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Mouthwash Use and the Prevention of Plaque, Gingivitis and Caries

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Executive Summary

Careful quantitative assessment of data regarding use of mouthwash and risk of common oral conditions reveals that there is a clear evidence of benefit from use in terms of reducing the risk of dental plaque, gingivitis, dental caries and that there are no major adverse effects including no evidence of an increased risk of oral cancer among users of mouthwash containing alcohol.

Despite limitations in the quality of many studies conducted, there is a significant reduction of both dental plaque and gingivitis associated with use of mouthwash preparations containing *chlorhexidine* or *essential oils* as an adjunct to standard care. The effect of mouthwash containing *essential oils* on both plaque and gingivitis is less than *chlorhexidine* in studies of less than 3 months duration but improves with increasing duration of use and equals or exceeds the effect of *chlorhexidine* when use is 6 months of longer. Mouthwash preparations containing *cetylpyridinium* or *triclosan* may also be effective, but less than the two former, while mouthwashes containing *delmopinol* are not effective for plaque and gingivitis control. However, there is a large degree of heterogeneity and strong evidence of publication bias: there is a lack of small studies with a small effect of mouthwash. This results in a biased estimate of effect (over-estimated) because there is a tendency to publish mainly positive studies (those showing a strong decrease).

Compared with fluoride toothpaste used alone, topical fluorides (mouthrinses, gels or varnishes) used in addition to fluoride toothpaste reduce caries by 10% on average. Topical fluorides (mouthrinses, gels, or varnishes) used in addition to fluoride toothpaste achieve a modest reduction in caries compared to toothpaste used alone. No conclusions about any adverse effects can be reached, because such data were rarely reported in the trials. It is possible to conclude that mouthwash containing fluoride is a useful adjunct to fluoridated toothpaste in reducing caries in children.

As regards oral malignancy, quantitative analysis of mouthwash use and oral malignancy revealed no statistically significant association between mouthwash use and risk of oral cancer including no significant trend in risk with increasing daily use; and no association between use of mouthwash containing alcohol and oral cancer risk.

Most recent estimates of the population attributable risk for alcohol consumption and oral cancer put the figure at 1%. The dose of acetaldehyde from mouthwash is minute compared to that from other sources notable cigarette smoking, alcohol drinking and certain foodstuffs including yoghurt and peas. It is extremely unlikely from a theoretical viewpoint that mouthwash could be a cause of oral cancer and this is substantiated from the lack of evidence of carcinogenicity found in epidemiological studies in humans.

In summary, there is evidence supporting the use of mouthwashes in terms of preventing or reducing the risk of developing a number of common conditions notably dental plaque, gingivitis and dental caries without any adverse effects. There is also evidence that mouthwash use does not increase the risk of oral cancer even when it contains a significant percentage of alcohol. Mouthwash use makes a significant contribution to public health.

Summary

Generally when there is talk of public health, dental public health is largely ignored despite the mouth being the gateway to the human body for a wide range of viruses, bacteria and pathogens. Current concentration on non-communicable disease worldwide focuses on the *big four*: cancer, diabetes, cardiovascular disease and respiratory diseases. In many respects, this is a tragic situation with more common, but less high-profile and less lethal, non-communicable diseases not high on the international agenda. Benign urinary problems effect ageing men and women much more frequently than any of the four diseases mentioned above. Similarly, with arthritis, dementia and other conditions, importantly among them dental disease. While these latter conditions may be benign in terms of mortality, they can frequently be malignant in terms of their impact on quality of life.

This century should be focused on prevention and dental disease is a key target where much could be accomplished in this domain. There are several known ways to prevent oral diseases notably by eradication or reduction of tobacco smoking. This is associated with an increased risk of periodontal disease and oral malignancy. It would be an important initiative to reduce tobacco use in terms of a reduction in the burden of disease in the mouth.

In this report, the literature on intervention trials dealing with mouthwash (or mouth rinse) is evaluated by a series of meta-analyses. The effect of mouthwash use on the risk of dental plaque, gingivitis, dental caries and oral malignancy is quantified. The impact on Dental Public Health is significant, as will become apparent.

A cautionary note is necessary. This entire field of research suffers from poor methodology, small studies which are frequently poorly described and an absence of harmonized methodology. While guidelines and published articles on recommendations provide useful guidance on many topics related to research conduct, principally on clinical aspects and quantitative indices, they have largely failed to address fundamental issues such as how data should be collected and analysed, how randomization should be done, techniques for blinding of endpoint assessors (or to minimize bias), minimization of between-assessor reproducibility and how final design and results should be reported. In addition, many of the key studies are old and have been conducted in an era when study methodology was not so advanced, or such an issue, as it is in the modern environment.

Careful quantitative assessment of data regarding use of mouthwash and risk of common oral conditions reveals that there is a clear benefit from its use in terms of reducing the risk of dental plaque, gingivitis, dental caries and that there are no adverse effects including no risk of oral cancer among users of mouthwash containing alcohol. Of course, mouthwash is not designed to be employed in isolation and has been tested in combination with tooth brushing and flossing.

Despite limitations in the quality of many studies conducted, there is a significant reduction of both dental plaque and gingivitis associated with use of mouthwash preparations containing *chlorhexidine* or *essential oils* as an adjunct to standard care. The effect of mouthwash containing *essential oils* on both plaque and gingivitis is less than *chlorhexidine* in studies of less than 3 months duration but improves with increasing duration of use and equals or exceeds the effect of *chlorhexidine* when use is 6 months of longer. Mouthwash preparations containing *cetylpyridinium* or *triclosan* are also effective, but less than the two former, while mouthwashes containing *delmopinol* are not effective for plaque and gingivitis control. However, there is a large amount of heterogeneity and strong indications of publication bias present: there is a lack of small studies with a small effect of mouthwash. This results in a biased estimate of effect (over-estimated) because there is a tendency to publish mainly positive studies (those showing a strong decrease).

Compared with fluoride toothpaste used alone, topical fluorides (mouthrinses, gels, or varnishes) used in addition to fluoride toothpaste reduce caries by 10% on average. Topical fluorides (mouthrinses, gels, or varnishes) used in addition to fluoride toothpaste achieve a modest reduction in caries compared to toothpaste use alone. No conclusions about any adverse effects could be reached, because such data were rarely reported in the trials. It is possible to conclude that mouthwash containing fluoride is a useful adjunct to fluoridated toothpaste in reducing caries in children.

Based on a substantial body of evidence, tobacco use, in all its forms, and alcohol consumption have both identified as human carcinogens. Both tobacco smoking and alcohol drinking have been strongly associated with the risk of developing oral malignancy. As regards alcohol consumption, ethanol has been identified as being carcinogenic to humans. In addition, there is substantial mechanistic evidence in humans who are deficient in aldehyde dehydrogenase that acetaldehyde derived from the metabolism of ethanol in alcoholic beverages contributes to the causation of malignant oesophageal tumours.

In October 2009, an IARC Working Group, assembled for Monograph Volume 100E, reviewed "Alcohol drinking" as a Group-1 carcinogenic agent. This Working Group considered that acetaldehyde is a genotoxic compound that is detoxified by aldehyde dehydrogenases (ALDH); that the *ALDH2*2* variant allele, which encodes an inactive enzyme, is prevalent in up to 30% of east-Asian populations; and that heterozygous carriers, who have about 10% enzyme activity, accumulate ac-

etaldehyde and have considerably higher relative risks for alcohol-related oesophageal and head and neck cancers compared with individuals with the common alleles. The Working Group concluded that "acetaldehyde associated with alcoholic beverages" is *carcinogenic to humans.* It is important to note that this applies to acetaldehyde derived from alcohol drinking and predominantly to east Asian populations.

The possibility of alcohol in mouthwash being converted to acetaldehyde in the oral cavity, which then may cause DNA damage and lead to mutations, cannot be concluded without additional studies designed to address this specific issue and to fully characterize the possibility that large inter-individual variability may exist in humans with regards to acetaldehyde formation. The existing evidence is based on a study with four subjects (see section 6.5). It is of great importance to bear in mind that the recent review conducted by the German authorities (*Gesundheitliche Bewertung von Acetaldehyd in alkoholischen Getränken Aktualisierte Stellungnahme Nr. 022/2010 des BfR vom 04. Mai 2010*) concluded after a review of all the evidence, that "alcohol in mouthwash solutions is not regarded as a risk to health with regard to the formation of acetaldehyde".

A recent large, multi-centric study from Europe, concluded that tobacco and alcohol are major risk factors for upper aerodigestive tract (UADT) cancer and significant variation was observed in UADT cancer rates across Europe. It was estimated that tobacco smoking and alcohol consumption together explained 73% of UADT cancer burden of which nearly 29% was explained by smoking alone, less than 1% due to alcohol on its own and 44% by the joint effect of tobacco and alcohol.

These recent estimates of the population attributable risk for alcohol consumption on its own and oral cancer put the figure below 1%. The dose of carcinogen from alcohol containing mouthwash is minute compared to that derived from other sources notable cigarette smoking, alcohol drinking and certain foodstuffs including yoghurt and peas. It is extremely unlikely from a biological perspective that mouthwash could be a cause of oral cancer and this is substantiated from the lack of evidence of carcinogenicity found in epidemiological studies in humans. Quantitative analysis of mouthwash use and oral malignancy revealed no statistically significant association between mouthwash use and risk of oral cancer including no significant trend in risk with increasing daily use; and no association between use of mouthwash containing alcohol and oral cancer risk.

In summary, there is evidence for the value of using mouthwash in terms of preventing or reducing the risk of developing a number of common conditions notably dental plaque, gingivitis and dental caries without any adverse effects. There is also evidence that mouthwash use does not increase the risk of oral cancer even with the use of mouthwash containing a significant percentage of alcohol. Mouthwash use makes a significant contribution to Public Health.

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List of abbreviations

There will be a number of abbreviations used throughout this text. Some of the most commonly employed are listed below.

CHX:	chlorhexidine
COT:	cross-over trial
CPC:	cetylpyridinium
DEL:	delmopinol
EO:	essential oils
GI:	same as L&S1963
HA:	hydro-alcoholic solution
L&S1963:	Loë & Silness (1963) gingivitis score
MGI:	modified gingival index (Lobene et al., 1986)
P1I:	same as S&L1964
RCT:	randomised controlled trial
RETD:	relative end-of-trial difference
SRD:	summary relative difference
S&L1964:	Silness & Loë (1964) plaque score
TM:	Turesky modification of the Quigley-Hein Index for plaque evaluation (Turesky et al., 1970)
TRI:	triclosan
95% CI:	95% confidence interval
s.d.:	standard deviation
SRD:	summary relative difference
RR:	relative risk
HR:	hazard ratio
OR:	odds ratio
SRR:	summary relative risk

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Chapter 1

Introduction and background

1.1 Preamble

The United Nations General Assembly, in its resolution A/RES/65/238, decided to hold an extraordinary, high-level meeting of the General Assembly on the prevention and control of non-communicable diseases in September 2011 at its headquarters in New York. This meeting was called to address the prevention and control of non-communicable diseases worldwide, with a particular focus on developmental and other challenges, and social and economic impacts, particularly for developing countries.

The World Health Organization derived an Action Plan (Appendix I) which was employed in the preparation of this meeting. It was stated that 'working in partnership to prevent and control the four non-communicable diseases – cardiovascular diseases, diabetes, cancers and chronic respiratory diseases and the four shared risk factors – tobacco use, physical inactivity, unhealthy diets and the harmful use of alcohol' (Anantharaman *et al*, 2011).

It is a tragedy that this unique opportunity did not consider more common but less high-profile and less lethal non-communicable diseases. Benign urinary problems effect ageing men and women much more frequently than any of the four diseases mentioned above, similarly with arthritis, dementia and other conditions among them dental disease. While these conditions may be benign in terms of mortality, they are frequently malignant in terms of their impact on quality of life.

It is, however, symptomatic of how little attention is given to the problems of benign conditions of the mouth such as plaque, gingivitis and caries, and even oral malignancy, despite their ubiquitous presence. Interestingly, unlike many other of the conditions considered at this Summit, there have been studies investigating the prevention of these dental conditions and there are several means identified which reduce their frequency and burden.

Sadly, in general when there is talk of public health, dental public health is largely ignored despite the mouth being the gateway to the human body of a wide range of viruses, bacteria and a wide range of pathogens. In this report, the literature on intervention trials dealing with mouthwash (or mouth rinse) is evaluated, and by a series of meta-analyses, the effect of mouthwash use on the risk of gingivitis, dental plaque, dental caries and oral malignancy is quantified. The impact of dental public health will become apparent.

1.2 Periodontal disease

Periodontal disease is extremely common and encompasses a number of conditions that affect one or more of the periodontal tissues. While many different diseases affect the tooth-supporting structures, plaque-induced inflammatory lesions make up the great majority of periodontal diseases (Page and Schroeder, 1976) and have traditionally been grouped into either *gingivitis* or *periodontitis*. Gingivitis always precedes periodontitis although in some individuals gingivitis may never progress to periodontitis.

Most periodontal disease arises from, or is aggravated by, accumulation of plaque. Calculus (tartar) is formed by calcification of plaque above or below the gum line. The plaque that collects on calculus exacerbates inflammation, and this inflammatory reaction is associated with progressive loss of periodontal ligament and alveolar bone, with resultant mobility and loss of teeth (Coventry *et al*, 2000).

Investigation into the causes and characteristics of periodontal diseases began in the 19th century with pure clinical observation (Riggs, 1876), and this remained the primary form of investigation well into the 19th century. During this time, the signs and symptoms of periodontal diseases were firmly established.

The advent of microscopy allowed later studies performed at the turn of the 19th century to report the histological structures and features of periodontal lesions, but were limited initially to advanced stages of the disease. Progress in microscopy in the 1960s allowed researchers to focus on earlier stages of inflammatory processes, while the innovation of experimentally induced periodontal disease in both human and animal models allowed for more detailed research into the temporal progression of the pathogenesis of plaque-induced periodontal disease.

Importantly, the development of gingivitis can be observed by a trained clinical observer and does not rely uniquely on a biochemical biomarker or pathological examination. It makes epidemiological study of these phenomena possible and meaningful.

1.3 Dental plaque

Plaque, also known referred to as a *biofilm*, is the sticky, colourless film that constantly forms on teeth and is ubiquitous in human beings. When examined under a microscope, it is comprised of thousands of bacteria. Naturally, bacteria are always present in the oral cavity. However, when plaque is not removed on a daily basis, its accumulation frequently leads to the development of disease in the mouth. It is the main cause of cavities and gum disease.

Initially, the biofilm is soft enough to come off by scraping with a finger nail. It starts to harden within 48 h, and in about 10 days, the plaque becomes dental calculus (tartar) which is hard and difficult to remove. Dental plaque can give rise to dental caries (tooth decay) and periodontal problems such as gingivitis and chronic periodontitis.

1.4 Causes and prevention of periodontal disease

Periodontal disease is extremely common in man. The disease process starts with plaque and proceeds to gingivitis, which is thought to effect 90% of the population at some point in their life (Coventry *et al*, 2000). If diagnosed and treated, the prognosis is favourable but if left unchecked,

it can lead to periodontitis and tooth mobility and loss. Chronic periodontitis (inflammation of the gingival and periodontal membrane) can be a sequel of chronic gingivitis developing through accumulation of plaque and calculus. The gingiva detaches from the tooth, the membrane and bone are damaged, a gap develops between the tooth and gum and, consequently, the tooth may loosen gradually and eventually be lost.

Prevention relies on identification of the determinants of the disease in question. There are genetic determinants of disease risk with individuals born with a propensity to develop particular conditions. The development of disease in such individuals may proceed without an external stimulus, but frequently such may be necessary. In considering periodontal disease and oral malignancy, this is indeed the case and tobacco smoking is one of the best identified lifestyle factors in determining risk of diseases of the mouth.

Tobacco use is a risk factor for oral cancer, oral mucosal lesions, periodontal disease and impaired healing after periodontal treatment, gingival recession, and coronal and root caries (Winn, 2001). Cigarette smoking is a significant determinant of risk of developing periodontal disease.

The extent and severity of periodontal attachment loss were described in a random sample of 690 dentate community-dwelling adults, aged 65 or over, residing in five counties in North Carolina (Beck *et al*, 1990). African American subjects had an average of 78% of their sites with attachment loss, and the average level of loss in those sites was approximately 4 mm, as compared with 65% and 3.1 min for white subjects. The logistic regression model for African American subjects found that tobacco use was associated with a odds ratio of 2.9 (Beck *et al*, 1990).

Tomar and Asma (2000) conducted a study to examine the relationship between cigarette smoking and periodontitis and to estimate the proportion of periodontitis in the US adult population which could be attributable to cigarette smoking. They examined data derived from the Third National Health and Nutrition Examination Survey, a nationally representative multipurpose health survey conducted between 1988 and 1994. Participants were interviewed about tobacco use and examined by dentists trained to use standardized clinical criteria. Analysis was limited to dentate persons aged 18 years and older with complete clinical periodontal data and information on tobacco use and important covariates (n = 12 329). Periodontitis was defined as the presence of one or more sites with clinical periodontal attachment level $\geq$4 mm apical to the cementoenamel junction and probing depth $\geq$4 mm. Current cigarette smokers were those who had smoked more than 100 cigarettes over their lifetime and smoked at the time of the interview; former smokers had smoked more than 100 cigarettes but did not currently smoke; and never smokers had not smoked more than 100 cigarettes in their lifetime.

The study found that 27.9% [95% confidence interval (CI) $\pm$1.8] of dentate adults were current smokers and 23.3% (95% CI $\pm$1.2) were former smokers. Overall, 9.2% (95% CI $\pm$1.4) of dentate adults met the case definition for periodontitis, which projects to about 15 million cases of periodontitis among US adults. Modelling with multiple logistic regression revealed that current smokers were about four times as likely as persons who had never smoked to have periodontitis [prevalence odds ratio (ORp) = 3.97; 95% CI 3.20–4.93], after adjusting for age, gender, race/ethnicity, education and income/poverty ratio. Former smokers were more likely than persons who had never smoked to have periodontitis (ORp = 1.68; 95% CI 1.31–2.17). Among current smokers, there was a dose–response relationship between cigarettes smoked per day and the odds of periodontitis ($P < 0.000001$), ranging from ORp = 2.79 (95% CI 1.90–4.10) for <9 cigarettes per day to ORp = 5.88 (95% CI 4.03–8.58) for more than 31 cigarettes per day. Among former smokers, the odds of periodontitis declined with the number of years since quitting, from ORp = 3.22 (95% CI 2.18–4.76) for 0–2 years to ORp = 1.15 (95% CI 0.83–1.60) for more than 11 years. Applying standard epidemiological formulas for the attributable fraction for the population, it was calculated that 41.9% of periodontitis cases (6.4 million cases) in the US adult population were attributable to current cigarette smoking and 10.9% (1.7 million cases) to former smoking. Among current smokers, 74.8% of their periodontitis was attributable to smoking (Tomar and Asma, 2000).

Tomar and Asma (2000) concluded that tobacco smoking is a major risk factor for periodontitis and may be responsible for more than half of periodontitis cases among adults in the United States. A large proportion of adult periodontitis may be preventable through prevention and cessation of cigarette smoking.

Bergström (2004) conducted a thorough and thoughtful review and clearly identified tobacco smoking as the main risk factor associated with chronic destructive periodontal disease. No other known factor can match the strength of smoking in causing harm to the periodontium. The harmful effects manifest themselves by interfering with vascular and immunological reactions, as well as by undermining the supportive functions of the periodontal tissues. The typical characteristic of smoking-associated periodontal disease is the destruction of the supporting tissues of the teeth, with the ensuing clinical symptoms of bone loss, attachment loss, pocket formation, and eventually tooth loss. A review of the international literature that has accumulated over the previous 20 years offers convincing evidence that smokers exhibit greater bone loss and attachment loss, as well as more pronounced frequencies of periodontal pockets, than non-smokers do. In addition, tooth loss is more extensive in smokers. Smoking considerably increases the risk of destructive periodontal disease. Depending on the definition of disease and the exposure to smoking, the risk is 5- to 20-fold elevated for a smoker compared with a never smoker. For a smoker exposed to heavy long-life smoking, the risk of attracting destructive periodontal disease is equivalent to that of developing lung cancer (Bergström, 2004).

The outcome of periodontal treatment is less favourable or even unfavourable in smokers. Although long-term studies are rare, available studies concur that treatment failures and relapse of disease are predominantly seen in smokers. This contention is valid irrespective of treatment

modality, suggesting that smoking will interfere with an expected normal outcome following commonplace periodontal therapies. The majority of available studies agree that the subgingival microflora of smokers and non-smokers are no different given other conditions. As a consequence, the elevated morbidity in smokers does not depend on particular microflora. The mechanisms behind the destructive effects of smoking on the periodontal tissues, however, are not well understood. It has been speculated that interference with vascular and inflammatory phenomena may be one potential mechanism. Nicotine and carbon monoxide in tobacco smoke negatively influence wound healing. Smoking research over the past two decades has brought new knowledge into the domains of periodontology. Even more so, it has called into question the prevailing paradigm that the disease is primarily related to intraoral factors such as supragingival and subgingival infection. Smoking research has revealed that environmental and lifestyle factors are involved in the onset and progression of the disease. Being the result of smoking, destructive periodontal disease shares a common feature with some 40 other diseases or disorders. As a consequence, periodontal disease should be regarded as a systemic disease in the same way as heart disease or lung disease. Thus, chronic destructive periodontal disease in smokers is initiated and driven by smoking. Its progression may or may not be amplified by unavoidable microbial colonization (Bergström, 2004).

