – environmental and occupational hazards. J Dent 2004; 32:359-365.

Huang CF, Liu SH, Hsu CJ, Shiau SY. Neurotoxicological effects of low-dose methylmercury and mercuric chloride in developing offspring mice. Toxicol Lett 2011; 201:196-204.

Hume WR, Gerzina TM. Bioavailability of components of resin-based materials which are applied to teeth. Crit Rev Oral Biol Med 1996; 7:172-179.

Ilie N, Bucuta S, Draenert M. Bulk-fill resin-based composites: An in vitro assessment of their mechanical performance. Oper Dent 2013; 38:618-25.

Ilie N, Hickel R. Investigations on mechanical behavior of dental composites. Clinical Oral Investigations 2009; 13:427-438.

Imai Y, Komabayashi T., Elution of Bisphenol A from Composite Resin: A Model Experiment. Dental Materials Journal 2000; 19 (2): 133-138.

Imai, Y., Comments on "Determination of Bisphenol A and Related Aromatic Compounds Released from Bis-GMA-Based Composites and Sealants by High Performance Liquid Chromatography". Environmental Health Perspectives 2000; 108 (12), 545.

International Agency for Research on Cancer (IARC) A review of human carcinogens. Part D: Radiation / IARC Working Group on the Evaluation of Carcinogenic Risks to Humans 2009, Lyon, 2012. http://monographs.iarc.fr/ENG/Monographs/vol100D/mono100D.pdf

International Agency for Research on Cancer (IARC). Beryllium, Cadmium, Mercury, and Exposures in the Glass Manufacturing Industry: Summary of Data Reported and Evaluation. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. 1997; Volume 58.

International Agency for Research on Cancer: IARC monographs on the evaluation of carcinogenic risks to humans – list of IARC evaluation. IARC, Lyon 1996, pp 1–40.

International Electrotechnical Commission (IEC). Medical electrical equipment – Part 2-57: Particular requirements for the basic safety and essential performance of non-laser light source equipment intended for therapeutic, diagnostic monitoring and cosmetic/aesthetic use, IEC 60601-2-57:2011, IEC, Geneva, 2011.

Issa Y, Duxbury AJ, Macfarlane TV, Brunton PA. Oral lichenoid lesions related to dental restorative materials. Br Dent J 2005; 198:361-6.

Jacobsen N, Aasenden R, Hensten-Pettersen A. Occupational health complaints and adverse patient reactions as perceived by personnel in public dentistry. Community Dent Oral Epidemiol 1991; 19:155-9.

Jacobsen N, Hensten-Pettersen A. Changes in occupational health problems and adverse patient reactions in orthodontics from 1987 to 2000. Eur J Orthod 2003; 25:591-8.

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

Jandt KD, Mills RW., A brief history of LED photopolymerisation. Dent Mater. 2013 Jun; 29(6):605-17

Janke V, von Neuhoff N, Schlegelberger B, Leyhausen G, Geurtsen W. TEGDMA causes apoptosis in primary human gingival fibroblasts. J Dent Res 2003; 82:814-8.

Jedrejko M, Skoczyńska A. Color vision impairment in workers exposed to mercury vapour. Med Pr. 2011; 62(3):227-35.

Jensen JS, Trap B., Skydsgaardk. Delayed contact hypersensitivity and surgical glove penetration with acrylic bone cements. Acta Orthop Scand 1991; 62:24-28.

Johnsson C, Schütz A, Sällsten G. Impact of consumption of freshwater fish on mercury levels in hair, blood, urine, and alveolar air. J Toxicol Environ Health A. 2005; 68(2):129-40.

Joly B, Cluzel R.,[The role of heavy metals and their derivatives in the selection of antibiotics resistant gram-negative rods]. Ann Microbiol (Paris). 1975 Jul-Aug;126B(1):51-61.

Jones DW, Exposure or absorption and the crucial question of limits for mercury. J Can Dent Assoc 1999, 65(1):42–46.

Jones L, Bunnell J, Stillman J. A 30 year follow-up of residual effects on New Zealand school dental nurses from occupational mercury exposure. Hum Exp Toxicol 2007; 26:367-74.

Joskow R, Boyd Barr D, Barr RR, Calafat AM, Needham LL, Rubin C. Exposure to bisphenol A from bis-glycidyl dimethacrylate-based dental sealants. J Amer Dent Assn 2006; 137:353-62.

Julvez J, Smith GD, Golding J, Ring S, Pourcain BS, Gonzalez JR, Grandjean P. Prenatal methylmercury exposure and genetic predisposition to cognitive deficit at age 8 years. Epidemiology 2013; 24(5): 643-650.

Julvez J., Grandjean P., Genetic susceptibility to methylmercury developmental neurotoxicity matters. Front Genet 2013;4:278.

Kallus T, Mjör IA. Incidence of adverse effects of dental materials. Scand J Dent Res 1991; 99:236-40.

Kanerva L, Alanko K. Stomatitis and perioral dermatitis caused by epoxy diacrylates in dental composite resins. J Am Acad Dermatol 1998; 38:116-20.

Kanerva L, Rantanen T, Aalto-Korte K. A multicenter study of patch test reactions with dental screening series. Am J Contact Dermatol 2001; 12:83-7.

Karagas MR, Choi AL, Oken E, Horvat M, Schoeny R, Kamai E, Cowell W, Grandjean P, Korrick S. Evidence on the human health effects of low-level methylmercury exposure. Environ Health Perspect 2012; 120: 799-806.

Kaufman JS, Poole C. Looking back on "causal thinking in the health sciences". Annu Rev Public Health. 2000; 21:101-19. Review.

Kermanshahi S, Santerre JP, Cvitkovitch DG, Finer Y. Biodegradation of resin-dentin interfaces increases bacterial microleakage. J Dent Res 2010; 89(9): 996-1001.

Khalichi P, Cvitkovitch DG, Santerre JP. Effect of composite resin biodegradation products on oral streptococcal growth. Biomaterials 2004; 25:5467-72.

Khalichi P, Singh J, Cvitkovitch DG, Santerre JP. The influence of triethylene glycol derived from dental composite resins on the regulation of Streptococcus mutans gene expression. Biomaterials 2009; 30(4): 452-459.

Khamaysi Z, Bergman R, Weltfriend S. Positive patch test reactions to allergens of the dental series and the relation to clinical presentations. Contact Dermatitis 2006; 55:216-8.

Kingman A, Albers JW, Arezzo JC, Garabant DH, Michalek JE. Amalgam exposure and neurological function. Neurotoxicology 2005; 26:241-55.

Kingman A, Hyman J, Masten SA, Jayaram B, Smith C, Eichmiller F, Arnold MC, Wong PA, Schaeffer JM, Solanki S, Dunn WJ. Bisphenol A and other compounds in human saliva and urine associated with the placement of composite restorations. J Am Dent Assoc. 2012

Klaassen, CD. editor. Casarett and Doull's toxicology. The basic science of poisons. New York: McGraw-Hill Medical Publishing Division; 2001.

Krämer N, Lohbauer U, García-Godoy F, Frankenberger R. Light curing of resin-based composites in the LED era. Am J Dent. 2008 Jun; 21(3):135-42.

Krifka S., Hiller K.A., Spagnuolo G., Jewett A., Schmalz G., Schweikl H. The influence of glutathione on redox regulation by antioxidant proteins and apoptosis in macrophages exposed to 2-hydroxyethyl methacrylate (HEMA). Biomaterials 2012; 33: 5177-5186.

Laeijendecker R, Dekker SK, Burger PM, Mulder PG, Van Joost T, Neumann MH. Oral lichen planus and allergy to dental amalgam restorations. Arch Dermatol 2004; 140:1434-38.

Langendijk PS, Kulik EM, Sandmeier H, Meyer J, van der Hoeven JS. Isolation of Desulfomicrobium orale sp. nov. and Desulfovibrio strain NY682, oral sulfate reducing bacteria involved in human periodontal disease. Int J Syst Evol Microbiol. 2001 May; 51(Pt3):1035-44.

Lau JC, Jacksin-Boeters L, Daley TD, Wysocki GP, Cherian MG. Metallothionein in human gingival amalgam tattoos. Arch Oral Biol 2001; 46:1015-20.