Torrungruang *et al* (2005) conducted a study in Thailand to determine the effect of cigarette smoking on the severity of periodontitis in a cross-sectional study of older Thai adults. The study population consisted of 1960 subjects (age 50–73 years old). All subjects received both medical and dental examinations. Periodontal examinations, including plaque score, probing depth and clinical attachment level, were carried out on all teeth present in two diagonal quadrants. Socio-demographic characteristics and smoking status were obtained by questionnaire. Multinomial logistic regression was used to address the association between cigarette consumption and mean clinical attachment level. Torrungruang *et al* (2005) found that 48.7% were non-smokers, 14.4% were current smokers, and 36.9% were former smokers. Current smokers had higher percentage of sites with plaque, deeper mean probing depth and greater mean clinical attachment level than former smokers and non-smokers. The odds of having moderate and severe periodontitis for current smokers were 1.7 and 4.8 times greater than non-smokers, respectively. Former smokers were 1.8 times more likely than non-smokers to have severe periodontitis. Quitting smoking reduced the odds of having periodontitis. For light smokers (<15 pack-years), the odds for severe periodontitis reverted to the level of non-smokers when they had quit smoking for more than 10 years. For moderate and heavy smokers (≥15 pack-year), the odds of having severe periodontitis did not differ from those of non-smokers when they had quit smoking for more than 20 years. Torrungruang *et al* (2005) concluded that there was a strong association between cigarette smoking and the risk of periodontitis among older Thai adults. Quitting smoking appears to be beneficial to periodontal health.

Razali *et al* (2005) compared the periodontal disease severity of adult heavy smokers and never smokers referred for assessment and treatment of chronic periodontitis. A random sample of patients with at least 20 teeth, stratified for smoking and age (5-year blocks, 35–55 years), was selected from an original referral population of 1221 subjects with chronic adult periodontitis. Adequate records for 59 never smokers and 44 subjects who smoked at least 20 cigarettes per day were retrieved. The percentage of alveolar bone support was measured from dental panoramic radiographs with a Schei ruler at $\times 3$ magnification with the examiner unaware of the smoking status. Probing depths at six sites per tooth were obtained from the initial consultation. There was no significant difference in age between groups. Smokers had fewer teeth ($P < 0.001$), fewer shallow pockets ($P < 0.001$) and more deep probing depths ($P < 0.001$). The differences were greater in subjects 45 years of age and over. In this age group, smokers had approximately 13% more bone loss, 15% more pockets in the 4–6 mm category and 7% more pockets in the ≥7 mm category than in never smokers. Razali *et al* (2005) concluded that smokers had evidence of more severe periodontal disease than never smokers. The differences increased with age confirming an exposure-related response.

Tanner *et al* (2005), reviewing risk indicators for early periodontitis in adults, concurred with Tomar and Asma (2000) that periodontal diseases affected over half the adults in the United States, disproportionately affecting minority populations. They aimed to evaluate associations between clinical and other risk indicators of early periodontitis in a cross-sectional evaluation of 225 healthy and early periodontitis adults aged 20–40 years. More periodontal attachment loss was detected in African American and Hispanic subjects compared with Asian and Caucasian subjects. Smoking history was associated with attachment loss.

Warnakulasuriya *et al* (2010) reviewed the accumulated evidence regarding the effects of tobacco use and tobacco use cessation on a variety of oral diseases and conditions. Exposures considered include cigarette and bidi smoking, pipe and cigar smoking, and smokeless tobacco use. Oral diseases and disorders considered include oral cancer and precancer, periodontal disease, caries and tooth loss, gingival recession and other benign mucosal disorders as well as implant failure. Warnakulasuriya *et al* (2010) concluded that robust epidemiological evidence exists for adverse oral health effects of tobacco smoking and other types of tobacco use. In addition, there is compelling evidence to support significant benefits of tobacco use cessation with regard to various oral health outcomes. Substantial oral health benefits can be expected from abstention and successful smoking cessation in a variety of populations across all ages.

1.5 Toothbrushing and flossing: frontline against dental plaque

Gingivitis is widely accepted as the first stage in a chronic degenerative process which resulted in the loss of both gums and bone tissue surrounding the teeth. However, this condition can be reversed by effective oral hygiene

practices on the part of the individual. No specific public health measure has been developed to prevent gingivitis other than the instruction of groups and individuals on how to remove the bacterial plaque from around the teeth and gums with a toothbrush and floss.

The net effect derived from a variety of sources including instructional actions given by health professionals assisted by commercial advertising, and a general increase in the standard of living seems to have resulted in mouths being generally cleaner and showing less signs of inflammation. This is particularly true in higher resource countries.

Severe periodontal disease can manifest itself in 5–10% of the population even though moderate disease affects the majority of adults. The rate of progression of this disease process in an individual is dependent on the virulence of the plaque and the efficiency of the local and systemic responses in the host.

Current research suggests that the host responses are influenced by specific environmental and genetic factors which can determine the susceptibility of the host generally to periodontal disease or the susceptibility of a particular site (tooth) within the mouth. In this regard, it is common for more severe forms of periodontal disease to present in individuals with compromised immune systems, for example in Diabetes, HIV infection, Leukaemia and Down's Syndrome. There is increasing evidence that smoking causes an acceleration of the disease process and a particular virulent type of periodontal disease. Acute necrotizing ulcerative gingivitis (Vincent's infection) occurs almost exclusively in smokers.

Most adults in high-resource countries suffer from some form of periodontal disease. The primary method of limiting periodontal disease is by plaque control directed to maintaining gingival health. This must be considered at two levels – what people can do for themselves by way of plaque control on a daily basis and what dentists and hygienists can do to eliminate plaque retention factors and to advise the individual on the most appropriate home care. The vast majority of gum disease can be prevented by thorough daily plaque removal once a day, and this is postulated to be achievable by toothbrushing and flossing.

Recent quantitative evidence. Berchier *et al* (2008) conducted a quantitative review designed to assess systematically the adjunctive effect of both flossing and toothbrushing *vs* toothbrushing alone on plaque and gingivitis. They found titles and abstracts of 1166 MEDLINE–PubMed and 187 Cochrane papers resulted in 11 publications that met the eligibility criteria. Mean values and s.d. were collected by data extraction. The majority of the studies did not show a benefit for floss on plaque and clinical parameters of gingivitis. A meta-analysis was performed for the plaque index and gingival index. Berchier *et al* (2008) concluded that a routine instruction to use floss is not supported by scientific evidence.

Matthews (2012) addressed the same issue using information gleaned from a variety of sources including the Cochrane Oral Health Group Trials Register, the Cochrane Central Register of Controlled Trials (CENTRAL) MEDLINE, EMBASE, CINAHL, LILACS, ZETOC Conference Proceedings, Web of Science Conference Proceedings, Clinicaltrials.gov and the metaRegister of Controlled Clinical Trials databases were searched. Manufacturers of dental floss were also contacted to identify any trials.

Matthews (2012) selected randomized controlled trials comparing toothbrushing and flossing with only toothbrushing in adults. Meta-analysis was conducted using random-effects models, the main effect measure being standardized mean difference (SMD) with 95% confidence intervals (CI). Potential sources of heterogeneity were examined, and a sensitivity analysis conducted omitting trials at high risk of bias.

Matthews (2012) found 12 trials that fit the criteria established *a priori*. These included a total of 582 participants in flossing plus toothbrushing (intervention) groups and 501 participants in toothbrushing (control) groups. Seven trials had an unclear risk of bias, and five had a high risk of bias. All the trials reported the outcomes of plaque and gingivitis. Flossing plus toothbrushing showed a statistically significant benefit compared with toothbrushing in reducing gingivitis.

The 1-month estimate translates to a 0.13 point reduction in a 0- to 3-point scale for Loe–Silness Gingivitis Index, and the 3- and 6-month results translate to 0.20 and 0.09 reductions in the same scale. Overall, there is weak, very unreliable evidence which suggests that flossing plus toothbrushing may be associated with a small reduction in plaque at 1 or 3 months. None of the trials included reported data for the outcomes of caries, calculus, clinical attachment loss or quality of life. There was some inconsistent reporting of adverse effects.

Matthews (2012) concluded that there is some evidence from 12 studies that flossing in addition to toothbrushing reduces gingivitis compared with toothbrushing alone. In addition, there is weak, 'very unreliable evidence' from 10 studies that flossing plus toothbrushing may be associated with a small reduction in plaque at 1 and 3 months. No studies reported the effectiveness of flossing plus toothbrushing for preventing dental caries.

So, while regular (at least daily) toothbrushing can reduce plaque (and hence periodontal disease and caries), the effect of adjunct flossing appears to be slight if at all. An adjunctive method of plaque control is the use of antiseptics, of which chlorhexidine is the most effective although its tendency to stain teeth and impair taste makes it generally unacceptable for long-term use.

1.6 Mouthwash

It is clear that there are significant lifestyle risk factors for many oral diseases, including the common ones such as periodontal diseases. Importantly, there is a body of evidence which confirms that prevention is possible given the example of smoking cessation. It is a relevant question to investigate whether active prevention with a mouthwash can impact on preventing or reducing periodontal disease.

The use of antimicrobial mouthrinses is not viewed in its independent effect but as an adjunct to toothbrushing and flossing. These mouthrinses (mouthwash) are employed to prevent and treat oral diseases including

caries, via remineralization and antiplaque effects, and gum diseases, via antiplaque effects and, potentially, anti-inflammatory effects. Cosmetic benefits include an effect on bad breath via antiplaque effects and chemical neutralization of malodorous compounds of bacterial origin.

To summarize, oral bacteria exist in the mouth in two forms: (i) free-floating (planktonic) bacteria in saliva and (ii) dental plaque 'biofilm', a tridimensional community of up to 700 different bacterial species, adherent to all oral surfaces. Dental plaque is the main cause of oral diseases and can be removed mechanically by 'effective' brushing and flossing. However, a very large proportion of plaque on teeth is left behind by most individuals and soft tissues largely untouched mechanical means of plaque control. This plaque, which can be initially removed by brushing or by scraping with a finger, hardens and then poses a danger to the development of gingivitis and eventually to mobility and loss of teeth. It is essential to bear in mind that more than three of four people have some sort of periodontal (gum) disease at some point in their lives.

Residual plaque if left undisturbed leads to plaque regrowth and maturation towards more pathogenic composition (gram-negative anaerobes). A major advantage which a mouthwash has is that antimicrobial mouthrinses can reach virtually all residual plaque and the overall antiplaque efficacy of an oral antimicrobial depends on a high activity to kill germs, substantivity (i.e. the capacity to adhere to tooth surfaces so that antibacterial activity can be locally maintained) and biofilm penetration ability.

Therefore, when devising a brief for a formulator of antiplaque mouthrinses, essential elements must include

- the use of antibacterial agents with (a) antiplaque properties demonstrated in long-term clinical trials and (b) proven safety at effective dose levels for the intended period of use (e.g. chlorhexidine, essential oils combination, CPC, triclosan etc);
- mixing with at least 50% of water;
- adding solubilizers for non-water soluble ingredients, that is, ethanol or emulsifiers such as surfactants.
- making the solution palatable and likeable (add flavour oils –sweeteners, *ethanol*, colourants, etc.).
- making it stable, mainly by preventing precipitation (add stabilizers such as surfactants, solubilizers such as *ethanol,* pH adjusters, etc.).
- making it *stay* stable for shelf-life (add preservatives such as antibacterials and antifungals, e.g. *ethanol* etc.).
- confirming safety and antiplaque efficacy of the final solution.

Ethanol has an important role to play in the formulation of mouthwash. Ethanol can act: (i) as a solubilizer (i.e. to dissolve non-water-soluble ingredients, such as antiseptic essential oils or flavour oils, into the aqueous base); (ii) as a stabilizer and preservative working in combination with other excipients and parameters to give an adequately preserved product; and, (iii) as a sensory cue, helping provide unique organoleptic cleanliness and a distinctive taste, which is much appreciated by consumers.

An important role of ethanol is its ability to operate as an 'antiplaque' efficacy enhancer (adjuvant effect), while ethanol *per se* has negligible antiplaque efficacy, unless present at concentrations of >50–70%, which is an incompatible level for use on oral mucosa as a rinse (but used in skin disinfectants liquids, gels, etc.). The compatible range of concentrations for oral use is <30%. There is evidence that an adjuvant effect exists for 18–27% ethanol combined with essential oils, whereas there is evidence that no adjuvant effect exists for 6–8% when combined with chlorhexidine, CPC and triclosan

Why is there such an effect of ethanol? Essential oils are small lipophilic molecules, with high potential for deep penetration in dental plaque (as opposed to chlorhexidine and CPC, which are 4–10 times larger and electrically charged molecules). It has been widely shown that the addition of ethanol at concentrations between 18% and 27% maximizes the transport of essential oils into the dental plaque biofilm within the recommended 30-s rinse period while preserving the bioavailability of these lipophilic molecules without the sequestration effects that could lead to partial or total disactivation of essential oils (as observed with alternative solubilizers).

In summary, antimicrobial mouthrinses are formulated to control the formation, growth and maturation of oral biofilms, which are highly organized tridimensional communities of bacteria. Once formed, they are difficult to penetrate and require mechanical disruption and chemotherapeutic treatment. Ethanol enhances the antiplaque efficacy of essential oil-based mouthwash by helping to penetrate the biofilm. Ethanol in mouthwash has negligible germ killing activity at levels below 30%.

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Chapter 2

A systematic review with meta-analysis of mouthwash use for the prevention of supragingival plaques and gingivitis: objectives, methods, data retrieval and trial characteristics

Objective
The principal objective was to summarize and quantify the efficacy of the most frequently used mouthwash products for the control of supragingival dental plaque and of gingivitis, when they are employed as complements to usual oral hygiene measures (e.g. toothbrushing and flossing) by the general adult population.

The objective assumes primarily that the quantification of 'efficacy' is based on the results of randomized controlled trials (RCT). Second, the efficacy is assessed over and above efficacy of usual oral hygiene. Hence, trials are eligible for the meta-analysis only if subjects included in both the intervention and in the control group followed usual oral hygiene measures, including toothbrushing and/or dental flossing.

Secondary objectives were (i) the evaluation of the quality of randomized controlled trials (trials) of mouthwash for plaque and gingivitis control and (ii) the assessment of the influence of selected factors (e.g. trial design) on the efficacy of mouthwash.

Methods
This meta-analysis was conducted following the PRISMA guidelines for systematic review and meta-analysis of randomized controlled trials (Moher *et al*, 2009) (Appendices II and III).

Definition of efficacy
In the context of this review, efficacy is understood as the impact of mouthwash use on plaque growth and on gingivitis severity over clinically relevant periods of use: that is, the time between a professional oral inspection at baseline, before start of the intervention and the last professional oral inspection. The optimal time interval between the baseline and the last oral inspections for evaluation of mouthwash efficacy was defined as 6 months by the American Dental Association [ADA, 1999, 2008; Council on Dental Therapeutics (CDT), 1986].

The evaluation of plaque and gingivitis extent and severity had to be based on quantitative scoring systems (or indexes – a brief outline of these scores is in Appendix IV). The most frequently utilized scoring systems are

- for plaque evaluation:
  - the Silness–Loe Plaque Index (S&L1964) that has four levels (Silness & Loë, 1964);
  - the Turesky Modification of the Quigley–Hein plaque index (TM, Turesky *et al*, 1970) that has six levels and is based on the Quigley–Hein Index (Quigley and Hein, 1962).
- for gingivitis evaluation:
  - the Loe–Silness Gingival Index (L&S1993) that has four levels (Loë & Silness, 1963);
  - the Lobene Modified Gingival Index (MGI) by Lobene *et al* (1986) that has five levels. This modification of the Loe–Silness Gingival Index (Loë and Silness, 1963) was developed to increase sensitivity in the low region of the scoring scale.

Mouthwash preparations selected for the meta-analysis
The following mouthwashes were included in this review:

- Chlorhexidine (CHX) – for example, Peridex®, Hibitane®, Eludril®, Chlorhexamed®. Preparations containing <0.06% of chlorhexidine were not selected.
- Essential oils (EO) preparations (Listerine®);
- Cetylpyridinium (CPC);
- Delpominol (DEL);
- Triclosan (TRI) [with zinc sulphate or Gantrex copolymer (TG)].

Most commercially available mouthwash preparations, with the exception of Cetylpyridinium, contain ethanol at variable concentrations.

The following substances were *not* included in the review:

- Mouthwashes prepared with *sanguinarine* ('Canadian bloodroot extract' in Viadent®) that were generally tested and marketed with toothpastes also containing this compound. Only one eligible trial was reported that tested sanguinarine mouthwashes alone (Grossman *et al*, 1989). In 2001, because of safety concerns, sanguinarine was removed from Viadent® and the brand disappeared recently (Vlachojannis *et al*, 2012).
- Mouthwashes containing fluoride compounds (e.g. Meridol®) are often tested and marketed together with fluoride toothpastes. A single randomized trial was found that reported a 2–2.5 lower efficacy of amine fluoride and stannous fluoride mouthwashes than 0.10% CHX preparations in the prophylaxis of plaque and gingivitis (Hoffmann *et al*, 2001).
- The mouthwash that had the commercial denomination of Plax®: this brand name had a formulation which varied from country to country. For instance, Plax formulations utilized sodium benzoate as the active ingredient in the United States and triclosan with copolymer of methoxyethylene and maleic acid in Europe [triclosan was not approved for mouthwash formation by the United States Food and Drug Administration (FDA)]. Often, the exact formulation of Plax was not reported in publications. One meta-analysis of trials on Plax found that the clinical magnitude and benefit on oral health associated with these products were likely to be small (Angelillo *et al*, 2002). Nonetheless, published articles reporting trials with Plax preparations including cetylpyridinium or triclosan were reviewed for the meta-analysis.
- Mouthwash preparations for which only few data from randomized trials is available, for instance, rinses prepared with *Lippia sidoides* ('*alecrim pimenta*')-based essential oil or with *Azarirachta indica* (neem), or the

sugar alcohol xylitol, or hydrogen peroxide and glycerol, or the soluble b-1,3/1,6-glucan, or alexidine·2HCl, or cationic histidine-rich peptides (histidines), probiotics (i.e. live microorganisms which confer a health benefit on the host), or 'total formulations' (i.e. mouthwashes combining several active compounds like potassium citrate plus sodium fluoride plus cetylpyridinium).

Literature search

The literature published in English was searched, without time restriction.

1 Manual search in references of review articles (see reference list).
2 MSeH Keywords:

« mouthwash » and « gingivitis »
« mouthwash » and « dental plaque »
« mouthwash » and « prevention and control »

Searches were conducted with and without limits set for « clinical trials ». Articles related to eligible or apparently eligible articles were also explored.

In addition, manual searches were undertaken in reference lists of review papers (Adams and Addy, 1994; Eley, 1999; Paraskevas, 2005; Gunsolley, 2006; Addy *et al*, 2007; Stoeken *et al*, 2007; Haps *et al*, 2008; Teles and Teles, 2009; Berchier *et al*, 2010; Gunsolley, 2010; Van Leeuwen *et al*, 2011).

Trial selection

Trials were selected based on the randomized controlled design (RCT) with parallel groups published in peer-reviewed journals that had the following characteristics:

- To be of at least 1 month duration,
- Inclusion of healthy subjects 18 years or older, representative of the average mouthwash users.
- Testing of an agent against a preparation taken by at least one control group consisting in
  - the product without the active substance, for example the vehicle solution; or an hydro-alcoholic solution at 21% of ethanol; or an HA 5%; or,
  - a placebo (coloured and/or flavoured water); or,
  - no substitute for the rinse;
- use of a standard scoring system for evaluation of plaque an gingivitis extent and severity;
- maintenance or reinforcement of usual oral hygiene methods (toothbrushing and/or dental floss).

Some trials used an 'active control group', that is, a group of subjects using CHX. Active control groups were considered as being intervention groups.

Exclusion criteria

1 Unpublished trials (e.g. « data-on-file ») were not included. We did not seek for unpublished data owned by companies producing or marketing mouthwash products.
2 Trials of <1-month duration;
3 Trials in subjects <18 years of age;
4 Trials restricted to elderly (≥65 years of age);
5 Trials without a control group receiving a preparation without the active ingredients (i.e. the 'positive control');
6 Trials in which usual oral hygiene methods were not maintained or reinforced;
7 Mouthwash used for the treatment of an oral condition;
8 Subjects with mouth disease or surgical intervention on head and neck;
9 Trials without a control group using a preparation not containing the active substance [trials that tested two active agents without a control group (head-to-head trials) were not eligible].
10 Trials that tested one of five substances (CHX, L, CPC DEL or T) as part of a toothpaste formulation without a group with a mouthwash with that substance and without the toothpaste.

Table 2.1 Trial characteristics considered by the review conducted as described in the text of Chapter 2

Place (country) where the trial took place	Description
Subjects	Adults, representative/adults, not representative/adolescents/children
Endpoints	Plaque/gingivitis and scoring method
Inclusion & exclusion criteria	Description
Intervention(s)	Active substance(s) tested
Type of control	Passive (vehicle): HA 20% or 5% Placebo (e.g. coloured water) No mouthrinse
Baseline and in-trial assessment of lesions	Schedule
Scoring methods	Plaque, gingivitis, bleeding
Randomization method	None reported/computer/lottery/etc…
Blinding	None/single/double
Professional prophylaxis (plaque removal) at baseline	No/yes
Method, instructions for rinsing	Oral, written, internet, training Products provided *ad libitum* or in specific amounts
Supervision of rinsing	None/partial/total
Daily oral hygiene maintained or reinforced	Toothbrushing at least 2 times per day
Method, instruction for usual oral hygiene	Oral, written, internet, training Provision of tooth brushes and/or of tooth paste
Trial duration	6+ months/3–5.9 months/1–2.9 months

Data abstraction

For each agent, a database was constructed which included those data items abstracted from the selected articles. Tables 2.1 and 2.2 summarize data items that were extracted from articles. Data abstraction was done by Alice Koechlin and verified by Philippe Autier. After abstraction ended, a synthetic table was derived for statistical analysis with « R » or SAS software.

Statistical analysis

Trial designs on mouthwashes were typically of the form:

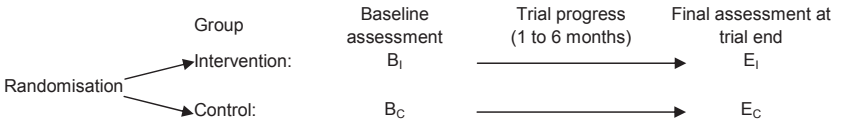

Table 2.2 Data abstracted and utilized in the meta-analysis conducted as described in the text of Chapter 2

Number of subjects randomized in intervention and control groups	Numbers at baseline
Lost-to-follow-up (LTFU)	Reported by group Reported for all subjects only Not reported
Age of subjects	Mean, median, range by group
Sex	% Males in each group
Baseline mean	Value
Baseline dispersion parameter (s.d., s.e., 95% CI)	Value
Final mean	Value
Final dispersion parameter (s.d., s.e., 95% CI)	Value
Intention-to-threat analysis	No/Yes
Analysis of covariance (ANCOVA)	No/Yes
Absolute difference	Value
% Difference (relative ratio of difference)	Value
Relative difference in %	Value

Where E stands for score value at trial end, B stands for score value at baseline, subscript I stands for « intervention group » and subscript C stands for « control group ». Published trials did not use a uniform metric to describe the *effect* of the intervention. Therefore, analyses reported herein had to rely on mean index and standard deviation (s.d.) at baseline in randomization groups and after intervention in randomization groups. Hence, the *main effect* could be calculated using four mean values and four standard deviations combined in various ways:

Absolute end-of-trial difference: $E_I - E_C$
Absolute end-of-trial ratio: E_I/E_C or $(E_I - E_C)/E_C$
Relative end-of-trial difference: $(E_I - E_C)/[(B_I + B_C)/2]$
Relative end-of-trial ratio: $(E_I/E_C)/([B_I + B_C]/2)$

Key methodological concern. The Figures 2.1, 2.2 and 2.3 displays scatter plots of intervention *vs* control group of plaque or gingivitis scores at trial end against baseline score values, calculated as $([B_I + B_C)]/2)$. These figures were constructed using data from 48 trials that reported scores at baseline using one of the four aforementioned scoring systems.

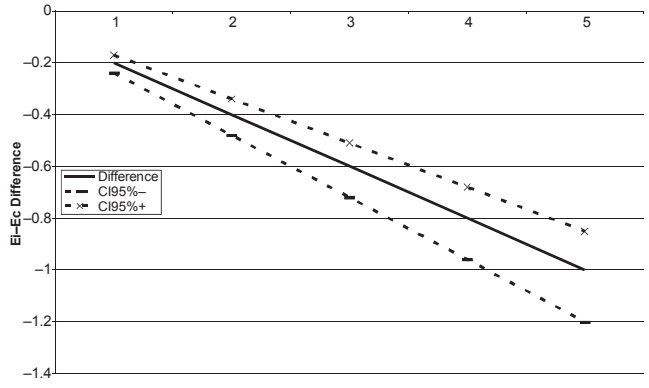

Figure 2.1 Difference in gingivitis scores at trial end ($E_I - E_C$) according to gingivitis scores at baseline in 48 randomized controlled trials of mouthwash

The absolute value of $E_I - E_C$ or the ratio E_I/E_C increased with increasing amounts of lesions at baseline, that is, the greater the gingivitis score at baseline, the greater the difference between the intervention and control groups at trial end (Figures 2.1 and 2.2). The ratio or the difference between intervention and control at the end of follow-up strongly depends on baseline values. As a consequence, when using either the ratio or the difference of endpoints measured at trial end, the main effect becomes strongly dependent on both the scoring method used for plaque or gingivitis evaluation and on the baseline value of these scores. This is an extraordinarily important point to bear in mind when interpreting such studies and to take care of when combining such studies.

In response to this situation, a composite main effect was calculated that took all information available in published papers:

Relative end-of-trial difference (RETD): $(E_I - E_C)/([B_I + B_C]/2)$

The baseline value is the mean of values of control and intervention group at baseline. Figure 2.3 shows that with utilization of the RETD, the correlation between the endpoint evaluation at trial end and at baseline fades away.

Statistical handling of individual trials. The RETD was always computed using the last evaluation *vs* the evaluation at baseline, most usually the evaluation at 6 months *vs* that at baseline. For the rare trials of more than 6-month duration, results reported at 6 months were retrieved. If the 6-month evaluation could not be retrieved, then the last evaluation was used instead.

For trials that tested different agents or different concentrations of an agent (e.g. CHX, 0.06%, 0.12% or 0.2%), the main effect was computed for each agent or concentration separately. As a consequence, a trial that tested CHX 0.12% and CPC against a control was classified among trials that tested CHX and also among trials that tested CPC.

Five trials had two control groups (Lamster *et al*, 1983; Gordon *et al*, 1985; Charles *et al*, 2001; Sharma *et al*, 2002; Sharma *et al*, 2004). In each case, the main effect was computed against the control deemed to be the less efficient.

For instance,

- if one group used the vehicle solution and another group used a coloured water placebo (Lamster *et al*, 1983; Gordon *et al*, 1985), the coloured water group was taken as control group;

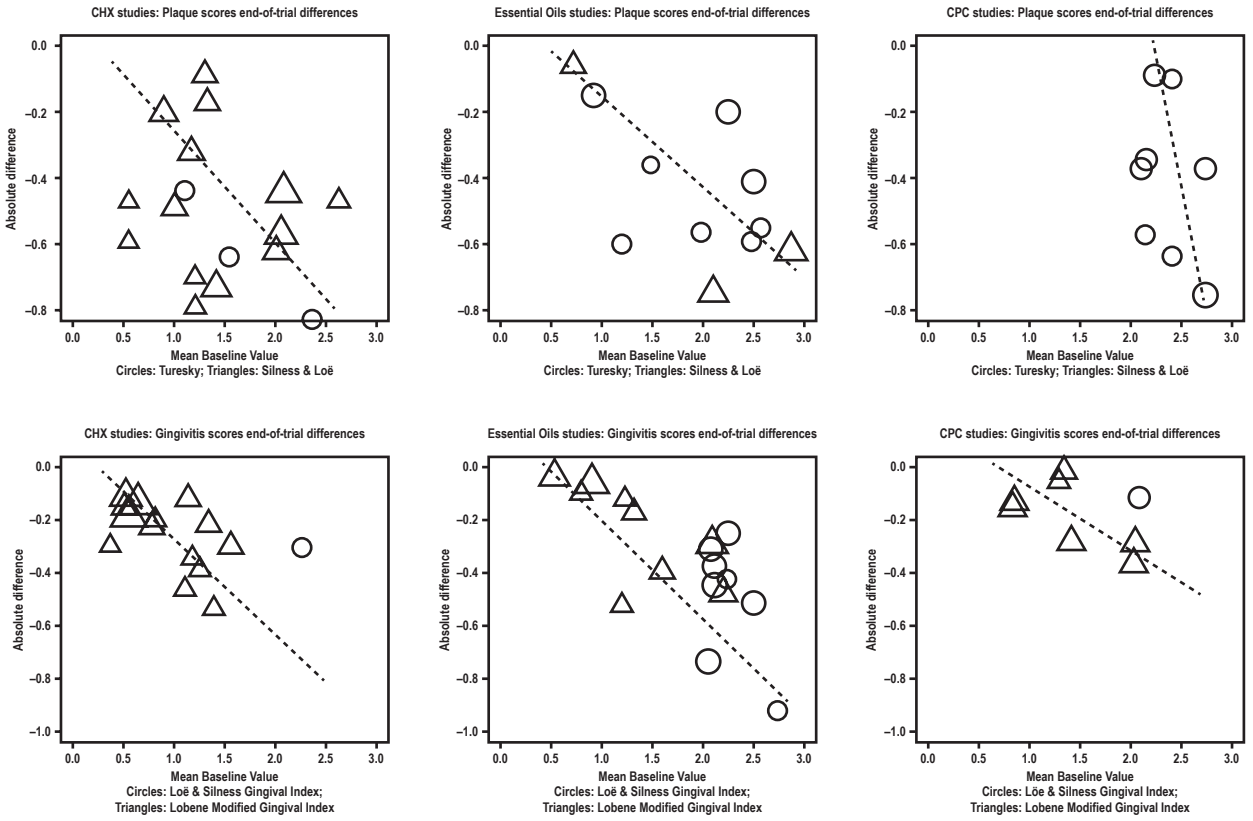

Figure 2.2 Difference between the intervention and the control group in plaque or gingivitis scores at trial end according to score values at baseline

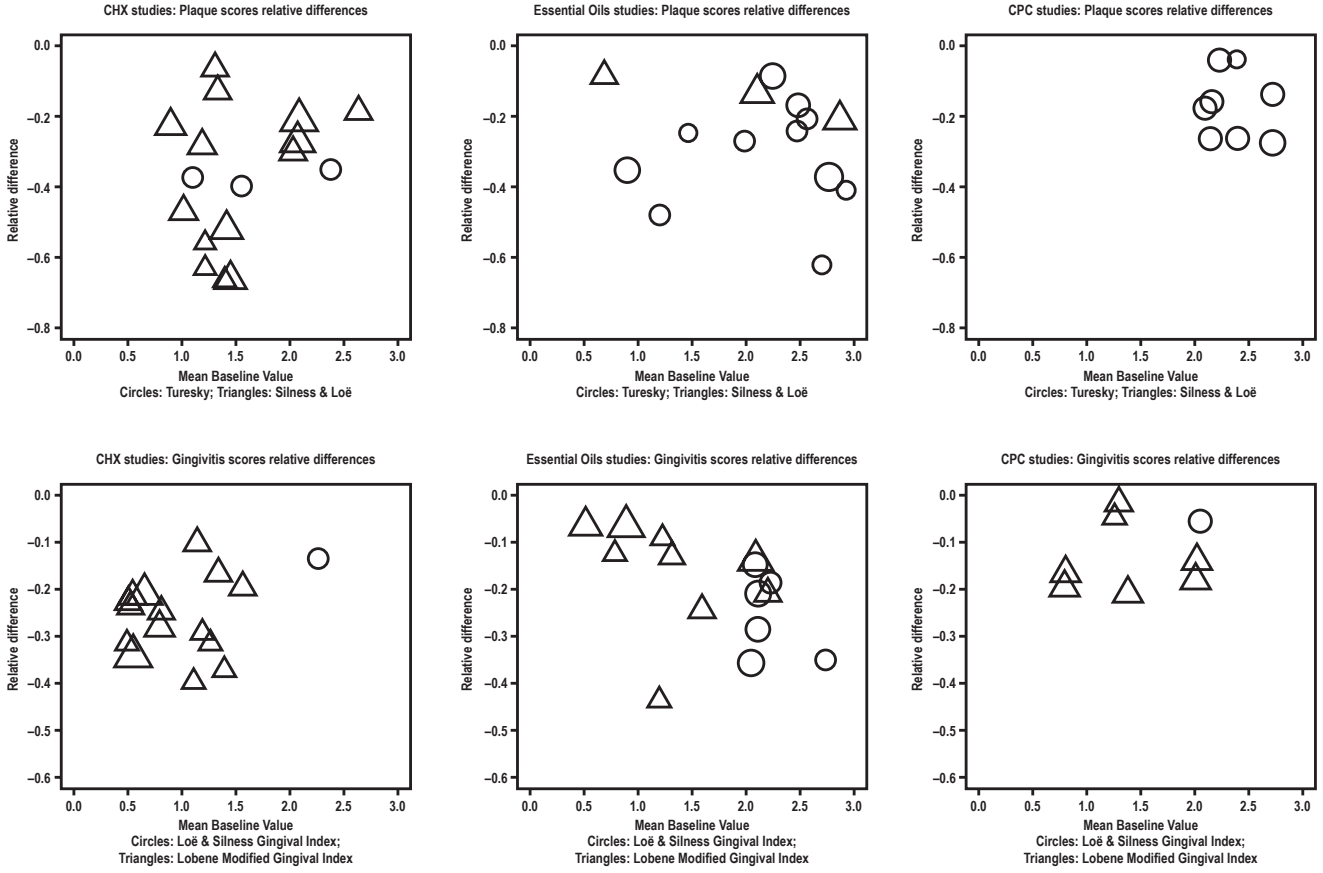

Figure 2.3 Relative difference between the intervention and the control group in plaque or gingivitis scores at trial end according to score values at baseline

- if one group used a 5% hydro-alcoholic solution together with a control toothpaste and another used 5% hydro-alcoholic solution together with a fluoride toothpaste (Charles *et al*, 2001), the former group was taken as control group.

Sometimes, selection and assumption had to be made when the variance was not available or computable and the average variance from all studies was used instead (Wiebe *et al*, 2006). The variance for the relative difference was estimated via a Delta method (method of errors propagation; Oehlert, 1992).

Meta-analysis method. A meta-analysis of all these studies was undertaken using a random-effects model with appropriate tests for statistical significance, publication bias and heterogeneity.

The meta-analysis resulted in a summary of the main effect computed from relative end-of-trial differences from by each trial and named 'summary relative difference (SRD)'. The SRD can be interpreted as a percentage change from the baseline (i.e. before use of a mouthwash) values of plaque or gingivitis scores. A SRD of say, −0.3 for plaque control, means that the plaque score will be reduced by 30%. If the baseline value was 4, then after intervention the score will be 2.8 on average. Hence, the SRD values ranges from −1.0 to +1.0. Of note, some 95% confidence intervals can have values below −1.0 or above +1.0 because the meta-analysis regression is based on a linear modelling which assumes a normal distribution of SRDs. Calculation of exact confidence intervals taking into account the real distribution properties of SRDs would require considerable mathematical developments, and exact confidence intervals are not likely to improve the interpretation of results.

Meta-analysis estimates were first pooled for all studies and then separately by product (chlorhexidine, essential oils, cetylpyridinium, delmopinol, triclosan).

Random-effect modelling was employed based on methodology proposed by van Houwelingen *et al* (2002). Calculation of 95% confidence intervals was based on the t-distribution (because of the large degree of heterogeneity on the study findings). Appendix V outlines calculations done for the variance of the summary relative difference. Sensitivity analyses were carried out to evaluate the influence of each study on the overall estimate from the meta-analysis (leave-one-out analysis). Publication bias was initially investigated graphically with a funnel plot. To complete this visual analysis, the three main publication bias tests were computed, namely Begg test (rank correlation test) (Begg and Mazumdar, 1994), Egger test (weighted linear regression test for funnel plot symmetry) (Egger *et al*, 1997) and Macaskill test (Macaskill *et al*, 2001). The Egger and Macaskill tests are relatively similar, but the latter is more reliable when <20 trials are considered. Nevertheless, statistical methods for assessing publication bias are rough, and the rule of thumb is to consider that publication bias is likely when one of the three tests is statistically significant (i.e. $P < 0.05$).

Heterogeneity in results between trials was assessed by Higgins and Thompson's I^2 (Higgins and Thompson, 2002). I^2 represents the proportion of the heterogeneity which cannot be explained by chance alone. The I^2 parameter values range from 0% to 100%, zero meaning that relative risks of the different studies included in the meta-analysis are homogeneous, that is, that these relative risks are consistent with each other. An I^2 tending to 100% indicates that relative risks of the different studies included in the meta-analysis are heterogeneous, that is, that these relative risks are not consistent with each other; for instance, an example would be where for ten studies of similar size, five would have relative risks well below unity and five would have relative risks well above unity.

All statistical analyses were carried out in programming language *R* (version 2.13.1, 2011) and package metafor. A result was labelled as statistically significant if the 95% confidence interval did not include zero.

Data retrieval and trial characteristics

Retrieval and selection of articles. Figure 2.4 summarizes the literature search. The searches returned a total of 2626 articles, including 260 for « mouthwash » and « gingivitis », 555 for « mouthwash » and « dental plaque » and 1811 for « prevention and control ». Two articles (Flötra *et al*, 1972; Nelson *et al*, 1991) were found in two journals that did no longer exist in 2011.

After checking of titles and abstracts for relevance (e.g. mouthwash or not), study design, redundancy and characteristics like study duration, 122 full-text articles were retrieved and read in detail. Some articles, although being apparently eligible, had to be excluded. For instance, the trial by Zimmer *et al* (2006) tested CPC and CHX mixed with other substances. The trials by Witt *et al* (2005) and Albert-Kiszely *et al* (2007) compared cetylpyridinium to essential oil preparations, without a control group (head-to-head trials).

The final selection included 51 articles reporting on independent trials eligible for the meta-analysis (Appendix VI; Table 2.3): 17 on CHX, 16 on EO, 7 on CPC, 4 on

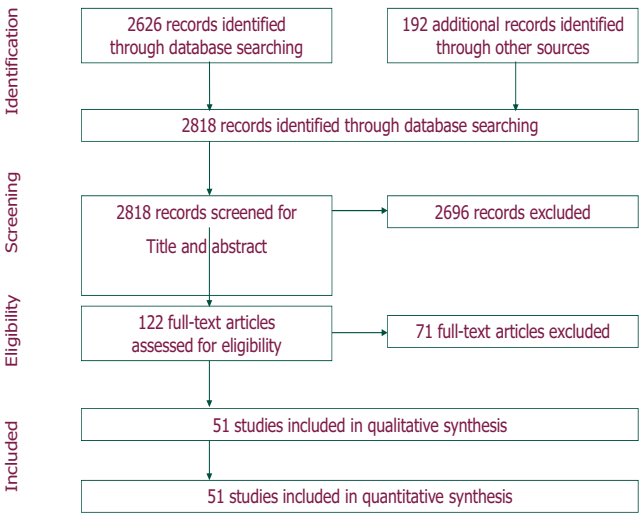

Figure 2.4 Outcome of literature searches of randomized controlled trials of mouthwash

Table 2.3 Number of randomized controlled trials selected for use in the metaanalysis described in Chapter 2

Substance (abbreviation)	*No. Articles*	*No. intervention groups*	*No. control groups*
Chlorhexidine (CHX)	17	20	17
Essential oils (EO)	16	16	21
Cetylpyridinium (CPC)	7	8	7
Delmopinol (DEL)	4	5	4
Triclosan (TRI)	7	8	7
All substances	51	57	56

DEL and 7 on TRI. These 51 articles reported results related to 57 intervention groups, partly because some trials tested preparations containing different concentrations of a substance, that is, three trials on chlorhexidin (Segreto *et al*, 1986, 0.12% and 0.20%; Axelsson *et al*, 1987, 0.1% and 0.2%; Hoffmann, 2001, 0.06% and 0.1%), one on cetylpyridinium (Stookey *et al*, 2005, 0.08% and 0.1%), one on delmopinol (Claydon *et al*, 1996, 0.1% and 0.2%), one on triclosan (Schaeken *et al*, 1996, 0.15% TRI in 15% HA solution and 0.15% TRI in 7.5% HA solution).

Five trials with Listerine had two control groups: Charles *et al* (2001, HA5% plus control toothpaste and HA5% plus fluoride toothpaste), Gordon *et al* (1985, vehicle HA 21.6% and water), Sharma *et al* (2004, HA5 and HA5% plus dental floss), Lamster *et al* (1983, vehicle HA 21.6% and water).

Characteristics of trials and reporting on design aspects. The first trial that lasted for at least 1 month tested CHX among Swedish soldiers (Flötra *et al*, 1972).