Lauterbach M, Martins IP, Castro-Caldas A, Bernardo M, Luis H, Amaral H, Leitão J, Martin MD, Townes B, Rosenbaum G, Woods JS, Derouen T. Neurological outcomes in children with and without amalgam-related mercury exposure: seven years of longitudinal observations in a randomized trial. J Am Dent Assoc. 2008 Feb; 139(2):138-45.

Lefeuvre M1, Bourd K, Loriot MA, Goldberg M, Beaune P, Périanin A, Stanislawski L.TEGDMA modulates glutathione transferase P1 activity in gingival fibroblasts. J Dent Res. 2004 Dec;83(12):914-9.

Lehtinen R, Kuusilehto A. Absorption of UVA light by latex and vinyl gloves. Scand J Dent Res. 1990; 98: 186-8.

Leistevuo J, Leistevuo T, Helenius H, Pyy L, Huovinen P, Tenovuo J. Mercury in saliva and the risk of exceeding limits for sewage in relation to exposure to amalgam fillings. Arch Environ Health. 2002 Jul-Aug; 57(4):366-70.

Leistevuo J, Leistevuo T, Helenius H, Pyy L, Osterblad M, Huovinen P, Tenovuo J. Dental amalgam fillings and the amount of organic mercury in human saliva. Caries Res. 2001; 35(3):163-6.

Li Y, Carrera C, Chen R, Li J, Lenton P, Rudney JD, Jones RS, Aparicio C, Fok A. Degradation in the dentin-composite interface subjected to multi-species biofilm challenges. Acta Biomater 2014; 10(1): 375-383.

Lin CY, Liou SH, Hsiech CM, Ku MC, Tsai SY. Dose-response relationship between cumulative mercury exposure index and specific uptake ratio in the striatum on Tc-99m TRODAT SPECT. Clin Nucl Me 2011; 36: 689-93.

Lind PO. Oral lichenoid reactions related to composite restorations. Preliminary report. Acta Odontol Scand 1988; 46:63-5.

Lindbohm ML, Ylöstalo P, Sallmén M. Occupational exposures in dentistry and miscarriage. Occup Environ Med 2007; 64:127-33.

Link B, Gabrio T, Zöllner I, Jaroni H, Piechotowski I, Schilling B, Felder-Kennel A, Flicker-Klein A, Konig M, Maisner V, Schick KH, Fischer G., Decrease of internal exposure to chlororganic compounds and heavy metals in children in Baden-Wurttemberg between 1996/1997 and 2008/2009. International Journal of Hygiene and Environmental Health, 2012; 215, 196-201.

Liu PT, Stenger S, Li H, Wenzel L, Tan BH, Krutzik SR, Ochoa MT, Schauber J, Wu K, Meinken C, Kamen DL, Wagner M, Bals R, Steinmeyer A, Zügel U, Gallo RL, Eisenberg D, Hewison M, Hollis BW, Adams JS, Bloom BR, Modlin RL. Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. Science. 2006 Mar 24;311(5768):1770-3.

Lohbauer, U., N. Krämer, G. Siedschlag, E. Schubert, B. Lauerer, F. Müller, A. Petschelt, J. Ebert. Strength and wear resistance of a dental glass-ionomer cement with a novel nanofilled resin coating. Am J Dent. 2011 Apr;24(2):124-8.

Luglie PF, Campus G, Chessa G, Spano G, Capobianco G, Fadda GM, Dessole S., Effects of amalgam fillings on the mercury concentration in human amniotic fluid. Arch Gynecol Obstet 2005; 271:138-142.

Luiz AC, Hirota SK, Dal Vechio A, Reis VM, Spina R, Migliari DA.: Diagnosing oral lichenoid contact reaction: clinical judgment versus skin-patch test. Minerva Stomatol. 2012 Jul-Aug; 61(7-8):311-7.

Lukacinova A, Racz O, Lovasova E, Nistiar F. Effect of lifetime low dose exposure to heavy metals on selected serum proteins of Wistar rats during three subsequent generations. Ecotoxicol Environ Saf. 2011; 74(6): 1747-55.

Lukacinova A, Benacka R, Sedlakova E, Lovasova E, Nistiar F. Multigenerational lifetime low-dose exposure to heavy metals on selected reproductive parameters in rats. J Environ Sci Health A Tox Hazard Subst Environ Eng. 2012; 47(9):1280-7.

Lutz E, Lind B, Herin P, Krakau I, Bui TH, Vahter M. Concentrations of mercury, cadmium and lead in brain and kidney of second trimester fetuses and infants. J Trace Elem Med Biol. 1996; 10, 61-67.

Lygre GB, Gjerdet NR, Björkman I. A follow-up study of patients with subjective symptoms related to dental materials. Community Dent Oral Epidemiol 2005; 33:227-34.

Lygre GB, Gjerdet NR, Grönningsaeter AG, Björkman L. Reporting on adverse reactions to dental materials – intraoral observations at a clinical follow-up. Community Dent Oral Epidemiol 2003; 31:200-6.

Lygre GB, Sjursen TT, Svahn J, Helland V, Lundekvam BF, Dalen K, Björkman L. Characterization of health complaints before and after removal of amalgam fillings - 3-year follow-up. Acta Odontol Scand. 2012 Jul 2.

Lygre H, Hol PJ, Moe G. Organic leachables from polymer-based dental filling materials. Eur J Oral Sci 1999; 107:378-83.

Lygre H, Solheim E, Gjerdet NR, Berg E. Leaching of organic additives from dentures in vivo. Acta Odontol Scand 1993; 51:45-51.

Lygre H. Prosthodontic biomaterials and adverse reactions: a clinical review of the clinical and research literature. Acta Odontol Scand 2002; 60:1-9.

Mackert JR Jr. Randomized controlled trial demonstrates that exposure to mercury from dental amalgam does not adversely affect neurological development in children. J Evid Based Dent Pract. 2010 Mar; 10(1):25-9.

Mainster MA. Violet and blue light blocking intraocular lenses: photoprotection versus photoreception. Br J Opthalmol 2006; 90: 784-792.

MAK Kommission der Deutschen Forschungsgemeinschaft (DFG). Mercury and inorganic mercury compounds. In: Greim H, editor. Occupational Toxicants - Critical data evaluation for MAK values and classification of carcinogens by the commission for the investigation of health hazards of chemical compounds in the work area. München: Wiley-VCH; 1999; Volume 15: p.81-122.

Manhart J, Kunzelmann K-H, Chen HY, Hickel R. Mechanical properties and wear behavior of light-cured packable composite resins. Dental Materials 2000; 16:33-40.

Marino R, Capaccio P, Pignataro L, Spadari F., Burning mouth syndrome: the role of contact hypersensitivity. Oral Dis. 2009 May; 15(4):255-8.

Mariotti A, Söderholm KJ, Johnson S. The in vivo effects of bisGMA on murine uterine weight, nucleic acids and collagen. Eur J Oral Sci. 1998 Dec; 106(6):1022-7.

Maruyama K., Yorifuji T., Tsuda T., Sekikawa T., Nakadaira H., Saito H., Methyl Mercury Exposure at Niigata, Japan: Results of Neurological Examinations of 103 Adults. Journal of Biomedicine and Biotechnology. Volume 2012 (2012).

Marquardt W, Seiss M, Hickel R, Reichl FX, Volatile methacrylates in dental practices. J Adhes Dent. 2009 Apr; 11(2):101-7.

Maserejian NN, Trachtenberg FL, Hauser R, McKinlay S, Shrader P, Tavares M, Bellinger DC. Dental composite restorations and psychosocial function in children. Pediatrics. 2012 Aug; 130(2):e328-38.

Mazzaron Barcelos GR, de Marco KC, Grotto D, Valentini J, Garcia SC, Leite Braga GÚ, Barbosa F Jr. Evaluation of glutathione S-transferase GSTM1 and GSTT1 polymorphisms and methylmercury metabolism in an exposed Amazon population. J Toxicol Environ Health A. 2012; 75(16-17):960-70

McComb D. Occupational exposure to mercury in dentistry and dentist mortality. J Can Dent Assoc 1997; 63:372-76.