Table 2.4 Countries in which randomized controlled trials were conducted

Country	*No. RTCs*	*%*
Brazil	3	6
Canada	3	6
China	1	2
Germany	1	2
The Netherlands	1	2
Salvador	1	2
Spain	1	2
Sweden	6	12
Switzerland	2	4
Thailand	1	2
UK	7	14
USA	24	47
Total	51	100

Table 2.5 Number of subjects in the control groups of randomized trials of mouthwash use considered for meta-analysis

No. subjects in control groups	*CHX*	*EO*	*CPC*	*DEL*	*TRI*	*Total*
10–29	4	4	0	1	2	11
30–59	6	5	4	2	3	20
60–99	2	1	1	0	2	6
100–225	5	6	2	1	0	14
Total	17	16	7	4	7	51

Table 2.6 Duration of randomized controlled trials of different tyoes of mouthwash use

Trial duration (months)	*Substance*					
	CHX	*EO*	*CPC*	*DEL*	*TRI*	*Total*
1	0	0	0	1	1	2
1.5	2	2	3	0	1	8
3 or 4	4	1	0	0	1	6
6	11	12	4	3	3	33
6.5	0	0	0	0	1	1
9	0	1	0	0	0	1
Total	17	16	7	4	7	51

Table 2.7 Number of subjects in control groups of randomized controlled trials of mouthwash use according to trial duration

Duration (months)	*No. trials*	*Total subjects in control group*	*% Of all subjects*	*Mean no. of subjects*
1	2	28	0.8	14
1.5	8	278	7.8	35
3 or 4	6	436	12.2	73
6	34	2801	78.4	82
9	1	28	0.8	28
Total	51	3571	100.0	70

Trials have been conducted in 12 different countries, with nearly half done in the United States (Table 2.4). Twelve trials were carried out before 1990, 21 between 1990 and 1999, and 18 after 1999.

The number of subjects in the control groups (considering one group per trial – see the Methods) was <60 in 31 trials (Table 2.5). Two-third of trials were of at least 6-month duration (Table 2.6). Trials of 6-month duration included nearly 80% of all subjects and were also of larger size (Table 2.7).

Figures 2.1 and 2.2 shows that subjects included in trials had highly variable levels of plaque or gingivitis extent and severity at baseline: mean baseline scores ranged from less than or around 1, indicating that subjects originated from a population with a good oral hygiene on average, to 2.5 and more, indicating that subjects originated from a population with a suboptimal oral hygiene on average.

Randomization procedures were seldom reported and generally without much detail (1 or 2 sentences maximum). Table 2.8 summarizes randomization methods

Table 2.8 Method of randomization employed in randomized controlled trials of mouthwash use

Method of randomization	*No. RCTs*	*%*
Random number table	2	4
Computer	12	24
Code	8	16
Random permutations	3	6
Schedule	2	4
Lotery	1	2
Unknown	23	45
Total	51	100

Table 2.9 Reporting of randomisation procedure according to type of mouthwash evaluated

	CHX	*EO*	*CPC*	*DEL*	*TRI*	*Total*
Randomization procedure not reported	8	4	6	0	5	23
%	47	25	86	0	71	45
Total	17	16	7	4	7	51

Table 2.10 Controlled randomized trials of mouthwash that did not perform an analysis of covariance of the endpoint data

	CHX	*EO*	*CPC*	*DEL*	*TRI*	*Total*
Baseline evaluation not considerd for endpoint calculations	3	2	2	3	6	16
Total	17	16	7	4	7	51

used, and Table 2.9 details mouthwash-specific trials that did not report on the randomization method.

Eight trials implemented single-blinding, 38 implemented double-blinding, and 2 did not report about blinding. Most trials claiming blinding of endpoint assessor did not report procedures were used for securing blinding. Of note, blinding of assessors is often difficult, for instance because of teeth staining with CHX.

Oral prophylaxis (e.g. tooth polishing/scaling) after baseline assessment was carried out in two-third of trial, in 82% of trials on chlorhexidine preparations and in 87% of trials on essential oil preparations.

Reporting of trial results. Three trials did not report age (TRI: Schaeken, 1996; CHX: Banting *et al*, 1989; CPC: Moran and Addy, 1991). The average of reported mean ages ranged from 19.6 to 52 years, with a median of 34. The mouthwash-specific median age ranged from 32 to 37 years ($P = 0.68$).

Ten trials did not report gender distribution. Four trials reported overall proportions of men and women, without mentioning their group distribution. The median percentage of men ranged from 38% to 42% in trials on CHX, Listerine, CPC and triclosan. In delmopinol trials, 93% of subjects were men.

The lost-to-follow-up (LTFU) median was 6% of all subjects included in trials, ranging from 0.2% to 40% (Corbet *et al*, 1997). Twenty-seven trials reported LTFU by randomization group. Seventeen trials reported LTFU for all groups together, and four reported no data on LTFU. Because of the imperfect reporting on LTFU, it was impossible to assess whether use of a specific substance was associated with a higher dropout rate that could have be a signal for the presence of side effects.

Baseline evaluation data were not reported by two trials and dispersion parameters (s.d., s.e. or 95% CI) were not reported by eight trials, five of which were CHX trials.

All trials reported the quantitative scoring system they used for plaque and/or gingivitis evaluation. All trials used one of the four scoring systems described in the Methods section, but the trial by Ashley *et al* (1984, CPC) that used a non-standard semi-quantitative scoring tool (the 'Podshalley PHPI'). Ayad *et al* (1995, TRI) and Triratana *et al* (1995, TRI) used the Talbott–Mandel–Chilton modification of the Loe–Silness Gingival Index.

Sixteen trials performed an end-of-trial evaluation and thus did not take baseline values into consideration (Table 2.10). Three trials did not report how they analysed their data. Four trials said that intent-to-treat analysis using covariance modelling was undertaken. However, intent-to-treat analysis implies that results are computed on all randomized subjects, whereas covariance analysis implies that only subjects with both baseline and end-of-trial data are analysed (i.e. the per protocol analysis). As LTFU subjects existed in all trials, intent-to-treat analysis using covariance analysis was impossible.

Articles rarely reported information regarding side effects, most usually limited to tooth staining, or taste and smell issues (i.e. halitosis).

The overall quality of trial design, conduct and reporting is summarized in Table 2.11. On average, trials of delmopinol and essential oils mouthwashes were of better quality. Trials on chlorhexidine met the average of all trials. Trials on cetylpyridinium and triclosan were of lower quality than trials for other mouthwashes.

Discussion of methods and trial characteristics

Meta-analysis options. For the sake of being inclusive rather than exclusive, trials with durations of at least 1 month were accepted for the meta-analysis. The positive aspect was that trials of at least 6-month duration included 80% of all subjects part of the 51 meta-analysed trials.

The minimal trial duration of 1 month which was selected was effective in avoiding inclusion of a large number of small trials of short duration (ranging from a few days to 1–2 weeks) that would have merely contributed noise to an overall interpretation.

For trials with more than one control group, the control group with the preparation deemed to be the less effective

Table 2.11 Assessment of quality indicators of randomized controlled trials of mouthwash by type of mouthwash

	CHX	*EO*	*CPC*	*DEL*	*TRI*	*Total*	*%*
Total	17	16	7	4	7	51	
Sex not reported	3	3	3	0	2	11	22
Age not reported	1	0	1	0	1	3	6
Randomization not explained	8	4	6	0	5	23	45
Presumably unblinded	2	1	0	0	1	4	8
No LTFU reported	2	0	1	0	1	4	8
LTFU for all groups together	3	9	3	0	4	19	37
Baseline data not reported	3	1	0	0	0	4	8
Baseline data not considered for endpoint calculations	3	2	2	2	6	15	29
No dispersion paramater reported	5	1	1	0	1	8	16
Total limitations	30	21	17	2	21	91	
Mean no. limitations per RCT	1.8	1.3	2.4	0.5	3.0	1.8	

LTFU, lost-to-follow-up subjects.

for plaque or gingivitis control was systematically selected. A consequence of this analysis option was that the main effect of the product (that includes the substance) was evaluated and not of the substance itself.

Other indexes for mouthwash efficacy have been proposed, but a recent statistical reappraisal of these indexes showed that plaque and gingivitis indices remained the best parameters for monitoring plaques and gingivitis and that other parameters did not yield additional information about trial outcome (Lorenz *et al*, 2009).

Supervision of mouth rinsing—Supervision of the use of the mouthwash tested and of control preparation by an external observer was not considered as an inclusion criterion because supervision is an artificial add-on in trials that seldom exists in real-life conditions (Chilton & Fleiss, 1986). Also, trials of sufficient duration and mainly those lasting at least 6 months are more likely to reproduce real life circumstances during which these products are used as well as the usual way they would be used by most people.

Crossover trials (COTs)—A number of crossover trials (COTs) compared the alternative use of mouthwash and of a comparator by the same subjects during two successive periods. Typically, half the subjects started the trial using mouthwash and the other half started the trial using the comparator. After a certain number of days of mouthwash use, subjects had to interrupt use of the mouthwash or of the comparator. The period after this interruption is the 'wash-out' period during which the oral cavity environment (in terms, e.g. of microbial flora) was deemed to return to pretrial conditions. After that wash-out period, the mouthwash and the comparator were substituted and were used during the same number of days. Oral inspections for outcome assessment were done at the beginning and end of the first and at the beginning and end of the second period. In theory, the COT is appealing because no difference exists between subjects included in the trial. Also, it's statistical efficiency is high and requires less subjects than parallel group randomized trials. However, the COT design presents many disadvantages (Chilton & Fleiss, 1986):

- COTs require an adequate wash-out period between treatment periods to avoid carry-over effect, that is, changes in the microbiological flora induced during the first part of the COTs that somehow persist during the second part of the trial (CDT, 1986; Imrey *et al*, 1994). It is never certain that a carry-over effect may have affected trial results, and eliminating this effect may in fact prove to be insurmountable (Chilton & Fleiss, 1986).
- Most COTs were of small size and of short-term duration (few days or weeks);
- The COT is not suitable for the evaluation of efficacy and side effects associated with long-term use of mouthwash products (CDT, 1986);
- It is difficult to guarantee that a similar level of plaque and gingivitis control will be obtained at each treatment period (Imrey *et al*, 1994). Also, subjects may change their way to use the mouthwash over the trial duration, and the way to use a mouthwash at the beginning of the COT may differ from the way the other mouthwash is used after the crossover has happened.
- COTs are known to be prone to the Hawthorne effect (Barnett, 2003): as the COT progresses, subjects tend to change their behaviours according to investigator's expectations, and the longer the trial, the greater the Hawthorne effect. This effect is facilitated by the fact that the mouthwash and the comparator often differ in several ways (e.g. different taste). If the mouthwash and the comparator also differ in parallel group randomized trials, subjects use only one of them and the Hawthorne effect is equally present in the trial groups.

Statistical analysis—American and Australian guidelines recommend using absolute end-of-trial ratios [(last score in intervention group − last score in control group)/last score in control group] for calculating the difference in plaque and gingivitis scores at trial end (see below). The absolute end-of-trial ratio has the limitation of not taking into account the extent and severity of plaque and gingivitis at baseline. One could argue that because in most trials, professional scaling of teeth was done at trial inception, the baseline plaque scores would not influence the absolute end-of-trial results. Figures 2.1 and 2.2 demonstrates that this was not the case. The reasons why professional prophylaxis did not set some sort of 'zero plaque score' at baseline for all subjects included in trials need to be clarified. Probably that extent and severity of plaque and gingivitis at baseline reflected the usual oral hygiene habits and the oral microflora of subjects. Professional scaling was not meant to modify these parameters, but will contribute to removing dental plaque so that plaque growth after the trial start was easier to evaluate using quantitative indexes.

About the trial design and analysis. Methodologies used in trials were often poorly described in published reports. The quality of reporting on design and results was generally lower in trials on triclosan or delmopinol. Trials did not use uniform metrics to describe the effect of the intervention. Recourse to 'exotic' statistical tests like the 'least square *t*-test' (Nelson *et al*, 1991) was not rare.

The field suffers enormously from the lack of a harmonized methodology. If guidelines and articles on recommendations provide useful guidance on many topics, principally on clinical aspects and on quantitative indexes, they did not really address how data should be collected and analysed, how randomization should be carried out, techniques for the blinding of endpoint assessors (or to minimize this bias), maximization of between-assessor reproducibility and how trial design and results should be reported. These are crucial aspects of trials design and reporting.

The CDT of the ADA issued in 1986 the first guidelines for acceptance of chemotherapeutic products for the control of supragingival dental plaque and gingivitis (CDT, 1986), after which in 1988 the CDT officially accepted Peridex® (chlorexidine preparations with 0.12%

CHX in 11.6% HA solution) and Listerine® (essential oils in a 26.9% HA solution; CDT, 1988a,b). This initial guideline issued the key recommendations of 6-month duration trials for evaluating the efficacy of mouthwash on plaque and gingivitis control, and systematic use of quantitative indexes for the evaluation of plaque and gingivitis extent and severity. This guideline did not mention how to organize the data collection, how to perform the statistical analysis, which design details and data had to be reported in publications. It was also silent on the professional prophylaxis at trial inception.

A recommendation for professional prophylaxis at trial inception was issued by a revision of these guidelines (Imrey *et al*, 1994). This revision also issued a benchmark for efficacy, set as

1 the estimated proportionate reductions [i.e. (control–active)/control] have to be no <15% in favour of the active treatment with a confidence interval of $\pm$10% and statistically significant in each of at least two trials;
2 in addition, the (arithmetic) mean of the estimated proportionate reductions [i.e. (control–active)/control] across trials should be no <20%.

The 15% benchmark is computed as a absolute end-of-trial ratio and expressed as a percentage. This revision, however, did not provide more in-depth guidance on data collection, statistical analysis and reporting. Since then, ADA guidelines did not elaborate much on these aspects. The Australian guidelines also recommend the use of absolute end-of-trial ratios for computation of outcomes (Aust, 1991).

Other authors made recommendations for the design, analysis and interpretation of plaque and gingivitis trials (Chilton and Fleiss, 1986; Addy, 1995; Addy and Newcombe, 2005), but in our opinion, these texts did not really fill in gaps left by guidelines. Thus, in 2011, there is still room for improvement for the development, conduct and analysis of studies aiming at evaluating the efficacy of chemotherapeutic products for the control of supragingival dental plaque and gingivitis.

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Chapter 3

A systematic review with meta-analysis of mouthwash use for the prevention of dental plaque

The materials and methods have been outlined in chapter 2. Fifty-one trials tested the ability of mouthwash to control plaque development, producing a total of 57 intervention groups (see chapter 2 for trials that tested two mouthwash preparations).

Table 3.1 shows mean score values at baseline (weighted for trial size), according to the scoring system used. Subjects included in trials with essential oils and cetylpyridinium had slightly higher plaque scores than subjects included in chlorhexidin and delmopinol trials. For the triclosan trials, baseline scores varied much with the scoring system used.

Meta-analysis results are displayed in forest plots (Figure 3.1). The square represents the mean relative difference found in a specific trial, and the square size reflects the number of subjects included in a trial. The tails represent the 95% confidence interval. The summary relative difference (SRD) is shown on the bottom of the plot, with its 95% CI represented as a lozenge.

The SRD for trials on chlorhexidine was −0.362 (95% CI −0.571, −0.153; Figure 3.1), with no evidence that a trial was more influential than others on the SRD (Figure 3.2). The cumulative SRD computed from adding trial results to each other according to the year of publication

Table 3.1 Plaque scores at baseline in randomized controlled trials of mouthwash use by type of mouthwash

Substance	*Index*	*Weighted mean*	*Weighted s.d.*	*Range*
CHX	S&L1964	1.62	0.5	0.53–2.64
	T1970	1.72	0.64	1.09–2.38
EO	S&L1964	2.2	0.96	0.7–2.88
	T1970	2.25	0.7	0.91–2.94
CPC	S&L1964	No study		
	T1970	2.37	0.29	1.09–2.38
DEL	S&L1964	1.35	0.46	1.05–1.7
	T1970	1.6	0.36	0.51–1.67
TRI	S&L1964	1.52	0.01	1.52–1.53
	T1970	2.72	0.33	2.4–3.5

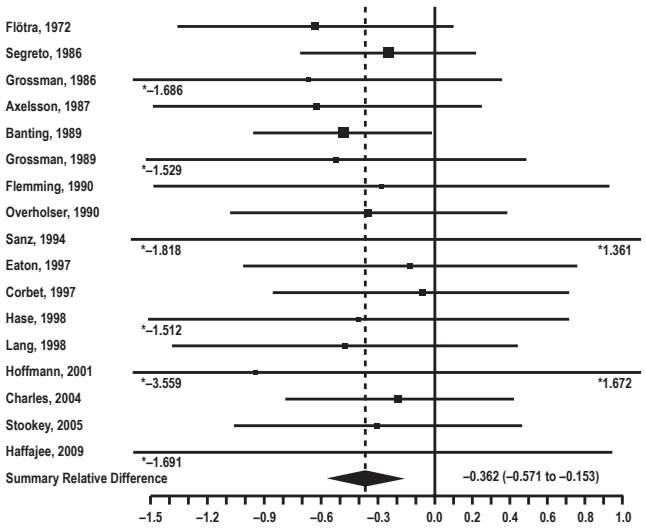

Figure 3.1 Forest plot of randomized controlled trials of use of chlorhexidine in prevention of dental plaque

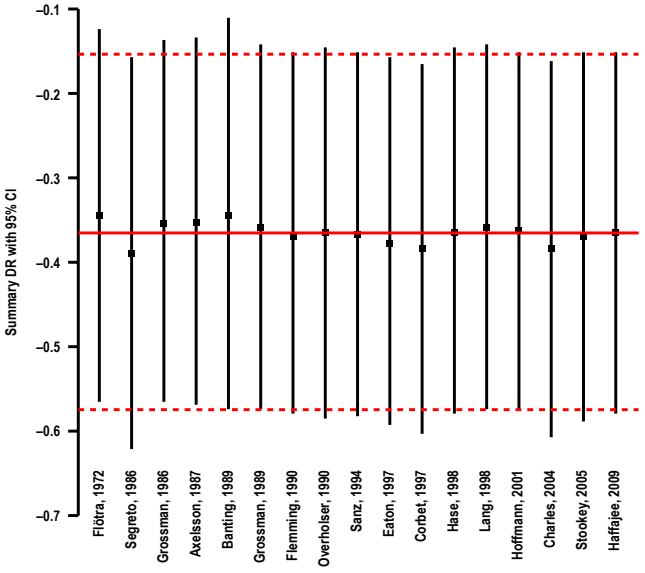

Figure 3.2 Sensitivity analysis (leave-one-out) of meta-analyses of randomized controlled trials of use of chlorhexidine in prevention of dental plaque

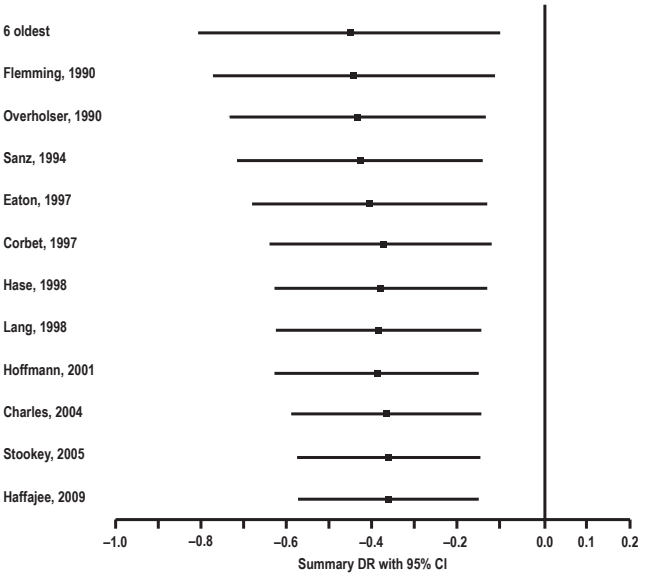

Figure 3.3 Cumulative meta-analysis of randomized controlled trials of use of chlorhexidine in prevention of dental plaque by temporal inclusion of new trials

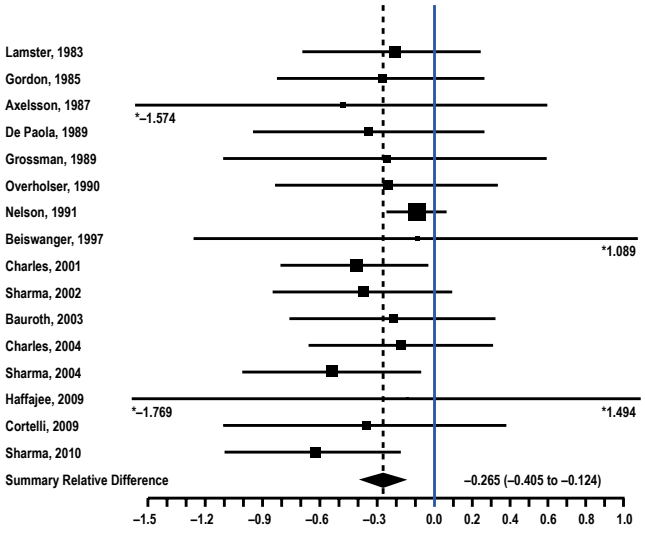

Figure 3.4 Forest plot of randomized controlled trials of use of essential oil mouthwash in prevention of dental plaque