McDowell MA, Dillon CH. F., Osterloh J., Bolger P.M., Pellizzari E., Fernando R., Montes de Oca R., Schober S.E., Sinks T., Jones R.L., Mahaffey K.R. Hair Mercury Levels in U.S. Children and Women of Childbearing Age: Reference Range Data from NHANES 1999–2000. Environ Health Perspect. 2004 Aug; 112(11): 1165-1171

McPharland H, Warnakulasuriya S. Oral lichenoid contact lesions to mercury and dental amalgam--a review. J Biomed Biotechnol. 2012; 2012: 589569.

Melchart D, Vogt S, Köhler W, Streng A, Weidenhammer W, Kremers L, Hickel R, Felgenhauer N, Zilker T, Wühr E, Halbach S. Treatment of health complaints attributed to amalgam. J Dent Res. 2008 Apr; 87(4):349-53.

Michelsen VB, Lygre H, Skalevik R, Tveit AB, Solheim E. Identification of eluates from four polymer-based dental filling materials. Eur J Oral Sci 2003; 111:263-71.

Michelsen VB, Moe G, Skalevik R, Jensen E, Lygre H. Quantification of organic eluates from polymerised resin-based dental restorative materials by use of GC/MS. J Chromatogr Analyt Technol Biomed Life Sci 2007; 850(issues 1-2):83-91. (Available online 28 November 2006).

Mickenautsch S, Yengopal V, Banerjee A. Atraumatic restorative treatment versus amalgam restoration longevity: a systematic review. Clin Oral Investig 2010; 14: 233-40.

Miller CS, Leonelli FM, Latham E. Selective interference with pacemaker activity by electrical dental devices. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1998; 85:33-36.

Mitchell RJ, Koike M, Okabe T, Posterior amalgam restorations – usage, regulation and longevity. Dent Clin N Amer 2007; 51:573-89.

Mittermüller P, Szeimies RM, Landthaler M, Schmalz G., A rare allergy to a polyether dental impression material. Clin Oral Investig. 2012 Aug; 16(4):1111-6.

Mjor, I. A. The location of clinically diagnosed secondary caries. Quintessence Int 1998; 29(5): 313-317.

Moen B, Hollund B, Riise T., Neurological symptoms among dental assistants: a cross-sectional study. J Occup Med Toxicol. 2008 May; 18;3:10

Moharamzadeh K, Van Noort R, Brook IM, Scutt AM. HPLC analysis of composites with different resin compositions using different extraction media. J Mater Sci Mater Med 2007; 18:133-7.

Moilanen LH, Dahms JK, Hoberman A. Reproductive toxicity evaluation of the resin monomer triethylene glycol dimethacrylate (TEGDMA) in mice. Int. J. Toxicol. 2014; 33(2):106-15.

Moilanen LH, Dahms JK, Hoberman A. Reproductive toxicity evaluation of the resin monomer BisGMA in mice. Int. J. Toxicol. 2013; 32(6):415-25.

Möller H. Dental gold alloys and contact allergy. Contact Dermatitis. 2002; 47:63-6.

Molin M , Bergman B, Marklund SL, Schütz A, Skerfving S. Mercury, selenium, and glutathione peroxidase before and after amalgam removal in man. Acta Odontol Scand. 1990 Jun; 48(3):189-202.

Montebugnoli L, Venturi M, Gissi DB, Cervellati F., Clinical and histologic healing of lichenoid oral lesions following amalgam removal: a prospective study. Oral Surg Oral Med Oral Pathol Oral Radiol. 2012 Jun; 113(6):766-72.

Moon HJ, Lee YK, Lim BS, Kim CW. Component elution from dental pit and fissure sealants. J Dent Res 2000; 79:191.

Morton J., Mason HJ., Ritchie KA., White M. Comparison of hair, nails and urine for biological monitoring of low level inorganic mercury exposure in dental workers. Biomarkers 2004; 9:47-55.

Mortada WL, Sobh MA, El-Defrawy MM, Farahat SE. Mercury in dental restoration: is there a risk of nephrotoxicity? J Nephrol. 2002 Mar-Apr; 15(2):171-6.

Moszner N, Gianasmidis A, Klapdohr S, Fisher UK, Rheinberger V. Sol-gel materials, 2. Light-curing dental composites based on ormocers of cross.linking alkoxysilane methacrylates and further nano-components. Dental Materials 2008; 24: 851-856.

Munksgaard EC. Toxicology versus allergy in restorative dentistry. Adv Dent Res 1992; 6:17-21.

Murray PE, Smith AJ, Windsor LJ, Mjor IA. Remaining dentine thickness and human pulp responses. Int Endo J 2003; 36(1):33-43.

Murray PE, Windsor LJ, Smyth TW, Hafez AA, Cox CF. Analysis of pulpal reaction to restorative procedures, materials, pulp capping and future therapies. Crit Rev Oral Biol Med 2002; 13(6):504-20.

Musanje M, Darvell BW. Polymerisation of resin composite restorative materials: exposure reciprocity. Dent Mat 2003; 19: 531-41.

Mutter J, Curth A, Naumann J, Deth R, Walach H. Does inorganic mercury play a role in Alzheimer's disease? A systematic review and an integrated molecular mechanism. J Alzheimers Dis. 2010; 22(2):357-74.

Myers DE. Hutz RJ. Current status of potential bisphenol toxicity in dentistry. [Review] General Dentistry. 2011; 59(4):262-5.

Naorungroj S, Slade GD, Beck JD, Mosley TH, Gottesman RF, Alonso A, Heiss G. Cognitive decline and oral health in middle-aged adults in the ARIC study. J Dent Res. 2013 Sep; 92(9):795-801.

Nathanson D, Lockhart P. Delayed extra-oral hypersensitivity to dental composite material. Oral Surg Oral Med Oral Pathol 1979; 47:329-33.

National Research Council. Science and decisions: advancing risk assessment. Washington, D.C.: 2009; National Academy Press.

Ngim CH, Foo SC, Boey KW, Jeyaratnam J. Chronic neurobehavioural effects of elemental mercury in dentists. British journal of industrial medicine. 1992; 49(11): 782-90.

Nicolae A., Ames H., Quiñonez C., Dental amalgam and urinary mercury concentrations: a descriptive study, BMC Oral Health 2013, 13:44.

Nielsen E, Larsen JC, Ladefoged O. Risk assessment of contaminant intake from traditional food items. Danmarks Fødevareforskning; 2006.

O'Brien WJ. Dental materials and their selection, Chicago: Quintessence Publishing Co., Inc.; 2002.

Oberländer H, Hiller KA, Thonemann B, Schmalz G. Clinical evaluation of packable composite resins in Class-II restorations. Clin Oral Investig. 2001 Jun; 5(2):102-7.

Olea N, Pulgar R, Pérez P, Olea-Serrano F, Rivas A, Novillo-Fertrell A, Pedraza V, Soto A M, Sonnenschein C. Estrogenicity of resin based composites and sealants used in dentistry. Env Health Perspec 1996; 104:298-305.

Opdam NJM, Bronkhorst EM, Loomans BAC, Huysmans M-CDNJM. 12-year survival of composite vs amalgam restorations. J Dent Res 2010:89:1063-7.

Opdam NJM, Bronkhorst EM, Roeters JM, Loomans BAC. A retrospective clinical study on longevity of posterior composite and amalgam restorations. Dent Mat 2007; 23:2-8.

Opdam NJM, van de Sande FH, Bronkhorst E, Cenci MS, Bottenberg P, Pallesen U, Gaengler P, Lindberg A, Huysmans MCDNJM, van Dijken JW. Longevity of posterior composite restorations. A systemativ review and meta-analysis. J Dent Res 2014; 93: 943-949.

Opländer C, Hidding S, Werners FB, Born M, Pallua N, Suschek CV. Effects of blue light irradiation on human dermal fibroblasts. J Photochem Photobiol B 2011; 103:118-125.

Örtengren U. On composite resin materials. Degradation, erosion and possible adverse effects in dentists. Swed Dent J 2000; Suppl 141:1-61.

Oysaed, H., Ruyter, I.E., Sjövik Kleven, I.J.: Release of formaldehydefrom dental composites. J Dent Res 1988; 67, 1289–1294.

Ozer F, Unlü N, Oztürk B, Sengun A. Amalgam repair: evaluation of bond strength and microleakage. Oper Dent 2002; 27(2): 199-203.

Palkovicova L, Ursinyova M, Masanova V, Yu Z, Hertz-Picciotto I. Maternal amalgam dental fillings as the source of mercury exposure in developing fetus and newborn. Journal of Exposure Science and Environmental Epidemiology 2008; 18, 326–331; doi:10.1038/sj.jes.7500606; published online 12 September 2007.

Pigatto PD, Guzzi G, Persichini P, Barbadillo S. Recovery from mercury-induced burning mouth syndrome due to mercury allergy. Dermatitis 2004; 15:75-77.

Piirilä P, Hodgson U, Estlander T, Keskinen H, Saalo A, *Voutilainen R, Kanerva L.* Occupational respiratory hypersensitivity in dental personnel. Int Arch Occup Environ Health 2002; 75:209-16.

Poiată A, Bădicuţ I, Indreş M, Biro M, Buiuc D. Mercury resistance among clinical isolates of Escherichia coli. Roum Arch Microbiol Immunol. 2000 Jan-Jun; 59(1-2):71-9.

Polydorou O, König A, Hellwig E, Kümmerer K. Long-term release of monomers from modern dental-composite materials. Eur J Oral Sci 2009; 117;68-75.

Polydorou O, Trittler R, Hellwig E, Kümmerer K. Elution of monomers from two conventional dental composite materials. Dent Mater 2007; 23(12):1535-41.

Powers J, Wataha J. Dental Materials: Properties and Manipulation. New York: Mosby; 2007.

Price R, Shortall A, Palin W. Contemporary issues in light curing. Oper Dent 2014; 39: 4-14.

Price RB., Avoiding pitfalls when using a light-curing unit. Compend Contin Educ Dent. 2013 Apr; 34(4):304-5.

Pulgar R., Olea-Serrano M. F., Novillo-Fertrell A., Rivas A., Pazos P., Pedraza V., Navajas J. M., Olea N.: Determination of bisphenol A an related aromatic compounds released from BisDMA-based composites and sealants by high performance liquid chromatography. Environ Health perspect 2000; 108, 21-27.

Raap U, Stiesch M, Reh H, Kapp A, Werfel T., Investigation of contact allergy to dental metals in 206 patients. Contact Dermatitis. 2009 Jun; 60(6):339-43206.

Ready D, Pratten J, Mordan N, Watts E, Wilson M., The effect of amalgam exposure on mercury- and antibiotic-resistant bacteria. Int J Antimicrob Agents. 2007 Jul; 30(1):34-9. Epub 2007 Apr 24.

Reichl FX, Durner J, Hickel R, Kunzelmann KH, Jewett A, Wang MY, Spahl W, Kreppel H, Moes GW, Kehe K, Walther U, Forth W, Hume WR. Distribution and excretion of TEGDMA in guinea pigs and mice. J Dent Res 2001a; 80:1412-5.

Reichl FX, Durner J, Hickel R, Spahl W, Kehe K, Walther U, Gempel K, Liebl B, Kunzelmann KH, Hume W. Uptake, clearance and metabolism of TEGDMA in guinea pigs. Dent Mater 2002a; 18:581-9.

Reichl FX, Durner J, Kehe K, Manhart J, Folwaczny M, Kleinsasser N, Hume WR, Hickel R., Toxicokinetic of HEMA in guinea pigs. J Dent 2002b; 30:353-8.

Reichl FX, Durner J, Kunzelmann KH, Hickel R, Spahl W, Hume WR, Moes GW, Kehe K, Walther U, Forth W. Biological clearance of TEGDMA in guinea pigs. Arch Toxicol 2001b; 75:22-7.

Reichl FX, Durner J, Manhart J, Spahl W, Gempel K, Kehe K, Liebl B, Walther UI, Hume WR, Hickel R., Biological clearance of HEMA in guinea pigs. Biomaterials 2002c; 23:2135-41.

Reichl FX, Seiss M, Kleinsasser N, Kehe K, Kunzelmann KH, Thomas P, Spahl W, Hickel R, Distribution and excretion of BisGMA in guinea pigs. J Dent Res. 2008 Apr; 87(4):378-80.

Ribeiro, D.A., Marques, M.E.A., Salvadori, D.M.F.: Genotoxicity and cytotoxicity of glass ionomer cements on Chinese hamster ovary (CHO) cells. J Mater Sci: Mater Med 2006; 17, 495–500.

Richardson GM, Wilson R, Allard D, Purtill C, Douma S, Graviere J. Mercury exposure and risks from dental amalgam in the US population, post-2000. Sci Total Environ. 2011; 409(20): 4257-68.

Ritchie KA1, Burke FJ, Gilmour WH, Macdonald EB, Dale IM, Hamilton RM, McGowan DA, Binnie V, Collington D, Hammersley R., Mercury vapour levels in dental practices and body mercury levels of dentists and controls. Br Dent J 2004; 197:625-32.

Ritchie KA1, Gilmour WH, Macdonald EB, Burke FJ, McGowan DA, Dale IM, Hammersley R, Hamilton RM, Binnie V, Collington D., Health and neuropsychological functioning of dentists exposed to mercury. Occupat Environ Med 2002; 59:287-93.

Roberts HW. The effect of electrical dental equipment on a vagus nerve stimulator's function. J Am Dent Assoc 2002; 133: 1657-1664.

Roberts MC, Leroux BG, Sampson J, Luis HS, Bernardo M, Leitão J., Dental amalgam and antibiotic- and/or mercury-resistant bacteria. J Dent Res. 2008 May;87(5):475-9.

Roedig JJ, Shah J, Elayi CS, Miller CS. Interference of cardiac pacemaker and implantable cardioverter-defibrillator activity during electronic dental device use. JADA 2010;141:521-26.

Roeters J, de Kloet H. Handboek voor Esthetische Tandheelkunde. Nijmegen: STI; 1998.

Rogalewicz R, Batco K, Voelkel A. Identificaton of organic extractables from commercial resin modified glass-ionomers using HPLC-MS. J Environ Monit 2006; 8:750-8.

Roggendorf MJ, Krämer N, Appelt A, Naumann M, Frankenberger R., Marginal quality of flowable 4-mm base vs. conventionally layered resin composite. J Dent. 2011 Oct; 39(10):643-7.

Roitt IM, Delves PT. Roitts Essential Immunology. London: Blackwells; 2006.

Rojas-Alcayaga G, Carrasco-Labra A, Danús P, Guzmán MA, Morales-Bozo I, Urzúa B, Ortega-Pinto A. Determination of susceptibility to sensitization to dental materials in atopic and non-atopic patients. Med Oral Patol Oral Cir Bucal. 2012 Mar 1;17(2):e320-4.

Rooney JP. The retention time of inorganic mercury in the brain--a systematic review of the evidence. Toxicol Appl Pharmacol. 2014. 1; 274:425-35. doi: 10.1016/j.taap.2013.12.011. Epub 2013 Dec 22.

Rooney JP. The retention time of inorganic mercury in the brain--a systematic review of the evidence. Toxicol Appl Pharmacol 2014; 274: 425-35.

Roos PM, Dencker L. Mercury in the spinal cord after inhalation of mercury., Basic Clin Pharmacol Toxicol. 2012; 111(2):126-32.

Rothwell JA, Boyd PJ. Amalgam dental fillings and hearing loss. Int J Audiol. 2008 Dec;47(12):770-6.

Rueggeberg, 2010, Ivoclar Scientific Documentation, January 2013 (Dr. Th. Völkel).

Ruyter, I.E. Physical and chemical aspects related to substances released from polymer materials in an aqueous environment. Adv Dent Res 1995; 9, 344–347.

Sallsten G, Barregard L, Schutz A. Clearance half life of mercury in urine after the cessation of long term occupational exposure: influence of a chelating agent (DMPS) on excretion of mercury in urine. Occup Environ Med. 1994; 51(5): 337-42.

Sallsten G, Thoren J, Barregard L, Schutz A, Skarping G. Long term use of nicotine chewing gum and mercury exposure from dental amalgam fillings. J Dent Res 1996; 75:594-8.