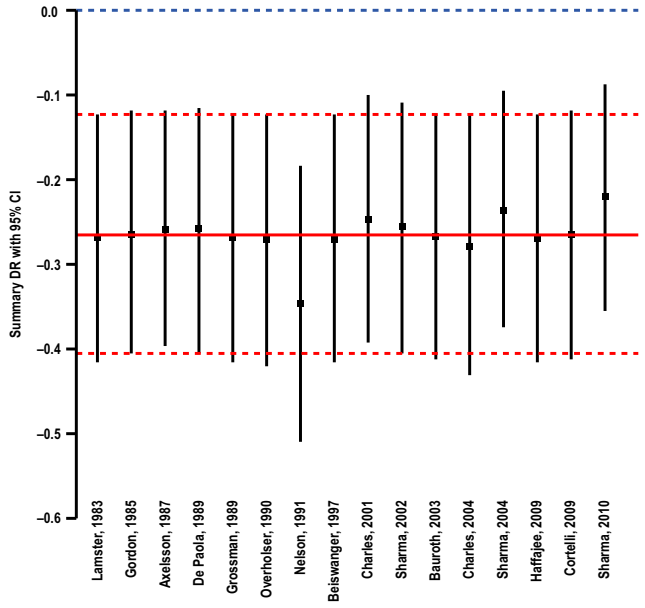

Figure 3.5 Sensitivity analysis (leave-one-out) meta-analyses of randomized controlled trials of use of essential oil mouthwash in prevention of dental plaque

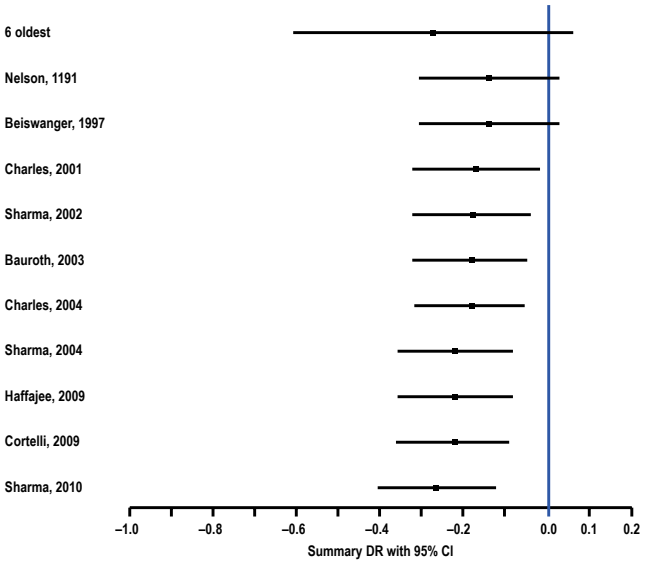

Figure 3.6 Cumulative meta-analysis of randomized controlled trials of use of essential oil mouthwash in prevention of dental plaque by temporal inclusion of new trials

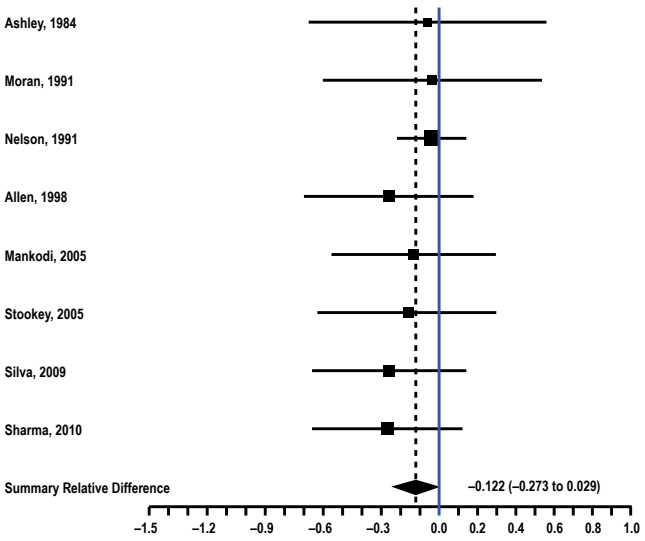

Figure 3.7 Forest plot of randomized controlled trials of use of cetylpyridinium mouthwash in prevention of dental plaque

(Figure 3.3) shows that the SRDs remained fairly stable over time, from earliest trials before 1990 to the most recent one published in 2009.

The summary relative difference (SRD) for trials on essential oils was −0.265 (95% CI −0.405, −0.124; Figure 3.4). The trial by Nelson *et al* (1991) was influential as the SRD computed while ignoring this trial decreased to −0.350 (Figure 3.5).

The cumulative SRD computed from adding trial results to each other according to the year of publication (Figure 3.6) shows that after publication of the Nelson *et al* trial in 1991, the SRDs steadily decreased as new trials obtained results compatible with a substantial effect of essential oil mouthwashes on plaques.

The summary relative difference (SRD) for trials on cetylpyridinium was −0.122 and statistically non-significant as the 95% CI ranging from −0.273 to 0.029 included zero (Figure 3.7). The trial by Nelson *et al* (1991) was influential as the SRD computed while ignoring this trial decreased to −0.200 (Figure 3.8).

The summary relative difference (SRD) for trials on delmopinol was −0.173 and statistically non-significant as the 95% CI ranging from −0.853 to 0.507 included

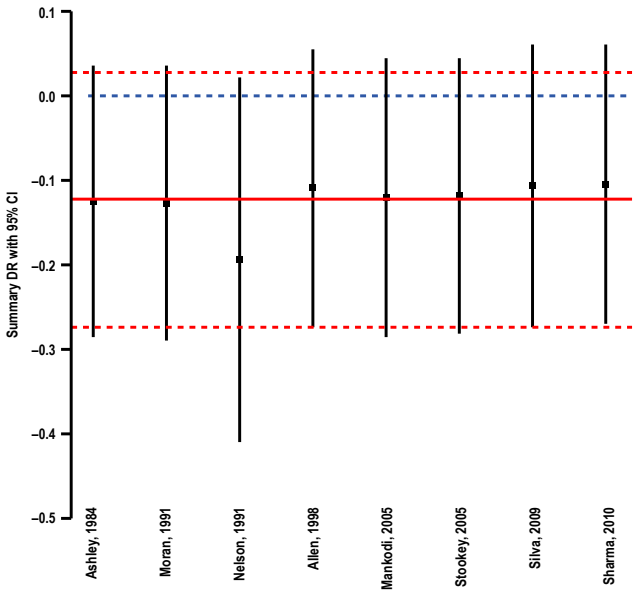

Figure 3.8 Sensitivity analysis (leave-one-out) of meta-analyses of randomized controlled trials of use of cetylpyridinium mouthwash in prevention of dental plaque

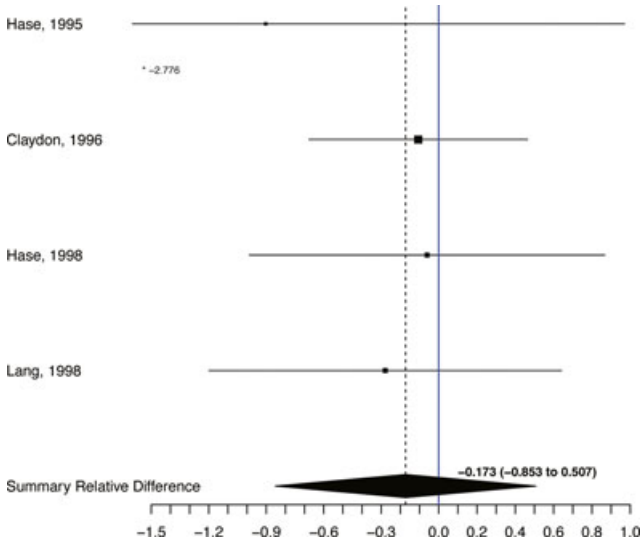

Figure 3.9 Forest plot of randomized controlled trials of use of delmopinol mouthwash in prevention of dental plaque

zero (Figure 3.9). This very large 95% confidence interval was due to the few number of subjects included in the four trials. There was no evidence that a trial was more influential than others on the SRR (data not shown).

The summary relative difference (SRD) for trials on triclosan was −0.209 and statistically non-significant as the 95% CI ranging from −0.436 to 0.017 included zero (Figure 3.10). There was no evidence that a trial was more influential than others on the SRR (data not shown).

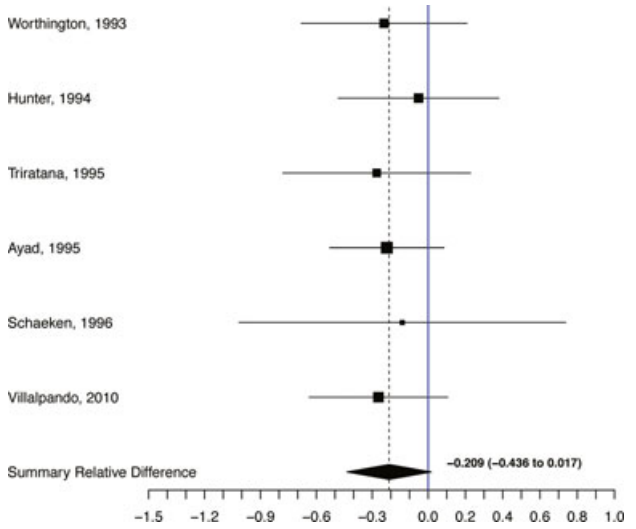

Figure 3.10 Forest plot of randomized controlled trials of use of triclosan mouthwash in prevention of dental plaque

The Table 3.2 summarizes results. If, overall, SRDs for all mouthwashes indicated a significant control on plaque development, the efficacy of the different products was variable. The Loe–Silness Plaque Index was used in 15 intervention groups and resulted in a summary relative difference of −0.291 (95% CI −0.571, −0.011). The Turesky Index was used in 41 intervention groups and resulted in a summary relative difference of −0.211 (95% CI −0.288, −0.134).

There was no evidence of heterogeneity in results as all I^2 were equal to zero.

Figure 3.11 is a funnel plot of all trials on plaque control. 'DR' means difference between the intervention and the control group, and '1/variance' provides an estimate of trial weight in the overall group of trials considered. Small size trials will tend to have a large variance and thus a low value for 1/variance; the reverse will happen with large trials.

The funnel plot shows numerous smaller size trials with 1/variance below 15, what indicates that many small size trials that had results suggesting no effect or a positive effect of mouthwash use on plaque extent did not publish their results. The funnel plot was about the same for all products, although tests in Table 3.2 suggest more publication bias for essential oil and cetylpyridinium trials. Small trial numbers for delmopinol did not allow proper examination of this aspect.

Figure 3.12 and the Table 3.3 show results of the meta-analysis restricted to the thirty-five trials that lasted 6 months or more and also reported results at 3 months. The difference in SRDs in Tables 3.2 and 3.3 is due to the fewer number of trials that reported results at 3 and 6 months. Overall, SRDs at 3 months were highly predictive of SRDs at 6 months (Figure 3.1). Table 3.3 shows results at 3 and 6 months per product. The closeness of 3- and 6-month SRDs was greatest for the chlorhexidine. If at 3 months, the SRD of essential oils

Table 3.2 Summary results of the meta-analysis of mouthwash for dental plaque control

Analysis	*No. of intervention groups*	*SDR (95% CI)*	I^2 *(%)*	P *(Begg)*	P *(Egger)*	P *(Macaskill)*
Main	57	−0.214 (−0.285 to −0.142)	0	0.94	<0.01	<0.01
Loe–Silness	15[a]	−0.291 (−0.571 to −0.011)	0	0.71	0.66	0.08
Turesky	41[a]	−0.211 (−0.288 to −0.134)	0	0.97	<0.01	<0.01
Chlorhexidine	20	−0.362 (−0.571 to −0.153)	0	0.87	0.65	1.00
Essential oil	16	−0.265 (−0.405 to −0.124)	0	0.79	<0.01	<0.01
Cetylpyridinium	9	−0.122 (−0.273 to 0.029)	0	0.62	0.02	0.06
Delmopinol	5	−0.173 (−0.853 to 0.507)	0	0.50	0.33	0.56
Triclosan	7	−0.209 (−0.436 to 0.017)	0	0.45	0.71	0.44

SRD, Summary relative difference.
[a]Sum does not add up to 57 because Ashley, 1984 (CPC), uses neither Silness–Loe nor Turesky Plaque Index, but 'Podshalley PHPI'.

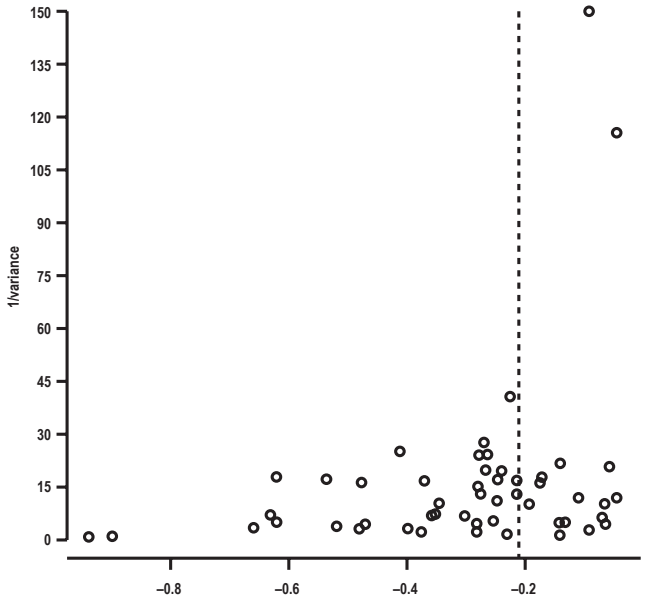

Figure 3.11 Funnel plot to investigate publication bias phenomenon in all randomized controlled trials of mouthwash in prevention of dental plaque

Discussion

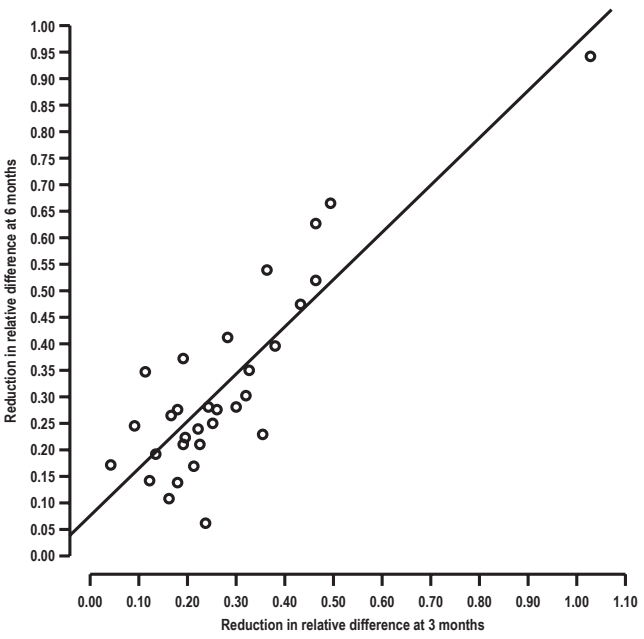

Figure 3.12 Correlation between effects reported after 3 and 6 months use of mouthwash in the prevention of dental plaque

was lower than that of the chlorhexidine, the 6-month SRDs were quite similar. For delmopinol and triclosan, SRDs at 6 months were equivalent (for triclosan) or lower (for delmopinol) than SRDs at 3 months.

Table 3.4 stratifies SRDs by trial duration. Trials <6 months lasted 1–3 months. There were fewer short-term trials that included 25% of all subjects included in the 51 trials selected for this meta-analysis (see Chapter 2). None of the SRDs computed from results of short-term trials was statistically significant as all 95% confidence intervals included zero. In trials that lasted 6 months, the changes in plaque scores were statistically significant and similar for chlorhexidine and essential oil products (comparison of SRDs of CHX 6-month trials *vs* SRDs of EO 6-month trials by meta-regression: $P = 0.95$).

Table 3.3 Summary relative differences of trials that provided results at 3 and 6 months

		SRD Plaque with 95% CI	
Substance	N	*3 months*	*6 months*
CHX	11	−0.316 (−0.603, −0.029)	−0.360 (−0.702, −0.018)
EO	11	−0.240 (−0.394, −0.086)	−0.350 (−0.525, −0.176)
CPC	5	−0.183 (−0.571, 0.205)	−0.214 (−0.562, 0.135)
DEL	4	−0.202 (−1.185, 0.781)	−0.134 (−1.078, 0.809)
TRI	4	−0.199 (−0.803, 0.406)	−0.22 (−0.752, 0.313)
ALL	35	−0.235 (−0.336, −0.134)	−0.286 (−0.392 −0.181)

SRD, summary relative difference; CHX, chlorhexidine; EO, essential oil; CPC, cetylpyridinium chloride; DEL, delmopinol; TRI, triclosan; *N*, number of intervention groups (not the number of trials).

Table 3.4 Meta-analysis SRDs and (95% CI) after stratification on trial duration: results for plaque control

	CHX		*EO*		*CPC*		*DEL*		*TRI*	
	N	*SRD*	N	*SRD*	N	*SRD*	N	*SRD*	N	*SRD*
<6 months	8	−0.32 (−0.714, 0.073)	4	−0.105 (−0.356, 0.145)	4	−0.075 (−0.325, 0.175)	1	−0.903 (−2.776, 0.971)	2	−0.175 (−2.000, 1.650)
≥6 months	12	−0.392 (−0.679, −0.105)	12	−0.346 (−0.517, −0.175)	5	−0.214 (−0.562, 0.135)	4	−0.134 (−1.078, 0.809)	5	−0.23 (−0.585, 0.125)
Overall	20	−0.362 (−0.571, −0.153)	16	−0.265 (−0.405, −0.124)	9	−0.122 (−0.273, 0.029)	5	−0.173 (−0.853, 0.507)	7	−0.209 (−0.436, 0.017)

SRD, summary relative difference; CHX, chlorhexidine; EO, essential oil; CPC, cetylpyridinium chloride; DEL, delmopinol; TRI, triclosan; *N*, number of interventions (*not* the number of studies).
Comparison CHX 6 months/EO 6 months: $P = 0.95$ (meta-regression).

The Nelson *et al*'s (1991) trial was influential on results obtained for essential oils and cetylpyridinium. This trial lasted for 6 weeks and included about 30 subjects per randomization group. The statistical analysis was based on an unknown test; the 'least square *t*-test' and adjusted results were provided, but there was no mention of factors that were adjusted for.

Reference cited in Chapter 3

Nelson RF, Rodasti PC, Tichnor A, Lio YL (1991). Comparative study of four over-the-counter mouthrinses claiming antiplaque and/or antigingivitis benefits. *Clin Prev Dent* **13**: 30–33.

Chapter 4

A systematic review with meta-analysis of mouthwash use for the prevention of gingivitis

The materials and methods have been described in chapter 2. Five trials reported results on plaques, but not on gingivitis control (Hase *et al*, 1998, CHX; Hase *et al*, 1995, DEL; Schaeken *et al*, 1996, TRI; Hunter *et al*, 1994, TRI; Ashley *et al*, 1984, CPC). A total of 49 intervention groups tested the ability of mouthwash to control gingivitis development, totalling fifty intervention groups, meaning that several trials tested two mouthwashes (see, chapter 2, for trials that tested 2 preparations).

Table 4.1 shows mean score values at baseline (weighted for trial size), according to the scoring system used. Subjects included in trials with chlorhexidin had lower gingivitis scores than subjects included in trials for other products.

Meta-analysis results are displayed in forest plots (Figure 4.1). The square represents the mean relative difference found in a specific trial, and the square size reflects the number of subjects included in a trial. The tails represent the 95% confidence interval. The summary relative difference (SRD) is shown on the bottom of the plot, with its 95% CI represented as a lozenge.

The SRD for trials on chlorhexidine was −0.223 (95% CI −0.412, −0.034; Figure 4.1), with no evidence that a trial was more influential than others on the SRR (Figure 4.2). The cumulative SRD computed from adding trial results to each other according to the year of publication (Figure 4.3) shows that the SRDs remained fairly stable over time, from earliest trials before 1990 to the most recent one published in 2009.

The SRD for trials on essential oils was −0.203 (95% CI −0.312, −0.093; Figure 4.4), with no evidence that a trial was more influential than others on the SRR (Figure 4.5). The cumulative SRD computed from adding trial results to each other according to the year of publication (Figure 4.6) shows that the SRDs remained fairly stable over time, from earliest trials before 1990 to the most recent one published in 2009.

The SRD for trials on cetylpyridinium was −0.126 and statistically non-significant as the 95% CI ranging from −0.312 to 0.059 included zero (Figure 4.7) no evidence that a trial was more influential than others on the SRD (data not shown).