Samir AM, Aref WM. Impact of occupational exposure to elemental mercury on some antioxidative enzymes among dental staff. Toxicol Ind Health. 2011 Oct;27(9):779-86.

Sandborgh-Englund G., Elinder C-G., Johanson G., Lind B., Skare I., Ekstrand J., The Absorption, Blood Levels, and Excretion of Mercury after a Single Dose of Mercury Vapor in Humans. Toxicology And Applied Pharmacology 1998; 150, 146–153 Article No. To 988400.

Sanfeliu C, Sebastià J, Cristòfol R, Rodríguez-Farré E. Neurotoxicity of organomercurial compounds. Neurotox Res. 2003; 5(4):283-305.

Santarsiero A, Settimo G, Dell'Andrea E. Mercury emissions from crematoria. Annali dell'Istituto Superiore di Santa 2006; 42:369-73.

Sasaki N1, Okuda K, Kato T, Kakishima H, Okuma H, Abe K, Tachino H, Tuchida K, Kubono K., Salivary bisphenol-A levels detected by ELISA after restoration with composite resin. J Mater Sci Mater Med 2005; 16:297-300.

Saxe SR1, Wekstein MW, Kryscio RJ, Henry RG, Cornett CR, Snowdon DA, Grant FT, Schmitt FA, Donegan SJ, Wekstein DR, Ehmann WD, Markesbery WR., Alzheimer's disease, dental amalgam and mercury. J Am Dent Assoc 1999; 130:191-199.

SCHER scientific opinion on the environmental risks and indirect health effects of mercury from dental amalgam (update 2014), 10 March 2014.

SCENIHR 2014. Preliminary Opinion on the safety of the use of bisphenol A in medical devices. http://ec.europa.eu/health/scientific_committees/emerging/docs/scenihr_o_040.pdf

SCENIHR (Scientific Committee on Emerging and Newly Identified Health Risks), Nanosilver: safety, health and environmental effects and role in antimicrobial resistance, 2013.

SCENIHR (Scientific Committee on Emerging and Newly- Identified Health Risks), Scientific opinion on the Safety of Dental Amalgam and Alternative Dental Restoration Materials for Patients and Users, 6 May 2008.

SCENIHR (Scientific Committee on Emerging and Newly- Identified Health Risks), Memorandum on the use of the scientific literature for human health risk assessment purposes – weighing of evidence and expression of uncertainty, 2012.

Schedle A, Franz A, Rausch-Fan X, Spittler A, Lucas T, Samorapoompichit P, Sperr W, Boltz-Nitulescu G., Cytotoxic effects of dental composites, adhesive substances, compomers and cements. Dent Mater 1998; 14:429–440.

Schedle A, Örtengren U, Eidler N, Gabauer M, Hensten A. Do adverse effects of dental materials exist? What are the consequences, and how can they be diagnosed and treated? Clin Oral Impl Res 2007; 18(suppl3):232-56.

Schedle, A., Samorapoompichit, P., Rausch-Fan, X.H., Franz, A., Füreder, W., Sperr, W.R., Sperr, W., Ellinger, A., Slavicek, R., Boltz-Nitulescu, G., Valent, P.: Response of L-929 fibroblasts, human gingival fibroblasts, and human tissue mast cells to various metal cations. J Dent Res 1995; 74, 1513–1520.

Schläwicke Engström K, Strömberg U, Lundh T, Johansson I, Vessby B, Hallmans G, Skerfving S, Broberg K.. Genetic variation in glutathione-related genes and body burden of methylmercury. Environ Health Perspect 2008; 116: 734-739.

Schmalz G, Krifka S, Schweikl H. Toll-like receptors, LPS, and dental monomers. Adv Dent Res 2011:302-6.

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

Schmalz G. The biocompatibility of non-amalgam dental filling materials. Eur J Oral Sci 1998; 106:696-706.

Schmalz G., Arenholt-Bindslev D.: Biocompatibility of dental materials. Springer, Berlin, Heidelberg (2009).

Schmalz G., Preiss A., Arenholt-Bindslev D.: Bisphenol-A content of resin monomers and related degradation products. Clin Oral Investig 1999; 3, 114 – 119.

Schmalz, G., Arenholt-Bindslev, D., Pfüller, S., Schweikl, H.: Cytotoxicity of metal cations used in dental cast alloys. ATLA 1997; 25, 323–330.

Schmalz, G., Thonemann, B., Riedel, M., Elderton, R.J.: Biological and clinical investigations of a glass ionomer base material. Dent Mater 1994; 10, 4–13.

Schneider LF, Cavalcante LM, Prahl SA, Pfeifer CS, Ferracane JL, Curing efficiency of dental resin composites formulated with camphorquinone or trimethylbenzoyl-diphenyl-phosphine oxide. Dent Mater. 2012 Apr;28(4):392-7.

Schuurs A, Exterkate R, ten Cate JM., Biological mercury measurements before and after administration of a chelator (DMPS) and subjective symptoms allegedly due to amalgam. Eur J Oral Sci. 2000 Dec; 108(6):511-22.)

Schwartz T. 25 years of UV-induced immunosuppression mediated by T-cells – from disregarded T suppressor cells to highly respected regulatory T cells. Photochem Photobiol 2008; 84:10-18.

Schweikl H, Altmannberger I, Hanser N, Hiller KA, Bolay C, Brockhoff G, Spagnuolo G, Galler K, Schmalz G. The effect of triethylene glycol dimethacrylate on the cell cycle of mammalian cells. Biomaterials. 2005; 26:4111-8.

Schweikl H, Hartmann A, Hiller KA, Spagnuolo G, Bolay C, Brockhoff G, Schmalz G. Inhibition of TEGDMA and HEMA-induced genotoxicity and cell cycle arrest by N-acetylcysteine. Dent Mater. 2007 Jun; 23(6):688-95.

Schweikl H1, Hiller KA, Bolay C, Kreissl M, Kreismann W, Nusser A, Steinhauser S, Wieczorek J, Vasold R, Schmalz G. Cytotoxic and mutagenic effects of dental composite materials. Biomaterials 2005; 26:1713-9.

Schweikl H, Schmalz G, Gottke C. Mutagenic activity of various dentine bonding agents. Biomaterials 1996b: 17:1451-6.

Schweikl H, Schmalz G, Rackebrandt K. The mutagenic activity of unpolymerised resin monomers in Salmonella typhimurium and V79 cells. Mutat Res 1998b; 415:119-30.

Schweikl H, Schmalz G, Spruss T. The induction of micronuclei in vitro by unpolymerised resin monomers. J Dent Res. 2001 Jul; 80(7):1615-20.

Schweikl H, Schmalz G, Weinmann W, Mutagenic activity of structurally related oxiranes and siloranes in Salmonella typhimurium. Mutat Res. 2002 Nov 26; 521(1-2):19-27.

Schweikl H, Schmalz G, Weinmann W. The Induction of Gene Mutations and Micronuclei by Oxiranes and Siloranes in Mammalian Cells in vitro. J Dent Res 2004; 83:17-21.

Schweikl H, Schmalz G. Glutaraldehyde-containing dentine bonding agents are mutagens in mammalian cells in vitro. J Biomed Mater Res 1997; 36:284-8.

Schweikl H, Schmalz G. Toxicity parameters for cytotoxicity testing of dental materials in two different mammalian cell lines. Eur J Oral Sci 1996a; 104:292-9.

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

Schweikl H., Schmalz G, Federlin M. Mutagenicity of the root canal sealer AHPlus in the Ames test. Clin Oral Invest 1998a; 2:125-9.

Schwengberg S1, Bohlen H, Kleinsasser N, Kehe K, Seiss M, Walther UI, Hickel R, Reichl FX., In vitro embryotoxicity assessment with dental restorative materials. J Dent 2005; 33:49-55.

Scott A, Egner W, Gawkrodger DJ, Hatton PV, Sherriff M, van Noort R, Yeoman C, Grummitt J., The national survey of adverse reactions to dental materials in the UK: a preliminary survey by the UK Adverse Reactions Reporting Project. Br Dent J 2004; 196:471-7.

Seldén AI1, Persson B, Bornberger-Dankvardt SI, Winström LE, Bodin LS., Exposure to cobalt-chromium dust and lung disorders in dental technicians. Thorax 1995; 50: 769-772.