The SRD for trials on delmopinol was −0.014 and statistically non-significant as the 95% CI ranging from −2.337 to 2.308 included zero (Figure 4.8). This very large 95% confidence interval was due to the small number of subjects included in the four trials. There was no

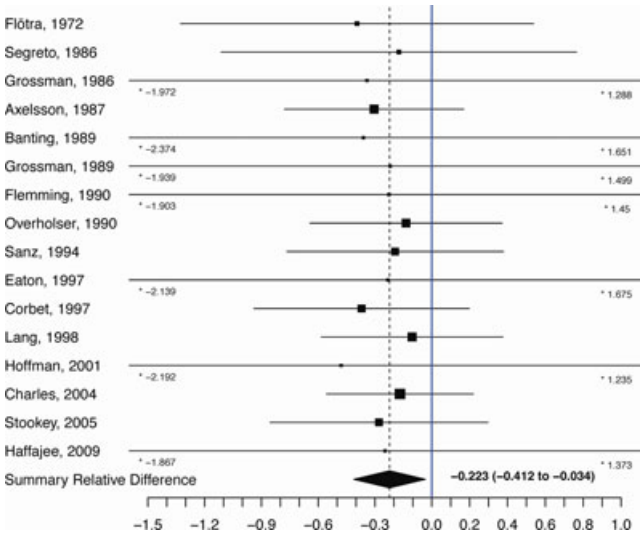

Figure 4.1 Forest plot of randomized controlled trials of use of chlorhexidine mouthwash in prevention of gingivitis

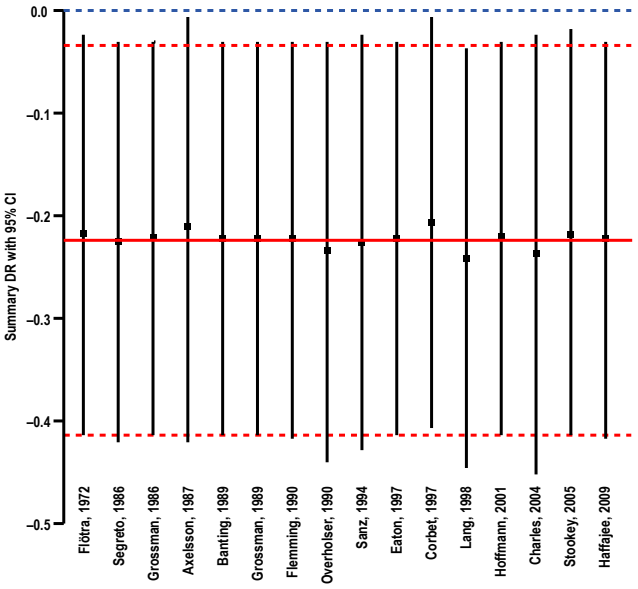

Figure 4.2 Sensitivity analysis (leave-one-out) of meta-analyses of randomized controlled trials of use of chlorhexidine mouthwash in prevention of gingivitis

Table 4.1 Gingivitis scores at baseline in randomized controlled trials of mouthwash use in prevention of gingivitis

Substance	*Index*	*Weighted mean*	*Weighted s.d.*	*Range*
CHX	L&S1963	0.78	0.36	0.38–1.57
	L1986		Only 1 study	
EO	L&S1963	1.28	0.67	0.52–2.2
	L1986	2.13	0.15	2.05–2.74
CPC	L&S1963	1.26	0.54	0.79–2.01
	L1986		Only 1 study	
DEL	L&S1963		Only 1 study	
	L1986	1.87	0.03	1.85–1.89
TRI	L&S1963		Only 1 study	
	L1986		No study	
	TMC1977	1.96	0.38	1.66–2.2

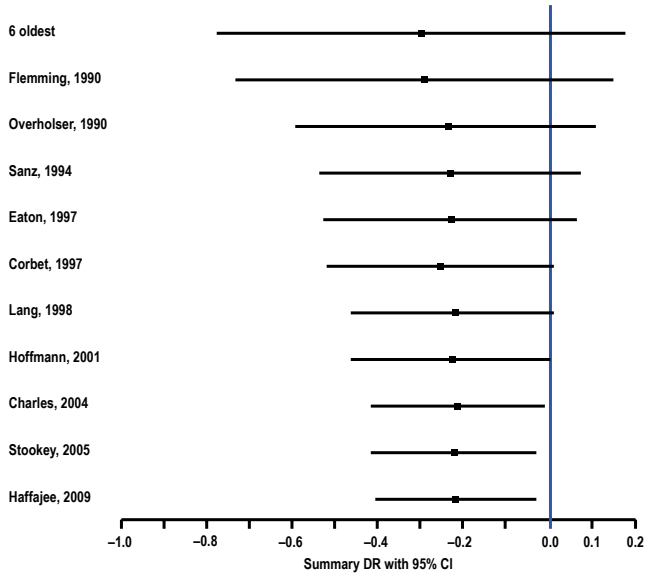

Figure 4.3 Cumulative meta-analysis of randomized controlled trials of use of chlorhexidine mouthwash in prevention of gingivitis by temporal inclusion of new trials

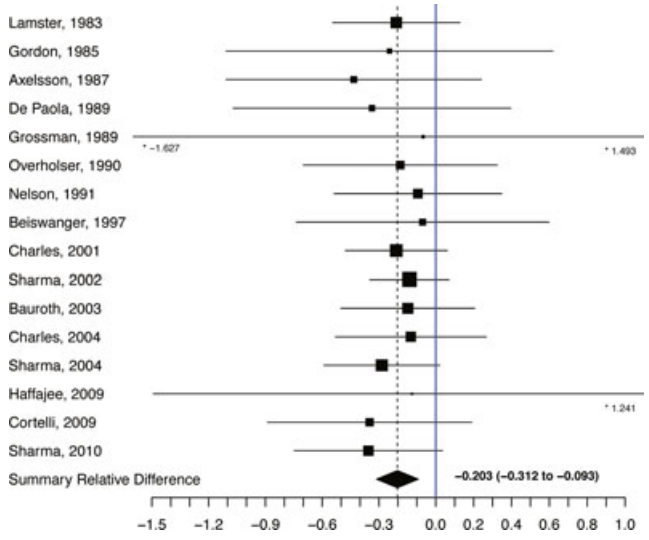

Figure 4.4 Forest plot of randomized controlled trials of use of essential oil mouthwash in prevention of gingivitis

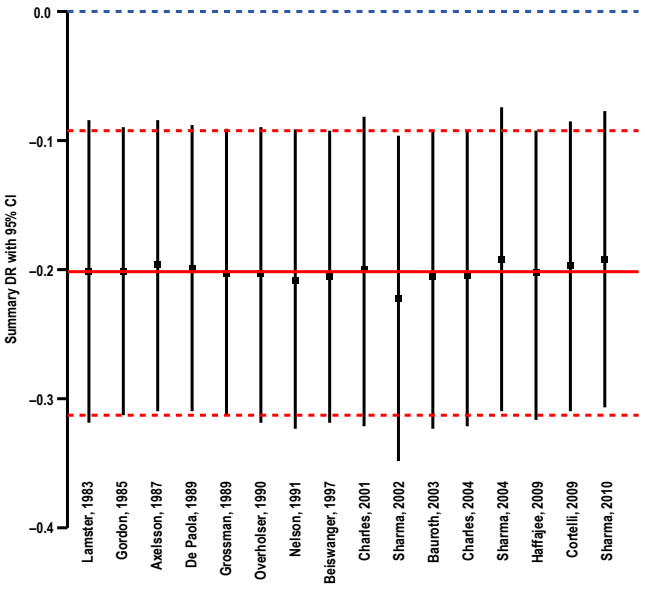

Figure 4.5 Sensitivity analysis (leave-one-out) of meta-analyses of randomized controlled trials of use of essential oil mouthwash in prevention of gingivitis

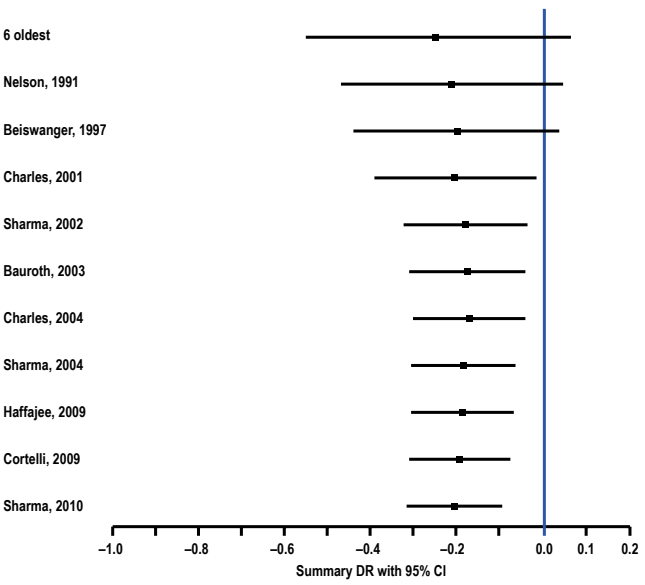

Figure 4.6 Cumulative meta-analysis of randomized controlled trials of use of essential oil mouthwash in prevention of gingivitis by temporal inclusion of new trials

evidence that any single trial was more influential than others on the SRR (data not shown).

The SRD for trials on triclosan was −0.210 and statistically non-significant as the 95% CI ranging from −0.539 to 0.120 included zero (Figure 4.9). There was no evidence that any single trial was more influential than others on the SRR (data not shown).

Table 4.2 summarizes results. If overall, SRDs for all mouthwashes indicated a significant control on gingivitis development, the efficacy of the different products was variable, although essential oil preparations were nearly as effective as chlorhexidine preparations. The Loe–Silness Gingivitis Index was used in 36 intervention groups, and the Lobene Index was used in 11 intervention groups. The two scoring systems indicated similar changes.

There was no evidence of heterogeneity in the results as all I^2 were equal to zero.

Figure 4.10 presents a funnel plot of all trials on gingivitis control. 'DR' means difference between the

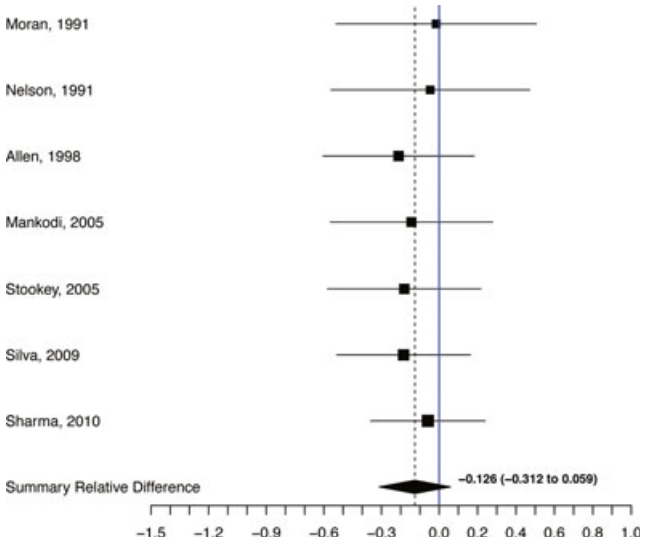

Figure 4.7 Forest plot of randomized controlled trials of use of cetylpyridinium mouthwash in prevention of gingivitis

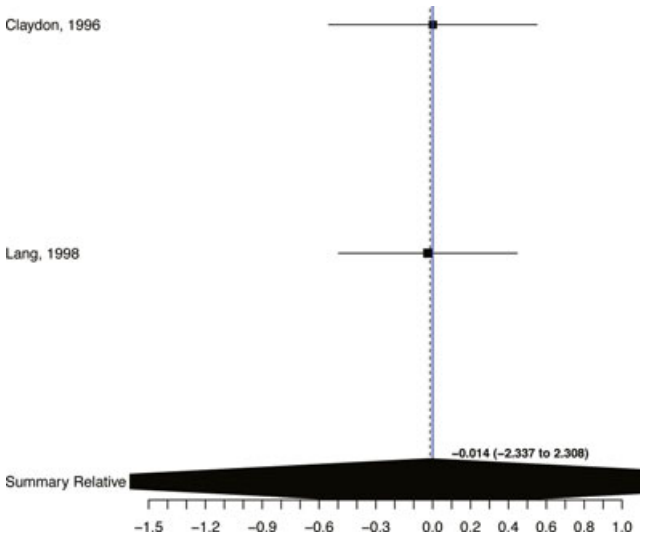

Figure 4.8 Forest plot of randomized controlled trials of use of delmopinol mouthwash in prevention of gingivitis

intervention and the control group, and '1/variance' provides an estimate of trial weight in the overall group of trials considered. Small size trials will tend to have a large variance and thus a low value for 1/variance; the reverse will happen with large trials. The funnel plot shows quite symmetric distribution of 1/variance values below and above the average difference for all trials, indicating no obvious presence of publication bias.

The Figure 4.11 and the Table 4.3 show results of the meta-analysis restricted to the thirty-five trials that lasted 6 months or more and also reported results at 3 months.

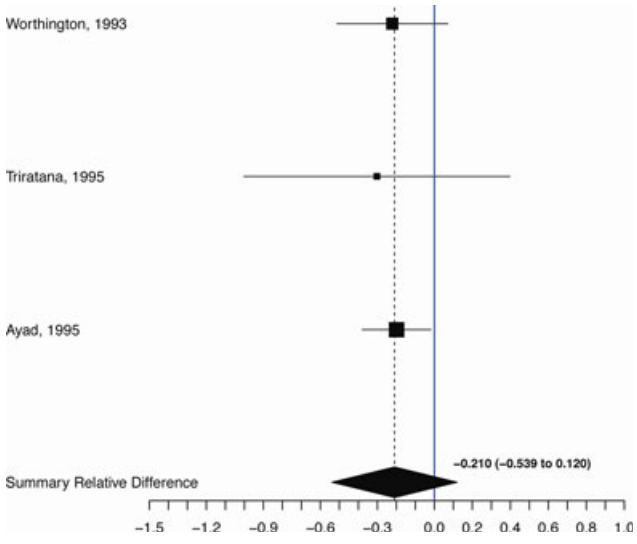

Figure 4.9 Forest plot of randomized controlled trials of use of triclosan mouthwash in prevention of gingivitis

The difference in SRDs in Tables 4.2 and 4.3 is due to the fewer number of trials that reported results at 3 and at 6 months. Overall, relative differences at 3 months only explain 30% of the variance of 6-month relative difference (Figure 4.11). Table 4.3 shows results at 3 and at 6 months per product. Changes at 3 and 6 months were about equivalent for all products but for the essential oils.

Table 4.4 stratifies SRDs by trial duration. Trials <6 months lasted 1–3 months. There were fewer short-term trials that included 25% of all subjects included in the 51 trials selected for this meta-analysis (see chapter 2). None of the SRDs computed from results of short-term trials was statistically significant as all 95% confidence intervals included zero. In trials that lasted 6 months, the changes in gingivitis scores were statistically significant and similar for chlorhexidine and essential oil products (comparison of SRDs of CHX 6-month trials *vs* SRDs of EO 6-month trials by meta-regression: $P = 0.85$).

Discussion

The severity of the plaque and gingivitis indices at baseline was not the same across products, with usually higher scores in essential oil trials then in chlorhexidine trials.

The Tureski and the Lobene Indexes are deemed to be more sensitive for showing changes in the low range of plaque and gingivitis scores, respectively. The findings here do not show greater changes in scores when these indexes are used instead of the Loe–Silness Index.

The decision to employ the relative differences between intervention and control groups has probably contributed to better control of statistical fluctuations associated with use of different scoring indexes and differences in scores at baseline between products.

At first sight, these trials provide evidence that chlorhexidine mouthwashes are the most effective. Essential oil mouthwashes also appear to be effective, but somewhat

Table 4.2 Summary results of the meta-analysis of mouthwash for gingivitis control

Analysis	*No. of intervention groups*	*SRD (95% CI)*	I^2 *(%)*	P *(Begg)*	P *(Egger)*	P *(Macaskill)*
Main	49	−0.184 (−0.251 to −0.116)	0	0.93	0.97	0.15
Loe–Silness	36[a]	−0.174 (−0.266 to −0.082)	0	0.95	0.23	0.45
Turesky	11[a]	−0.198 (−0.339 to −0.056)	0	0.79	0.79	0.13
Chlorhexidine	19	−0.223 (−0.412 to −0.034)	0	0.72	0.20	0.76
Essential oil	16	−0.203 (−0.312 to −0.093)	0	0.93	0.09	0.62
Cetylpyridinium	8	−0.126 (−0.312 to 0.059)	0	0.65	0.65	0.86
Delmopinol	3	−0.014 (−2.337 to 2.308)	0	NR	NR	NR
Triclosan	3	−0.210 (−0.539 to 0.120)	0	0.30	<0.01	0.49

SRD, summary relative difference.
aSum does not add up to 49 because Ayad, 1995 (triclosan) and Triratana, 1995 (triclosan) use neither Loe–Silness nor Lobene Gingival Indexes, but Talbott–Mandel–Chilton modification of the Loe–Silness Gingival Index.

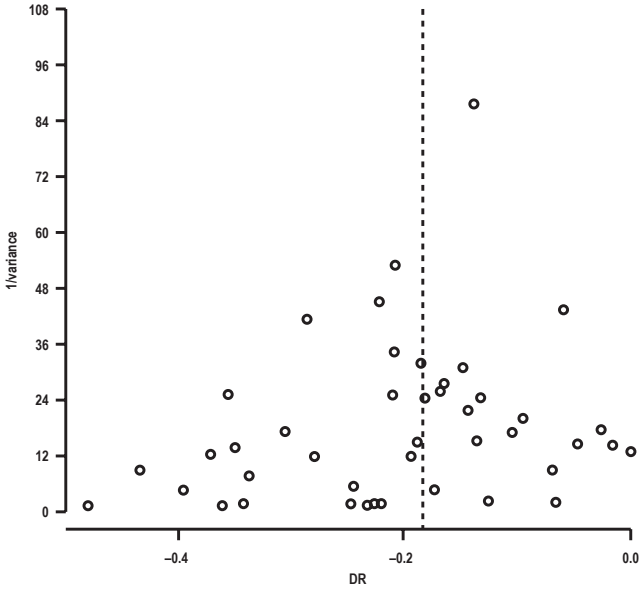

Figure 4.10 Funnel plot to investigate publication bias phenomenon in all randomized controlled trials of mouthwash in prevention of gingivitis

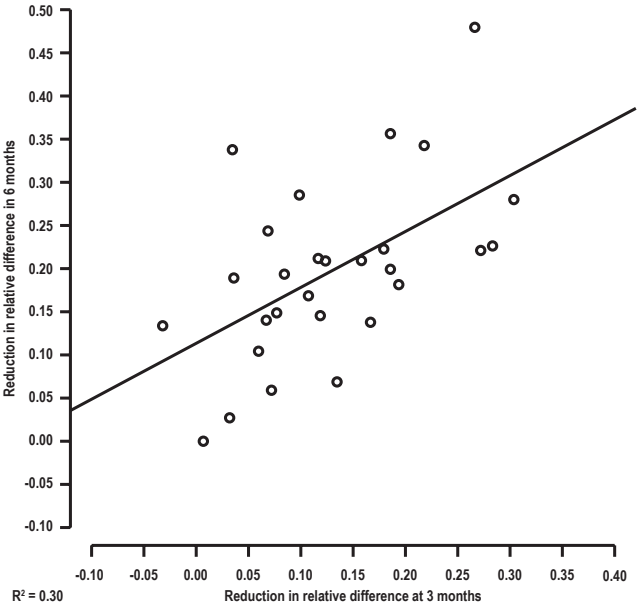

Figure 4.11 Correlation between effects reported after 3 and 6 months use of mouthwash in the prevention of gingivitis

less than chlorexidine. For delmopinol, the few small trials were not suggestive of an effect of this product on plaque and gingivitis. If SRDs for triclosan suggest some efficacy, more trials would be necessary for demonstrating it. Cetylpyridinium trials were not suggestive of a meaningful impact of this product. If the 20% benchmark of the American Dental Associations is considered (CDT, 1986), chlorhexidine at 0.06–0.12% concentration and essential oil preparations that have a constant formulation are the two types of mouthwashes that are effective for the control of supragingival plaque and gingivitis.

Stratification by trial duration and results after 3 and 6 months suggest that chlorhexidine and essential oil products would have the same efficacy, but essential oil products need more time of regular use before their full effect can be observed.

Table 4.3 Summary relative differences of trials that provided results at 3 and 6 months

		SRD Gingivitis with 95% CI	
Substance	N	*3 months*	*6 months*
CHX	10	−0.146 (−0.388, 0.096)	−0.178 (−0.427, 0.071)
EO	11	−0.105 (−0.189, −0.021)	−0.201 (−0.325, −0.077)
CPC	5	−0.112 (−0.355, 0.131)	−0.134 (−0.433, 0.165)
DEL	3	−0.014 (−2.523, 2.496)	−0.014 (−2.337, 2.308)
TRI	2	−0.185 (−1.177, 0.807)	−0.205 (−1.201, 0.791)
ALL	31	−0.119 (−0.179, −0.058)	−0.181 (−0.257, −0.104)

SRD, summary relative difference; CHX, chlorhexidine; EO, essential oil; CPC, cetylpyridinium chloride; DEL, delmopinol; TRI, triclosan; *N*, number of intervention groups (not the number of trials).