Sevkusic M, Schuster L, Rothmund L, Dettinger K, Maier M, Hickel R, Van Landhuyt KL, Durner J, Högg C, Reichl FX, The elution and breakdown behavior of constituents from various light-cured composites. Dent Mat 2014.

Shajii l, Santerre JP. Effect of filler content on the profile of released biodegradation products in microfilled bis-gma/tegdma dental composite resins. Biomaterials 1999; 20:1897-1908.

Shenker BJ, Maserejian NN, Zhang A, McKinlay S. Immune function effects of dental amalgam in children: a randomized clinical trial. J Am Dent Assoc. 2008 Nov; 139(11):1496-505.

Sherman LS1, Blum JD, Franzblau A, Basu N., New insight into biomarkers of human mercury exposure using naturally occurring mercury stable isotopes, Environ Sci Technol. 2013 Apr 2;47(7):3403-9.

Shokati B, Tam LE, Santerre JP, Finer Y. Effect of salivary esterase on the integrity and fracture toughness of the dentin-resin interface. J Biomed Mater Res B Appl Biomater 2010; 94(1): 230-237.

Sidhu SK, Glass-ionomer cement restorative materials: a sticky subject? Aust Dent J. 2011 Jun; 56 Suppl 1:23-30.

Sigusch BW, Pflaum T, Völpel A, Schinkel M, Jandt KD. The influence of various light curing units on the cytotoxicity of dental adhesives. Dent Mater. 2009 Nov; 25(11):1446-52.

Silbergeld EK, Silva IA, Nyland JF. Mercury and autoimmunity: implications for occupational and environmental health. Toxicol Appl Pharmacol 2005; 207(suppl 2): 282-92.

Sinclair NA, Thomson WH. Prevalence of self-reported dermatoses in New Zealand dentists. N Z Dent J 2004; 100:38-41.

Sjögren P, Halling A., Long-term cost of direct Class II molar restorations. Swed Dent J. 2002; 26(3):107-14.

Sjursen TT, Lygre GB, Dalen K, Helland V, Laegreid T, Svahn J, Lundekvam BF, Björkman L. Changes in health complaints after removal of amalgam fillings. J Oral Rehabil. 2011 Nov;38(11):835-48.

Skjelvik JM, and Schou Grytli E: Review of Norwegian experiences with the phase-out of dental amalgam use. Norwegian Climate and Pollution Agency 2012.

Sletvold, H., Svendsen, K., Aas, O., Syversen, T., Hilt, B. Neuropsychological function and past exposure to metallic mercury in female dentalworkers. Scandinavian Journal of Psychology 2012; 53, 136–143.

Small BW. A review of devices used for photocuring resin-based composites. Gen Dent 2001; 49:457-60.

Söderholm KJ. Degradation mechanisms of dental resin composites. In: Eliades G, Eliades T, Brantley W.A, Watts DC, editors. Dental Materials In Vivo. Aging and Related Phenomena. Chicago: Quintessence Publishing co, Inc; 2003. p.99-122.

Soncini JA, Maserejian NN, Trachtenberg F, Tavares M, Hayes C, The longevity of amalgam versus compomer/composite restorations in posterior primary and permanent teeth: findings From the New England Children's Amalgam Trial. J Am Dent Assoc. 2007 Jun; 138(6):763-72.

Sørensen FW, Larsen JO, Eide R, Schiønning JD., Neuron loss in cerebellar cortex of rats exposed to mercury vapor: a stereological study. Acta Neuropathol 2000; 100(1):95-100.

Spahl W, Budzikiewicz H, Geursten W. Determination of leachable components from four commercial dental composites by gas and liquid chromatography/mass spectrometry. J Dent 1998; 26:137-45.

Spranley TJ, Winkler M, Dagate J, Oncale D, Strother E. Curing light burns. Gen Dent 2012;60:e210-214.

Spulber S, Rantamäki T, Nikkilä O, Castrén E, Weihe P, Grandjean P, Ceccatelli S., Effects of maternal smoking and exposure to methylmercury on brain-derived neurotrophic factor concentrations in umbilical cord serum. Toxicol Sci. 2010 Oct; 117(2):263-9.

Stanislawski L, Daniau X, Lauti A, Goldberg M. Factors responsible for pulp cell cytotoxicity induced by resin-modified glass ionomer cements. J Biomed Mater Res 1999; 48:277-88.

Stanislawski L, Lefeuvre M, Bourd K, Soheili-Majd E, Goldberg M, Perianin A. TEGDMA-induced toxicity in human fibroblasts is associated with early and drastic glutathione depletion with subsequent production of oxygen reactive species. J Biomed Mater Res A 2003; 66:476-82.

Stanislawski L, Soheili-Majd E, Perianin A, Goldberg M. Dental restorative biomaterials induce glutathione depletion in cultured human gingival fibroblast: protective effect of N-acetyl cysteine. J Biomed Mater Res 2000; 51:469-74.

Stone ME, Cohen ME, Stone Debban, BA. Mercury vapour levels in exhaust air from dental vacuum systems. Dent. Mater. 2007; 23:527-32.

Stoz F, Aicham P, Jovanovic S, Steuer W, Mayer R., Effects of new dental amalgam fillings in pregnancy on Hg concentration in mother and child. With consideration for possible interactions between amalgam and precious metals]. [Article in German], Zentralbl Gynakol. 1995; 117(1):45-50.

Summers AO, Wireman J, Vimy M J, Lorscheider F L, Marshall B, Levy S B, Bennett S, Billard L. Mercury released from dental "silver" fillings provokes an increase in mercury- and antibiotic-resistant bacteria in oral and intestinal floras of primates. Antimicrob Agents Chemother. 1993 Apr;37(4):825-34.Sundström A, Bergdahl J, Nyberg L, Bergdahl M, Nilsson LG. Cognitive status in persons with amalgam-related complaints. J Dent Res. 2010 Nov;89(11):1236-40.

Sundström A, Bergdahl J, Nyberg L, Bergdahl M, Nilsson LG. Stressful negative life events and amalgam-related complaints. Community Dent Oral Epidemiol. 2011 Feb;39(1):12-8. doi: 10.1111/j.1600-0528.2010.00571.

Sunnegårdh-Grönberg K, Peutzfeldt A, van Dijken JWV. Flexural strength and modulus of a novel ceramic restorative cement intended for posterior restorations. Acta Odontol Scand 2003; 61:87-92.

Suñol C, Rodríguez-Farré E. In vitro models for methylmercury neurotoxicity: effects on glutamatergic cerebellar granule neurons. In Methylmercury and Neurotoxicity (S. Ceccatelli & M. Aschner Eds). Current Topics in Neurotoxicity 2012; 2: 259-270.

Suñol C, Rodríguez-Farré E. In vitro models for methylmercury neurotoxicity: effects on glutamatergic cerebellar granule neurons. In Methylmercury and Neurotoxicity (S. Ceccatelli & M. Aschner Eds). Current Topics in Neurotoxicity 2012; 2: 259-270.

Svendsen K, Rękojeść B. The agreement between workers and within workers in regard to occupational exposure to mercury in dental practice assessed from a questionnaire and an interview. Journal of Occupational Medicine and Toxicology 2011, 6:8.

Symanski E, Sällsten G, Chan W, Barregård L. Heterogeneity in sources of exposure variability among groups of workers exposed to inorganic mercury. Ann Occup Hyg. 2001; 45(8):677-87.

Takahashi Y, Imazato S, Russell RR, Noiri Y, Ebisu S. Influence of resin monomers on growth of oral streptococci. J Dent Res 2004; 83(4): 302-306.

Takeuchi T, Eto K. The pathology of Minamata Disease. A Tragic Story of Water Pollution. Fukuoka: Kyushu University Press, 1999.

Tamm C, Duckworth J, Hermanson O, Ceccatelli S. High susceptibility of neural stem cells to methylmercury toxicity: effects on cell survival and neuronal differentiation. J Neurochem. 2006; 97(1):69-78.

Tarumi H, Imazato S, Narimatsu M, Matsuo M, Ebisu S. Estrogenicity of fissure sealants and adhesive resins determined by reporter gene assay. J Dent Res. 2000 Nov; 79(11):1838-43.