Table 4.4 Meta-analysis SRDs and (95% CI) after stratification on trial duration: results for gingivitis

	CHX		*EO*		*CPC*		*DEL*		*TRI*	
	N	*SRD*	N	*SRD*	N	*SRD*	N	*SRD*	N	*SRD*
<6 months	8	−0.316 (−0.721, 0.089)	4	−0.24 (−0.724, 0.244)	3	−0.112 (−0.667, 0.442)	0	NA	0	NA
≥6 months	11	−0.18 (−0.423, 0.063)	12	−0.198 (−0.318, −0.077)	5	−0.134 (−0.433, 0.165)	3	−0.014 (−2.337, 2.308)	3	−0.21 (−0.539, 0.120)
Overall	19	−0.223 (−0.412, −0.034)	16	−0.203 (−0.312, −0.093)	8	−0.126 (−0.312, 0.059)	3	−0.014 (−2.337, 2.308)	3	−0.21 (−0.539, 0.120)

SRD, summary relative difference; CHX, chlorhexidine; EO, essential oil; CPC, cetylpyridinium chloride; DEL, delmopinol; TRI, triclosan; *N*, number of interventions (*not* the number of studies).
Comparison CHX 6 months/EO 6 months: $P = 0.85$ (meta-regression).

These findings support the notion that chlorhexidine products have rapid action on the oral microbiological flora and that in addition to their activity on the oral flora, essential oil products would have anti-inflammatory properties that take longer to show-up. These results also illustrate the relevance of the ADA's recommendation of having trails testing mouthwash products for plaque and gingivitis control lasting at least 6 months (CDT, 1986).

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Chapter 5

A systematic review with meta-analysis of fluoridated mouthwash use for the prevention of dental caries

Dental caries (tooth decay) is a disease of the hard tissues of the teeth. It is one of the most common conditions which afflicts humans and is caused by the interactions over time between certain microorganisms found in dental plaque (*cariogenic bacteria*) and dietary fermentable carbohydrates (principally sugars). This interaction produces organic acids which dissolve tooth substance via a process of demineralization. Progressive dental caries may result in cavities, pain and loss of teeth.

Prospects for primary prevention of caries have been proposed, and there is evidence that the process of caries development can be arrested by appropriate intervention. Fluoride has been viewed as one of the key factors which may prevent and arrest the process towards caries development. The predominant effect of fluoride is topical, that is, a direct effect when it interacts with the tooth enamel surface. Fluoride present in saliva and in dental plaque inhibits tooth enamel demineralization and promotes remineralization. When ingested, fluoride also works through a systemic effect by being built into the enamel during the development of the teeth prior to eruption, although this effect is minor compared with the topical one.

Fluoridation has been supported by the World Health Organization (WHO, 2004). Dental caries remains an extremely common disease which is strongly related to deprivation status: the incidence, prevalence and severity of caries being greater among economically disadvantaged children than among other groups. Although the disease is largely preventable, and despite substantial improvements in dental health over the last decades, it remains a public health problem in most industrialized countries, affecting 60–90% of school children and the vast majority of adults (Petersen, 2003).

The WHO has established a goal of 3.0 Diseased, Missing and Filled Teeth (DMFT) at 12 years of age (Beltran-Aguilar, 1999). In many developing countries, such as in the Latin American countries and Asia, caries prevalence has been notably high, reaching a prevalence of 95% prevalence over this WHO goal. Given the simultaneous lack of access to dental services in these regions, this emphasizes the need to establish methods of caries prevention in the community.

Water fluoridation has been the principal approach to community caries prevention for over half a century and is an important public health measure being able to intervene not only in the development of caries but also in the dynamic process of caries development. Water fluoridation involves adjusting the fluoride content of the water supply to an *optimal* level of one part of fluoride per million parts of water (1 ppm, or 1 mg l^{-1}) or defined levels in tropical environments (0.5–1.0 ppm depending on climate). The advantage of water fluoridation is that it reaches the entire population served by the water distribution system, including the socially deprived groups who constantly have the highest levels of caries. An exemplary systematic review on public water fluoridation (McDonagh *et al*, 2000) demonstrated that water fluoridation reduces the number of decayed, missing and filled teeth by, on average, two and a quarter teeth per child and on average increases the proportion of children completely free from tooth decay by 15%.

McDonagh *et al* (2000) noted the large degree of heterogeneity which existed between the findings in the studies considered in this review. McDonagh demonstrated that water fluoridation increases the risk of dental fluorosis (a form of enamel hypomineralization in the event of excessive ingestion of fluoride during tooth development) – the prevalence of fluorosis at a water fluoride level of 1 ppm was estimated to be 48%, and for fluorosis of aesthetical concern, 12.5% of exposed people would be affected. McDonagh *et al* (2000) concluded that there was little high quality research on fluoride and health and that findings have often been misinterpreted and have been used to support arguments on both sides of the water fluoridation debate.

Various political, geographical, financial and technical reasons have prevented the availability of water fluoridation to a large proportion of the world's population and alternative methods of fluoride therapy (topical and systemic) have been identified and introduced. While fluoridated toothpaste is the most widely used delivery method of fluoride nowadays, other methods of community fluoridation have been used, including fluoride-containing mouthwashes, ingested fluorides in milk and salt.

In this chapter, we review the evidence about fluoride mouthwashes and concomitant use of two oral fluoride preparations compared with a single oral fluoride preparations.

5.1 Fluoride mouthwashes

Adding fluorine salts to mouthwashes was a straightforward way to deliver fluorine to teeth. However, at first sight, fixation of fluorine in tooth enamel was less obvious than for instance, with toothpastes or fluoridated water. Randomized or quasi-randomized trials on the effect of fluorinated mouthwashes for caries prevention in children started in 1965 (Torell *et al*, 1965). Since then, 34 trials have been conducted that were inventoried by Marinho *et al* (2003c), of which 31 were published before 1990. All trials reported data on caries scoring at the end of the trial, comparing a group of children receiving the fluoridated mouthrinses *vs* a group receiving a negative control (most usually water). Fluoride concentrations were variables as well as the fluoride salt incorporated in the mouthwash (e.g. most often sodium fluoride or ammonium fluoride) (DePaola *et al*, 1977).

We performed a meta-analysis of randomized (or quasi-randomized) trials included in the review of Marinho *et al* (2003b), with the objective to ascertain the heterogeneity of results reported by these trials. For this purpose, we used exactly the same methods as those used for the meta-analyses on mouthwashes for the prevention of plaques and gingivitis (see sections 3 and 4). A difference, however, was that a number of trials did not report baseline caries scores, and thus, only end-of-trial results could be calculated.

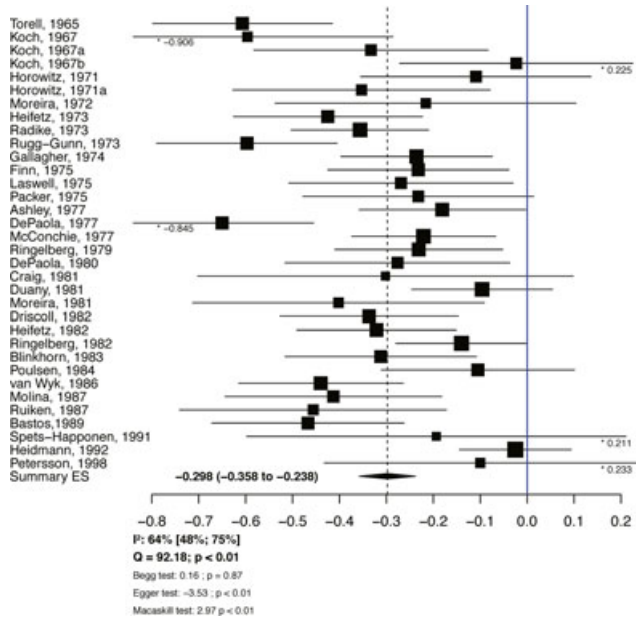

Figure 5.1 Forest plot of randomized controlled trials of use of fluoridated mouthwash in prevention of caries

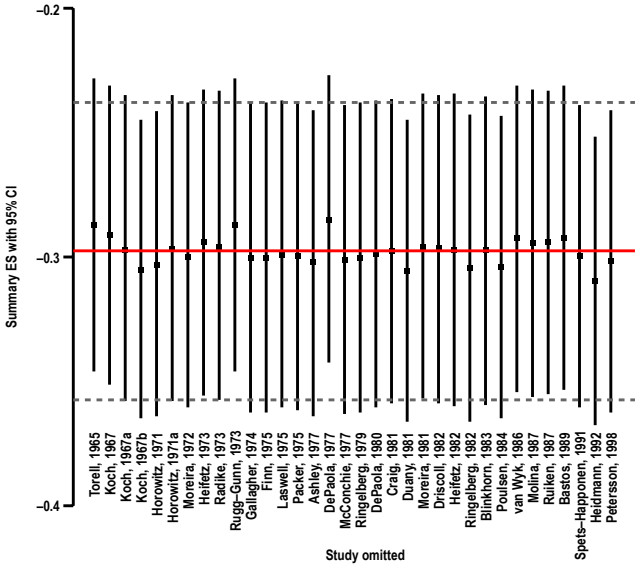

Figure 5.2 Sensitivity analysis (leave-one-out) of meta-analyses of randomized controlled trials of use of fluoridated mouthwash in prevention of caries

These trials included 8950 children allocated to intervention groups and 5713 children allocated to control groups. We found that use of fluoride mouthwashes reduced by 30% the increment in caries scores (Figure 5.1). This end-of-trial effect size is the same to that found by Marinho *et al* (2003b).

Figure 5.2 displays summary effect size for all trials with omitting one trial at a time. This method allows identifying trials whose results were influential on the summary effect size obtained by the meta-analysis. Four trials strongly influenced meta-analysis results towards greater effect size because they obtained considerable reductions in caries scores: these were Koch *et al* (1967), Torell *et al* (1965), Rugg-Gunn *et al* (1973), De Paola *et al* (1977). In contrast, four other trials strongly influenced meta-analysis results towards small or null effect size because they obtained effect sizes compatible with no reduction in caries scores: these were Koch *et al* (1967), Horowitz *et al* (1971), Duany *et al* (1981), Poulsen *et al* (1984), and Heidmann *et al* (1992). Of note, some trials organized by the same authors (Koch *et al*, 1967a,b) ended up in opposite results.

The immediate consequence of the juxtaposition of trials suggesting strong protection while others suggested no protection is the huge variability in results across trials. The heterogeneity in results that is visible in Figure 5.1 is denoted by a I^2 of 64%, that is, 64% of heterogeneity in results cannot be explained by statistical hazard. This heterogeneity contrasts with the absence of heterogeneity in trials on mouthwashes for the prevention of plaques and of gingivitis (see sections 3 and 4). Marinho *et al* (2003b) acknowledged the 'statistical evidence for heterogeneity', but judged that all being considered, there was not so much heterogeneity in results between studies.

Figure 5.3 suggests that a meta-analysis that would have included results of the trials as publications

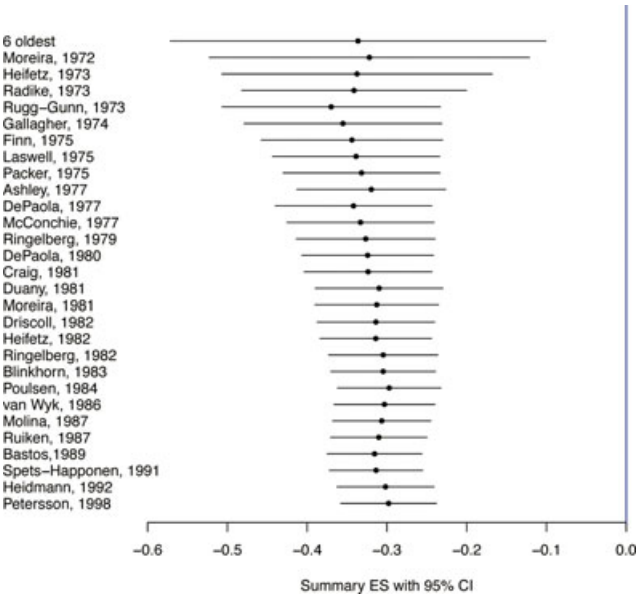

Figure 5.3 Cumulative meta-analysis of randomized controlled trials of use of fluoridated mouthwash in prevention of caries

proceeded would have found quite stable preventive effect over time. Hence, the heterogeneity in trial results was spread over time, which explains why the cumulative summary effect size in Figure 5.3 remains relatively stable over time.

In 1975, the US Council on Dental Therapeutics (CDT, 1975) classified the fluoridated mouthwashes as efficient, based on results of the fourteen randomized trials available at that time. Three trials had obtained results suggesting huge decreases in caries scores while two had obtained results suggesting no effect of fluorinated mouthwashes

(Figure 5.1). Most probably that the Council did not take note of trial validity issues denoted by the substantial heterogeneity in results that were already noticeable.

Each fluoridated mouthwash trial included on average 2.6 more control subjects than trials on mouthwashes for the prevention of plaque and gingivitis. Thus, the statistical power of trials with fluoridated mouthwashes was greater, which should have contributed to stabilize results across trials, but this was not the case. Several factors may explain the large degree of heterogeneity. First, designs of trials were variable, with use of different preparations, different study settings and randomization procedures. As for the trials on plaque and gingivitis, most trials did not report how subjects were allocated to the intervention and control groups. Many trials claimed being 'double-blinded', but no details were provided on how blinding of subjects and endpoint assessors was implemented.

Second, a high attrition rate has been observed with a mean of 32% dropout. However, few trials reported whether dropouts were associated with being allocated to an intervention or a control group.

Third, similarly to trials on plaques and gingivitis, most probably, the preventive effect of fluoridated mouthwashes was proportional to amounts of caries at baseline. A number of trials on fluorinated mouthwashes did not report baseline caries scores and it is likely that failure to take into account baseline caries scores in effect size computations (i.e. via relative end-of-trial results) also contributed to the heterogeneity in results.

A consequence of these considerations is whether the magnitude of the preventive effects of fluorinated mouthwashes on dental caries has not been overestimated. Future research should examine meta-analysis results according to trial quality, and perhaps, one could envision the organization of new randomized trials based on more robust designs.

5.2 Combination of topical fluoride compared with single topical fluoride for preventing dental caries

Various modes have evolved, each with its own recommended concentration, frequency of use and dosage schedule. The use of topically applied fluorides in particular, which are much more concentrated than the fluoride in drinking water, has increased over recent decades, and fluoride-containing toothpastes (dentifrices), mouthrinses, gels and varnishes are the modalities most widely used at present, either alone or in different combinations. By definition, the term 'topically applied fluoride' describes those delivery systems that provide fluoride to exposed surfaces of the dentition, at elevated concentrations, for a local protective effect and are therefore not intended for ingestion. Fluoride gels and varnishes are typical methods of professional topical fluoride application, and both delivery systems have been used in preventive programmes. Fluoride gels have also been used as a self-applied intervention in such programmes.

Fluoride mouthrinses and toothpastes are the main forms of self-applied fluoride therapy. The intensive use of fluoride mouthrinsing in school programmes has been discontinued in many developed countries because of doubts regarding its cost-effectiveness at a low prevalence of dental caries and is being replaced by selective fluoride therapy directed to high risk children. Such procedures usually involve the combined use of fluoride toothpastes with gels or varnishes. Toothpaste is by far the most widespread form of fluoride usage (Murray, 1991; Ripa, 1991), and the decline in the prevalence of dental caries in developed countries has been mainly attributed to its increased use (Glass, 1982; Rolla, 1991; Marthaler, 1994, 1996; O'Mullane, 1995).

However, there is currently a debate regarding the appropriate use of fluorides. The lower caries prevalence now prevailing in many countries and the widespread availability of fluoride from multiple sources have raised the question of whether topically applied fluorides are still effective in reducing caries, and safe, mainly in terms of the potential risk of fluorosis (mottled enamel; Ripa, 1991). In this context, even the need for selective professional fluoride applications has been questioned (Seppa, 1998). The persistence of this debate and the variations in the use of the main forms of topically applied fluorides suggest the need to search for meaningful ways to summarize the empirical findings on this topic systematically.

If topical fluorides remain effective, it will then become relevant to assess which form is best by directly comparing the various treatments currently used and to assess how much extra benefits topical fluoride treatments used together may actually have and whether the likely benefits are worth the effort considering potential negative effects such as fluorosis. Because the use of fluoride toothpaste is widespread in fluoridated and non-fluoridated areas, and supported by researchers and public health authorities as the method of choice among all topical fluoride interventions, there would be little justification for the use of professionally applied or supervised self-applied fluoride interventions if their combined use with toothpastes results in a marginal enhancement of effectiveness.

The unanswered question today, of how much extra caries protection comes from a professionally applied fluoride or a fluoride rinsing programme on top of that provided from the regular use of fluoride toothpaste, is of clear importance and needs to be formally investigated.

Over the past half-century, numerous clinical trials have investigated the anticaries effect of each topical fluoride intervention (Hausen, 2004). It appears that most of the trials have focused on topical fluoride in one form or another and that a small number of such trials have directly investigated increased effectiveness when two or more fluoride interventions are topically applied. Although the results of studies investigating the cariostatic efficacy of the combined use of various fluorides have been assessed before (Marthaler, 1971, 1990; Horowitz, 1980), there has been no systematic review of the available evidence until recently (Marinho *et al*, 2009b).

Topical fluorides in the form of toothpastes, mouthrinses, varnishes and gels are effective caries preventive interventions. The effectiveness of each of these has been fully assessed in four previous systematic reviews in this series (Marinho *et al*, 2002a,b, 2003a,c). In these and in a subsequent review which compiles the evidence from the previous four and exploits power with additional investigation of covariates across all topical fluoride therapies

(TFTs), Marinho *et al* (2003b) found no evidence that the effect of topical fluorides was dependent on background exposure to fluoridated water (Marinho *et al*, 2003c). However, there is uncertainty about the relative value of these interventions when used together. Marinho *et al* (2009) set out to compare the effectiveness of two topical fluoride modalities (TFMs) combined with one of them alone (mainly toothpaste) when used for the prevention of dental caries in children.

5.3 Materials and methods for the combination of topical fluoride compared to single topical fluoride for preventing dental caries

Marinho *et al* (2004) conducted a comprehensive search strategy involving journals and reference lists of articles as well as contacting selected authors and manufacturers. Randomized or quasi-randomized controlled trials with blind outcome assessment, comparing fluoride varnish, gel, mouthrinse or toothpaste in combination with each other in children up to 16 years during at least 1 year. The main outcome was caries increment measured by the change in decayed, missing and filled tooth surfaces (D(M)FS).

Inclusion decisions, quality assessment and data extraction were duplicated in a random sample of one-third of studies and consensus achieved by discussion or a third party. Authors were contacted for missing data. The primary measure of effect was the prevented fraction (PF) that is the difference in mean caries increments between the 'treatment' and 'control' groups expressed as a percentage of the mean increment in the control group. Random-effects meta-analyses were performed where data could be pooled.

All the 12 included studies used parallel group designs and with one exception (Arcieri, 1988), all had more than two relevant arms. In one of the 11 multiple arm trials (Triol, 1980), there was one group (study arm) of the single topical fluoride modality (toothpaste) and three groups of toothpaste and mouthrinse combined (where different concentrations of the same fluoride agent in the mouthrinse was tested); in another (Mainwaring and Naylor, 1978), there were two toothpaste study arms (testing different flavours of toothpaste) and one group of gel and toothpaste combined; and in another (Ringelberg *et al*, 1979), there were two groups of each, toothpaste or mouthrinse, and of these tested in combination (using different active fluoride agents). It should be noted that two of the included studies (Triol, 1980; Arcieri, 1988) had only one single fluoride modality being compared with this combined with another; that is, each study had one relevant comparison only; eight studies compared two different single topical fluoride modalities to a common group where both modalities were combined; that is, there were two relevant comparisons (with a common group) in each; and one study (Axelsson, 1987) with three relevant comparisons where both the single fluoride group and the combined fluoride group were alternatively common to two comparisons. This study has therefore been entered as two distinct studies (Axelsson, 1987) because mouthrinses or varnishes tested in combination with toothpaste, each combined regimen in a separate arm, were to be compared with a common toothpaste group in the main meta-analysis. All but one study (Arcieri, 1988) used inactive/placebo interventions for the single fluoride arm of the relevant comparisons. Study duration ranged from 2 to 3 years.

There are five trials comparing fluoride toothpaste plus mouthrinse with toothpaste alone (Ashley *et al*, 1977; Ringelberg *et al*, 1979; Triol, 1980; Blinkhorn *et al*, 1983; Axelsson, 1987) – and four comparing mouthrinse plus toothpaste with mouthrinse alone (Ashley *et al*, 1977; Ringelberg *et al*, 1979; Blinkhorn *et al*, 1983; Axelsson, 1987), followed by three comparing toothpaste plus gel with toothpaste alone (Marthaler *et al*, 1970; Mainwaring and Naylor, 1978) – and the same three comparing fluoride gel plus toothpaste with gel alone, two comparing toothpaste plus varnish with toothpaste alone (Petersson *et al*, 1985; Axelsson, 1987) – and one comparing fluoride varnish plus toothpaste with varnish alone (Petersson *et al*, 1985), two comparing gel plus mouthrinse with gel alone (DePaola *et al*, 1980; Arcieri, 1988) – and one comparing mouthrinse plus gel with mouthrinse alone (DePaola *et al*, 1980).