Thornhill MH, Pemberton MN, Simmons RK, Theaker ED. Amalgam contact hypersensitivity lesions and oral lichen planus. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2003; 95:291-99.

Thygesen LC, Flachs EM, Hanehøj K, Kjuus H, Juel K. Hospital admissions for neurological and renal diseases among dentists and dental assistants occupationally exposed to mercury. Occup Environ Med. 2011 Dec;68(12):895-901.

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

Urban P, Gobba F, Nerudová J, Lukás E, Cábelková Z, Cikrt M. Color discrimination impairment in workers exposed to mercury vapor. Neurotoxicology. 2003 Aug; 24(4-5):711-6.

Ursinyova M, Uhnakova I, Serbin R, Masanova V, Husekova Z, Wsolova L. The relation between human exposure to mercury and thyroid hormone status. Biol Trace Elem Res. 2012 Sep;148(3):281-91.

Vamnes JS, Eide R, Isrenn R, Höl PJ, Gjerdet NR., Diagnostic value of a chelating agent in patients with symptoms allegedly caused by amalgam fillings. J Dent Res. 2000 Mar; 79(3):868-74).

Vamnes JS, Lygre GB, Grönningsaeter AG, Gjerdet NR. Four years of clinical experience with an adverse reaction unit for dental biomaterials. Community Dent Oral Epidemiol 2004; 32:150-7.

Van der Hoeven JS, Van den Kieboom CWA, Schaeken MJM. Sulfate-reducing bacteria in the periodontal pocket. Oral Microbiol Immunol. 1995 Oct; 10(5):288-90.

van Dijken J WV. Durability of resin composite restorations in high C-factor cavities. A 12-year follow-up. J Dentistry 2010; 38:469-474.

van Dijken JW, Pallesen U. Four-year clinical evaluation of Class II nano-hybrid resin composite restorations bonded with a one-step self-etch and a two-step etch-and-rinse adhesive. J Dent. 2011 Jan; 39(1):16-25.

van Dijken JWV, Sunnegårdh-Grönberg K. A two-year clinical evaluation of a new calcium aluminate cement in Class II cavities. Acta Odontol Scand 2003; 61: 235-240.

van Dijken JWV, Hasselrot L. A prospective 15-year follow up of extensive dentin-enamel-bonded pressed ceramic coverages. Dental Mater 2010; 26:929-939.

van Dijken JWV. A 6-year prospective evaluation of a one-step HEMA-free self etching adhesive in Class II restorations. Dental Materials 2013; 29; 1116-1122.

Van Landuyt KL, Nawrot T, Geebelen B, De Munck J, Snauwaert J, Yoshihara K, Scheers H, Godderis L, Hoet P, Van Meerbeek B. How much do resin-based dental materials release? A meta-analytical approach. Dent Mater. 2011 ; 27:723-47.

Van Landuyt KL, Snauwaert J, De Munck J, Peumans M, Yoshida Y, Poitevin A, Coutinho E, Suzuki K, Lambrechts P, Van Meerbeek B, Systematic review of the chemical composition of contemporary dental adhesives. Biomaterials. 2007 Sep;28(26):3757-85.

Van Landuyt KL, Yoshihara K, Geebelen B, Peumans M, Godderis L, Hoet P, Van Meerbeek B., Should we be concerned about composite (nano-)dust? Dent Mater. 2012 Nov;28(11):1162-70).

van Noort R1, Gjerdet NR, Schedle A, Björkman L, Berglund A., An overview of the current status of national reporting systems for adverse reactions to dental materials. J. Dent. 2004; 32:351-358.

Vangstein A. Case report: Dental light-curing unit and brain stimulator electrodes - a risk? Nor Tannlegeforen Tid 2003; 113:337.

Vidnes-Kopperud S, Tveit AB, Espelid I: Changes in the treatment concept for approximal caries from 1983 to 2009 in Norway. Caries Research 2011;45:113-120.

Volk J, Engelmann J, Leyhausen G, Geurtsen W. Effects of three resin monomers on the cellular glutathione concentration of cultured human gingival fibroblasts. Dent Mater 2006;22:499-505.

Wada H, Tarumi H, Imazato S, Narimatsu M, Ebisu S. In vitro estrogenicity of resin composites. J Dent Res 2004 Mar;83(3):222-6.

Wang JY, Wicklund BH, Gustilo RB, Tsukayama DT. Titanium, chromium and cobalt ions modulate the release of bone-associated cytokines by human monocytes/macrophages *in vitro.*Biomaterials. 1996; 17(23):223-40.

Wang Y, Goodrich JM, Werner R, Gillespie B, Basu N, Franzblau A. 2012. An investigation of modifying effects of single nucleotide polymorphisms in metabolism-related genes on the relationship between peripheral nerve function and mercury levels in urine and hair. Sci Total Environ 417-418: 32-38.

Warwick R, O'Connor A, Lamey B.,Mercury vapour exposure during dental student training in amalgam removal. J Occup Med Toxicol. 2013 Oct 3;8(1):27.

Wataha JC, Rueggeberg FA, Lapp CA, Lewis JB, Lockwood PE, Ergle JW, Mettenberg DJ. In vitro cytotoxicity of resin-containing restorative materials after aging in artificial saliva. Clinical Oral Investigations 1999; 3:144-9.

Wataha JC, Schmalz G. Dentalegierungen. In: Schmalz G, Arenholt-Bindslev D, editors. Biokompatibilität zahnarztlicher Werkstoffe. München: Elsevier GmbH; 2005. p.212-44.

Wataha JC, Schmalz G.: Konzepte zur Biokompatibilität. [Concepts for biocompatibility] Zahnärztl Mitt 2001; 91, 1830–1834.

Watson GE, Lynch M, Myers GJ, Shamlaye CF, Thurston SW, Zareba G, Clarkson TW, Davidson PW. Prenatal exposure to dental amalgam: evidence from the Seychelles Child Development Study main cohort. J Am Dent Assoc. 2011 Nov; 142(11):1283-94.

Weidenhammer W, Hausteiner C, Zilker T, Melchart D, Bornschein S. Does a specific dental amalgam syndrome exist? A comparative study. Acta Odontol Scand. 2009; 67(4):233-9.

Weinmann W, Thalacker C, Guggenberger R., Siloranes in dental composites. Dent Mater. 2005 Jan; 21(1):68-74.

WHO (World Health Organisation). Concise International Chemical Assessment Document 50. Elemental mercury and inorganic mercury compounds: human health aspects. Geneva: World Health Organization; 2003.

WHO (World Health Organisation). Environmental Health Criteria 101, Methylmercury. Geneva: World Health Organisation, International Programme on Chemical Safety; 1990.

WHO (World Health Organisation). Environmental Health Criteria 118, Inorganic mercury. Geneva: World Health Organisation, International Programme on Chemical Safety; 1991.

WHO (World Health Organisation), Future Use of Materials for Dental Restoration, 2011.

WHO (World Health Organisation), Study on potential for reducing mercury pollution from dental amalgam and batteries, 2012.

Wilson AD, Kent BE. A new translucent cement for dentistry. The glass ionomer cement. Br Dent J 1972; 132:133-5.

Wilson AD, Prosser HJ, Powis DM. Mechanism of adhesion of polyelectrolyte cements to hydroxyapatite. J Dent Res 1983; 62:590-2.

Wong L, Freeman S. Oral lichenoid lesion (OLL) and mercury in amalgam fillings. Contact Dermatitis 2003; 48:74-79.

Woods JS, Echeverria D, Heyer NJ, Simmonds PL, Wilkerson J, Farin FM. The association between genetic polymorphisms of coproporphyrinogen oxidase and an atypical porphyrinogenic response to mercury exposure in humans. Toxicol Appl Pharmacol 2005; 206(2): 113-120.

Woods JS, Heyer NJ, Echeverria D, Russo JE, Martin MD, Bernardo MF, Luis HS, Vaz L, Farin FM. Modification of neurobehavioral effects of mercury by a genetic polymorphism of coproporphyrinogen oxidase in children. Neurotoxicol Teratol. 2012 Jul 2; 34(5):513-521. [Epub ahead of print]

Woods JS1, Heyer NJ, Echeverria D, Russo JE, Martin MD, Bernardo MF, Luis HS, Vaz L, Farin FM. Modification of neurobehavioral effects of mercury by a genetic polymorphism of coproporphyrinogen oxidase in children. Neurotoxicol Teratol 2012; 34(5): 513-521.

Woods JS, Heyer NJ, Russo JE, Martin MD, Pillai PB, Bammler TK, Farin FM. Genetic polymorphisms of catechol-o-methyltransferase modify the neurobehavioral effects of mercury in children. J Toxicol Environ Health A. 2014;77(6):293-312.

Woods JS, Heyer NJ, Russo JE, Martin MD, Pillai PB, Farin FM. Modification of neurobehavioral effects of mercury by genetic polymorphisms of metallothionein in children. Neurotoxicol Teratol 2013; 39C: 36-44.

Woods JS, Martin MD, Leroux BG, DeRouen TA, Bernardo MF, Luis HS, Leitão JG, Kushleika JV, Rue TC, Korpak AM. Biomarkers of kidney integrity in children and adolescents with dental amalgam mercury exposure: findings from the Casa Pia children's amalgam trial. Environ Res. 2008 Nov; 108(3):393-9.

Woods JS, Martin MD, Leroux BG, DeRouen TA, Bernardo MF, Luis HS, Leitão JG, Simmonds PL, Echeverria D, Rue TC. Urinary porphyrin excretion in children with mercury amalgam treatment: findings from the Casa Pia Children's Dental Amalgam Trial. J Toxicol Environ Health A. 2009; 72(14):891-6.

Woods JS, Martin MD, Leroux BG, DeRouen TA, Leitão JG, Bernardo MF, Luis HS, Simmonds PL, Kushleika JV, Huang Y. The Contribution of Dental Amalgam to Urinary Mercury Excretion in Children. Env Health Perspec 2007; 115(10): 1527- 1531.

Woods, J. S., Heyer, N. J., Russo, J. E., Martin, M. D., Farin, F. M. Genetic polymorphisms affecting susceptibility to mercury neurotoxicity in children: Summary findings from the Casa Pia Children's Amalgam Clinical Trial. Neurotoxicology 2014; 44C, 288–302.

Wrangsjö K, Swartling C, Meding B. Occupational dermatitis in dental personnel: contact dermatitis with special reference to (meth)acrylates in 174 patients. Contact Dermatitis 2001; 45:158-63.

Yap AU, Soh MS. Thermal emission by different light-curing units. Oper Dent 2003; 2.

Yap AY, Soh MS. Post-gel polymerisation contraction of "low shrinkage" composite restoratives. Operative Dentistry 2004; 29;182-7.

Ye X, Qian H, Xu P, Zhu L, Longnecker MP, Fu H fillings. Nephrotoxicity, neurotoxicity, and mercury exposure among children with and without dental amalgam. Int J Hyg Environ Health. 2009 Jul; 212(4):378-86.

Yilmaz A, Ozdemir CE, Yilmaz Y., A delayed hypersensitivity reaction to a stainless steel crown: a case report. J Clin Pediatr Dent. 2012 Spring; 36(3):235-8.

Yoshida M, Honda M, Watanabe C, Satoh M, Yasutake A. Neurobehavioral changes and alteration of gene expression in the brains of metallothionein-I/II null mice exposed to low levels of mercury vapor during postnatal development. J Toxicol Sci. 2011; 36(5):539-47.8:260-6.

Yoshida M, Satoh H, Sumi Y. Effect of ethanol pretreatment on mercury distribution in organs of fetal guinea pigs following in utero exposure to mercury vapor. Toxicology. 1997; 119(3): 193-201.

Zalups RK. Reductions in renal mass and the nephropathy induced by mercury. Toxicol Appl Pharmacol 1997; 143: 366-79.

Zhou J, Paul A, Bennani V, Thomson WM, Firth NA. New Zealand dental practitioners' experience of patient allergies to dental alloys used for prosthodontics. N Z Dent J. 2010 Jun; 106(2):55-60.

Zimmer B. Lee G, Balmer NV, Meganathan K, Sachinidis A, Studer L Leist M.*:* Evaluation of Developmental Toxicants and signalling pathways in a functionaln test based on the migration of human neural crest cells. Environ Health Perspect 2012; 120:1116–1122.

Annex I. Organic chemicals in resin-based restorative materials

The following list is based on a compilation by Schmalz and Arenholt-Bindslev (2009).

Bisphenol A dimethacrylate,
CAS number: 3253-39-2

bisphenol A diglycidyl methacrylate (Bis- GMA),
CAS number: 1565-94-2

ethoxylated Bisphenol-A (Bis-EMA).
BisphenolA ethoxylate dimethacrylate
CAS number 24448-20-2
(also: CAS Number 41637-38-1 for higher molecular substance)

Urethane dimethacrylate, UDMA
CAS number: 72869-86-4

urethane bisphenol-A-dimethacrylate UPGMA
nothing found!

Triethylene glycol dimethacrylate
CAS number: 109-16-0

triethylene glycol monomethacrylate (TEGMA)
CAS number: 39670-09-2
Mol wt. 246

Tetraethylene glycol dimethacrylate
CAS number: 109-17-1

Di(ethylene glycol) dimethacrylate (DEGDMA)
CAS number: 2358-84-1

Ethylene glycol dimethacrylate (EGDMA)
CAS number: 97-90-5

1,10-Decanediol dimethacrylate
CAS number 6701-13-9

1.6 Hexanediol Dimethacrylate
CAS number 6606-59-3

2-hydroxyethyl methacrylate
CAS Number 868-77-9
1,5-pentanediol dimethacrylate
CAS number: 13675-34-8

1,4-Butanediol dimethacrylate
CAS number 2082-81-7

BDDMA-methanol-adduct ½
Nothing found

BDDMA-auto-adduct ½
Nothing found

1,2-propanediol dimethacrylate
CAS number 7559-82-2)

bis(oxymethyl)tricyclo[5.2.1.02,6]decane
nothing found

Benzyl methacrylate
CAS number 2495-37-6

3-(trimethoxysilyl)propyl methacrylate
CAS number 2530-85-0

Trimethylolpropane trimethacrylate
CAS number 3290-92-4

Methyl methacrylate
CAS number 80-62-6

Methacrylic acid
CAS number 79-41-4

Additional substances analysed for in extracts from dental composite resins by Landuyt *et al.,* 2011.

Trivial name	**Chemical name Molecular**	**mass**
BADGE	Bisphenol A diglycidyl ether	340.45

BADGE, 2,2-bis(4-hydroxyphenyl)propane, *bisphenol A* diglycidyl ether (*BADGE*)

CAS No. 1675-54-3

BHT	Butylatedhydroxytoluene	220

2,6-Di-tert-butyl-4-methylphenol

CAS Number 128-37-0

BPA	Bisphenol A	228.29

2,2-Bis(4-hydroxyphenyl)propane,

CAS Number 80-05-7

CQ	Camphorquinone	166

2,3-Bornanedione

CAS Number: 10373-78-1

DMABEE	Ethyl4-(dimethylamino)benzoate	193

CAS Number: 10287-54-4

EBPA	Bisphenol A ethoxylate	316

CAS Number: 32492-61-8

HMBP	2-hydroxy-4-methoxybenzophenone	228.25

CAS Number: 131-57-7

HQ	Hydroquinone	110.1
CAS Number: 123-31-9		
Irgacure	1,2-Diphenyl-2,2-dimethoxyethanone	256.3
CAS Number:		
MEHQ	4-Methoxyphenol	124.14
CAS Number: 24650-42-8		
PBPA	Bisphenol A propoxylate	344
(propoxylated Bisphenol A)		
CAS Number: 37353-75-6		
Quantacure BEA		
2-n-butoxyethyl-4-dimethyl-aminobenzoat		
CAS Number: 67362-76-9		
TMA	3-(Trimethoxysilyl)-propylmethacrylate	248.35
CAS Number: 2530-85-0		
TIN P (drometrizole)	2-(2-Hydroxy-5-methylphenyl)benzotriazole	225.1
CAS Number: 2440-22-4		
TMPTMA	Trimethylolpropanetrimethacrylate	338.2
CAS Number: 3290-92-4		