Studies were generally large with only three allocating <200 children to relevant study groups; all but one study recruited children from school settings. In all but one trial testing fluoride toothpastes, the fluoride concentrations in the toothpastes were similar, ranging from 1000 to 1250 ppm F, and in three of these trials, toothbrushing was performed under supervision at school. In one of the trials testing fluoride varnish, the application frequency was semi-annual (concentration 22 600 ppm F) and in the other, testing a 22 600 ppm F (Duraphat) varnish; the frequency of application was four times a year. The fluoride concentration in all five trials testing a fluoride gel was also similar (12 300/12 500 ppm F), but frequency of gel application varied from twice (operator-applied) to 22 times a year (self-applied). There was variation in the fluoride concentration (100, 30/250 900 ppm F) in the trials testing fluoride mouthrinsing, but frequency of application was either daily (in two trials) or weekly (in the other five trials).

5.4 Findings for the combination of topical fluoride to single topical fluoride for preventing dental caries

Eleven studies contributed data suitable for meta-analysis of the effect on dental caries increment. Standard deviations (s.d.) of mean caries increment data (new D(M)FS) were missing in three of the 11 studies (Axelsson, 1987b; Arcieri, 1988). From the analysis of the 179 available treatment arms for the topical fluoride reviews with complete information (as of October 1999), we derived a regression equation log (s.d. caries increment) = 0.64 + 0.55 log (mean caries increment; R^2 = 77%). This equation was used to estimate missing standard deviations from mean D(M)FS increments for the meta-analyses.

The single study reporting caries increment in deciduous tooth surfaces (Petersson *et al*, 1985) did not provide standard deviations of mean caries increment (newdfs) either and is not included in the analysis of D(M)FS PF (no caries increment data for the permanent dentition).

For all nine trials that compared fluoride toothpaste plus any TFT *vs* toothpaste alone combined (one comparing fluoride toothpaste with varnish plus toothpaste, three

comparing toothpaste with gel plus toothpaste, and five comparing toothpaste with mouthrinse plus toothpaste; $n = 4026$), the D(M)FS prevented fraction pooled estimate from the random-effects meta-analysis was 0.10 (95% CI 0.02–0.17; $P = 0.01$), that is, a significant difference was detected in favour of toothpaste used in combination with other topical fluorides. Heterogeneity in results was not detected statistically ($\chi^2 = 11.75$ on 8 degrees of freedom, $P = 0.16$), although some inconsistency in treatment effects can be observed graphically and confirmed by the I^2 heterogeneity statistic ($I^2 = 32\%$). Nevertheless, the largest variation in D(M)FS PF (−0.15 and 0.48) accrues from the trials that carry the lowest weight in the meta-analysis.

Numbers of children needed to treat (NNT) to prevent one D(M)FS were calculated based on the pooled D(M)FS prevented fraction and on the caries increments in the single toothpaste groups of the nine trials in the meta-analysis. The overall caries inhibiting effect (% PF) derived from the pooled results of the trials was 10% (95% CI 2–17); the caries increments in the included trials ranged from 0.8 to 2.5 D(M)FS per year. In populations with a caries increment of 0.8 D(M)FS per year (at the lowest end of the results seen in the included studies), this implies an absolute caries reduction of 0.08 D(M)FS per year, equivalent to an NNT of 13 (95% CI 8–63): that is, 13 children need to use topical fluorides in combination with avoid one D(M)FS. In populations with a caries increment of 2.5 D(M)FS per year (at the highest range of the results seen in the included studies), this implies an absolute caries reduction of 0.25 D(M)FS per year, equivalent to an NNT of 4 (95% CI 3–20): that is, four children need to use combined TFT to avoid one D(M)FS.

Five trials (Ashley *et al*, 1977; Ringelberg *et al*, 1979; Triol, 1980; Blinkhorn *et al*, 1983; Axelsson, 1987) compared fluoride toothpaste in combination with mouthrinse *vs* toothpaste alone ($n = 2738$). The D(M)FS prevented fraction pooled estimate from the random-effects meta-analysis of all five trials combined was 0.07 (95% CI 0.00–0.13; $P = 0.06$), a just non-significant effect in favour of the combined regimen within a relatively narrow confidence interval for the pooled estimate of effect. Heterogeneity in the results could not be observed graphically nor statistically ($\chi^2 = 1.42$ on 4 degrees of freedom, $P = 0.84$; $I^2 = 0\%$).

Three trials (Mainwaring and Naylor, 1978; Marthaler *et al*, 1970) compared fluoride toothpaste in combination with fluoride gel *vs* toothpaste alone ($n = 1217$). The D(M)FS prevented fraction pooled estimate from the random-effects meta-analysis of the three trials combined was 0.14 (95% CI −0.09 to 0.38; $P = 0.23$), a non-significant effect in favour of the combined regimen within a relatively large confidence interval. Although no significant heterogeneity was detected ($\chi^2 = 5.12$ on 2 degrees of freedom, $P = 0.08$), as the test would have minimal power to detect heterogeneity in this meta-analysis involving very few trials, the inconsistency in treatment effects is in fact large according to the I^2 statistic ($I^2 = 61\%$).

There was one small trial (Axelsson, 1987) for this comparison ($n = 71$), estimating the relative effects in the permanent dentition, which showed a large and highly significant effect in favour of fluoride varnish in combination with toothpaste, and very wide confidence interval for the estimate of effect. The D(M)FS prevented fraction for this trial was 0.48 (95% CI 0.12–0.84; $P = 0.009$).

Numbers of children needed to treat (NNT) to prevent one D(M)FS were calculated based on the D(M)FS PF and on the caries increment in the toothpaste group of this trial. In populations with a caries increment of 0.8 D(M)FS per year (seen in this study), this implies an absolute caries reduction of 0.38 D(M)FS per year, equivalent to an NNT of 3 (95% CI 2–11): that is, three children need to use the combined regimen (rather than toothpaste alone) to avoid one D(M)FS.

Another trial (Petersson *et al*, 1985) comparing fluoride varnish combined with toothpaste *vs* toothpaste alone ($n = 173$) assessed the relative effect in terms of caries increment in deciduous surfaces only and provided no standard deviations or data from which these could be derived. It reported a dfs PF of 0.15 in favour of the combined therapy (CI not obtainable).

Only one trial (DePaola *et al*, 1980) compared fluoride gel in combination with mouthrinse *vs* mouthrinse alone ($n = 252$). It showed non-significant differences in effect. The D(M)FS prevented fraction was 0.02 (95% CI −0.20 to 0.24; $P = 0.86$), suggesting that there is insufficient evidence from this trial to confirm or refute a differential effect in caries reduction.

Two trials (DePaola *et al*, 1980; Arcieri, 1988) compared fluoride gel in combination with mouthrinse *vs* mouthrinse alone ($n = 497$). The D(M)FS prevented fraction pooled estimate from the random-effects meta-analysis of the two trials combined was 0.23 (95% CI 0.04–0.43; $P = 0.02$), a significant effect in favour of the combined regimen. Although heterogeneity in the results could not be detected by the standard chi-square test ($\chi^2 = 2.05$ on 1 degree of freedom, $P = 0.15$), this was not due to homogeneity but to the smaller number of studies ($I^2 = 51\%$).

Numbers of children needed to treat (NNT) to prevent one D(M)FS were calculated based on the pooled D(M)FS PF and on the caries increments in the gel groups of the trials that contributed data to the meta-analysis. The caries increments were 1.56 and 5.09 D(M)FS per year. In populations with a caries increment of 1.56 D(M)FS per year, this implies an absolute caries reduction of 0.36 D(M)FS per year, equivalent to an NNT of 3 (95% CI 2–16): that is, three children need to use the combined regimen (rather than fluoride gel alone) to avoid one D(M)FS. In populations with a caries increment of 5.09 D(M)FS per year, this implies an absolute caries reduction of 1.17 D(M)FS per year, equivalent to an NNT of 1 (95% CI 1–5): that is, one child need to use the combined regimen to avoid one D(M)FS.

Four trials (Ashley *et al*, 1977; Ringelberg *et al*, 1979; Blinkhorn *et al*, 1983; Axelsson, 1987) compared fluoride toothpaste in combination with mouthrinse *vs* mouthrinse alone ($n = 1678$). The D(M)FS prevented fraction pooled estimate from the random-effects meta-analysis of the four trials combined was 0.05 (95% CI −0.05 to 0.15; $P = 0.33$), a non-significant effect in favour of the combined regimen. Heterogeneity in the results could not be observed graphically nor statistically ($\chi^2 = 3.38$ on 3 degrees of freedom, $P = 0.34$; $I^2 = 11\%$).

Three trials (Marthaler *et al*, 1970; Mainwaring and Naylor, 1978) compared fluoride toothpaste in combination with fluoride gel *vs* gel alone ($n = 759$). The D(M)FS prevented fraction pooled estimate from the random-effects meta-analysis of the three trials combined was 0.10 (95% CI −0.01 to 0.21; $P = 0.06$), a just non-significant effect in favour of the combined regimen. Heterogeneity in the results could not be observed graphically nor statistically ($\chi^2 = 0.17$ on 2 degrees of freedom, $P = 0.92$; $I^2 = 0\%$).

The single trial (Petersson *et al*, 1985) comparing fluoride varnish combined with fluoride toothpaste *vs* varnish alone ($n = 186$) assessed the relative effect in terms of caries increment in deciduous surfaces only and provided no standard deviations or data from which these could be derived. It reported a dfs PF of 0.19 in favour of the combined therapy (CI not obtainable).

Data for unacceptability of treatment were reported in six trials that reported dropouts fully. Each of the six trials reported equivocal results for this outcome, that is, no demonstrated differential effect. The pooled estimate (random-effects meta-analysis) of the risk ratio (RR) of dropping out from the fluoride toothpaste group as opposed to the group where other fluoride treatment is in combination with toothpaste in the five trials that reported dropouts was 1.06 (95% CI 0.96–1.21), a non-significant effect ($P = 0.37$) slightly in favour of fluoride toothpaste. Heterogeneity was not detected in these results ($\chi^2 = 2.66$ on 4 degrees of freedom, $P = 0.62$; $I^2 = 0\%$). Using alternative measures of effect has given similar results [odds ratio (OR) = 1.09, CI 0.88–1.34; risk difference (RD) 0.00, CI −0.03 to 0.03].

The pooled estimate (random-effects meta-analysis) of the risk ratio (RR) of dropping out from the fluoride toothpaste group as opposed to the combined mouthrinse–toothpaste arm in the three trials (Ringelberg *et al*, 1979; Blinkhorn *et al*, 1983; Axelsson, 1987) that reported dropouts was 1.03 (95% CI 0.84–1.26). Heterogeneity was not detected in these results ($\chi^2 = 2.15$ on 2 degrees of freedom, $P = 0.34$), and the amount present was negligible ($I^2 = 8\%$). Using alternative measures of effect has given similar results (OR = 1.02, CI 0.74–1.40; RD = 0.00, CI −0.05 to 0.05).

The pooled estimate (random-effects meta-analysis) of the risk ratio (RR) of dropping out from the fluoride toothpaste group as opposed to the combined varnish–toothpaste arm in the two trials (Petersson *et al*, 1985; Axelsson, 1987) that reported dropouts was 1.29 (95% CI 0.61–2.71). Heterogeneity was not detected in these results ($\chi^2 = 0.24$ on 1 degree of freedom, $P = 0.62$; $I^2 = 0\%$). Using alternative measures of effect has given similar results (OR = 1.31, CI 0.57–3.05; RD = 0.01, CI −0.05 to 0.06).

Fluoride mouthrinse plus toothpaste *vs* mouthrinse alone pooled estimates of the RR of dropping out from the fluoride toothpaste group as opposed to the combined TFT arm could be obtained for the three trials comparing fluoride mouthrinse plus toothpaste *vs* mouthrinse alone. Results are again consistent with no difference in effect: 0.88 (95% CI 0.67–1.17), and heterogeneity is low ($I^2 = 24\%$).

The main question addressed by Marinho *et al* (2009) is how effective the simultaneous use of combined topical fluoride therapy (TFT) for the prevention of caries in children is compared with one topical fluoride treatment used alone. The 11 studies included in the seven meta-analyses (or in the nine comparisons) have not tested all combinations of possible practical value, and there are a small number of trials in each relevant comparison/meta-analysis. However, the randomized evidence that we have brought together is, as far as we can ensure, the totality of the available randomized evidence comparing the combined use of any two topical fluoride modalities with one of them used alone. Although there is a suggestion of a modest caries inhibiting effect with the combined use of topical fluorides in the permanent dentition for most of the comparisons, a general lack of statistical significance is apparent. Further, in a few comparisons, the confidence intervals are relatively wide and the variation among the results of the studies can be substantial. This calls for a cautious interpretation of the data.

Not all other combinations of possible practical value were tested in the included studies. The only other statistically significant result was in favour of the combined use of fluoride gel and mouthrinse in comparison with gel alone (pooled DMFS PF 23%; 95% CI, 4–43; $P = 0.02$), based on two trials. No other combinations of TFT were consistently superior to a single TFT.

Marinho *et al* (2009) concluded that compared with fluoride toothpaste used alone, topical fluorides (mouthrinses, gels or varnishes) used in addition to fluoride toothpaste reduce caries by 10% on average. Topical fluorides (mouthrinses, gels or varnishes) used in addition to fluoride toothpaste achieve a modest reduction in caries compared with toothpaste used alone. No conclusions about any adverse effects could be reached, because data were scarcely reported in the trials (Marinho *et al*, 2009).

5.5 Fluoridation of salt

Switzerland has had a long history of goitre recognized since the mid-1800s (Berghaus, 1863) and has tackled this problem by switching to iodized salt. Salt fluoridation began in Switzerland in 1955 and is far more common on mainland Europe than water fluoridation. It is widely employed in a number of countries including Germany, France and Switzerland, where some 30–80% of the marketed salt for domestic use is fluoridated (Marthaler, 2005) Fluoridated salt is currently used in over thirty countries worldwide. The Pan American Health Organization (PAHO), the WHO Regional of the Americas, has been active in developing strategies to implement caries prevention programmes in its region using both water and salt fluoridation (Gillespie, 1986). Salt fluoridation schemes have been implemented in more than ten countries in Central and South America (including Bolivia, Ecuador, Colombia, Peru, Jamaica, Costa Rica, Mexico, Uruguay, and Venezuela) and frequently involve all salt for human consumption (Estupinan-Day, 2005).

The use of salt fluoridation as a vehicle for providing additional fluoride has some important attractions, and the methods and procedures for community introduction of this vehicle appear to be more acceptable and less conflictive

than water fluoridation. Salt is an essential component of the diet, reaches all sectors of society, has worldwide distribution and is not dependent upon a limited distribution or treatment system. It is a viable public health intervention.

Fluoride in salt is also compatible with iodization of salt. Community-based caries prevention programmes indicate that salt provides a relatively cost-effective vehicle for fluoride in the prevention of dental caries (salt fluoridation may be carried outmore cheaply than water fluoridation). Salt is most commonly fluoridated at 250 parts per million (ppm; range 200–250 ppm) which means 250 mg of fluoride per kg of salt, depending on dietary practices. It is expected that the use of table (domestic) salt used at the table and in the kitchen can contribute 1–4 g of the daily salt intake. Thus, a person would take in 1 mg of fluoride a day at a salt intake of 4 g a day – 1 mg per day being the 'optimal' dose of fluoride intake (the same concept as in water fluoridation; Gillespie *et al*, 2007). Properly fluoridated salt produces levels of urinary fluoride excretion similar to those found in communities with fluoridated water.

One key issue with regard to salt fluoridations arises when there are multiple sources of drinking water in an area with high fluoride content. It is essential that the natural fluoride level of each major source of drinking water must be determined prior to implementation of a salt fluoridation programme. A secondary concern relates to reluctance to implement such a programme, because a high consumption of sodium is a risk factor for hypertension (high blood pressure) and people who must restrict their salt intake may not find salt fluoridation an acceptable method of receiving fluoride. There is also the belief that the availability of fluoridated salt may lead to increases in per capita salt consumption although water fluoridation did not increase water intake. The same is true with fluoridated salt; both are merely vehicles. Nevertheless, where only domestic salt (as opposed to all salt for human consumption) is fluoridated, it permits people the freedom of choice.

Approximately a quarter of salt consumption in many countries comes from domestic salt bought in shops, and any change in the amount purchased, either an increase or decrease, is likely to have little effect on the total consumption. It has been indicated, however, that when fluoridation of salt is implemented in a way to reach all consumers of a region, that is, when both domestic salt and bulk salt are fluoridated (used by commercial bakeries, restaurants, institutions and industrial food production), the caries-reducing effect of salt fluoridation may be marginally greater than when only domestic salt is fluoridated (Kunzel, 1993; Marthaler, 2000). Nevertheless, the impact of salt fluoridation depends not only on the potential benefits of this intervention (including reduced caries levels), but also on the potential benefits over that offered by the widespread use of common approaches to control caries (e.g. fluoridated toothpaste) and on the potential harms (including dental fluorosis and other possible effects on general health such as bone fractures and bone development problems).

Although a great deal of reviews on the efficacy of salt fluoridation have been undertaken, these reviews have not addressed the issues of benefit and harm in conjunction and in a systematic manner. As the use of fluoridated salt is growing and a clear evaluation of impact has not been made, a systematic review of its effect is therefore considered essential and undertaken (Gillespie *et al*, 2007). Gillespie *et al* (2007) primarily assessed the likelihood that use of salt fluoridation will reduce caries in children and adults. While randomized controlled trials (RCTs) are the strongest method for proving a causal effect of an intervention, as these provide the least biased estimates of effect, where such RCTs are lacking other study designs such as non-randomized studies with a concurrent control group (where the intention is experimental) may also provide weaker evidence of effect when the participants and exposure (salt fluoridation) match what is likely to happen in an intervention programme and when other confounding factors can be excluded [some of these factors may be related to the outcomes under investigation (such as initial caries level) and so will confound any observed relationship and thus should be controlled for in the analysis, and in the case of salt fluoridation, these are also likely to include age, gender, ethnicity, social class and other sources of fluoride exposure (e.g. toothpaste)].

Meta-regression or subgroup analysis was employed to explore the influence of prespecified study characteristics on outcome in an attempt to try to explain any heterogeneity between studies. Sensitivity analyses was also undertaken to examine the effect of key methodological quality aspects on the overall estimates of effect.

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Chapter 6

Mouthwash and oral malignancy

Summary
Alcohol drinking has been identified and accepted as a major risk factor for squamous cell carcinoma of the upper aerodigestive tract (UADT). While tobacco smoking is the most important risk factor for upper aerodigestive tract cancer, studies focusing on never smokers have demonstrated an independent effect of alcohol. The interaction between alcohol drinking and tobacco smoking on upper aerodigestive tract cancer risk is substantial, with attributable fractions suggesting that alcohol mainly plays an important role in carcinogenesis together with tobacco rather than alone. Most recently, alcohol drinking on its own has been attributed to cause 1% of oral cavity cancers. A quantitative meta-analysis of all published epidemiological studies of mouthwash use and oral malignancy revealed (i) no statistically significant association between mouthwash use and risk of oral cancer including no significant trend in risk with increasing daily use and (ii) no association between use of mouthwash containing alcohol and oral cancer risk.

6.1 Background
Upper aerodigestive tract (UADT) cancers include malignancies of the oral cavity, oropharynx, hypopharynx, larynx and oesophagus. Worldwide, more than 1 million UADT cases and 700 000 deaths due to UADT are estimated to occur each year (Ferlay *et al*, 2010). Smoking tobacco products including cigarettes, cigar, pipes is the major risk factor for UADT cancers (IARC, 2004a). Additional UADT cancer risk factors are chewing betel quid and areca nut for oral cavity cancers (IARC, 2004b), family history of cancer (Negri *et al*, 2009), asbestos and inorganic acid mists (occupational) for laryngeal cancer (Baan *et al*, 2009; Straif *et al*, 2009) and genetic variants in the alcohol metabolism genes ADH1B and ADH7 (Hashibe *et al*, 2008). The major histological type of oral cavity, pharyngeal and laryngeal cancers is squamous cell carcinoma (SCC), while the proportion of adenocarcinomas to SCC varies by geographical region for oesophageal cancers (Vizcaino *et al*, 2002; Bosetti *et al*, 2008). Although the UADT incidence rates have been decreasing with the decreasing prevalence of tobacco smoking in most regions over the last few decades, the incidence rates for tonsil and tongue cancers overall (Ryerson *et al*, 2008) and for the oral cavity and pharyngeal cancer among young women (Bleyer, 2009) have been increasing in the United States. The alarming trend for oropharyngeal cancer might be due to human papillomavirus (HPV) infection, a recognized cause of oropharyngeal cancer (IARC, 2007). In North America, 40–80% of oropharyngeal cancer cases are HPV positive (Marur *et al*, 2010). Oesophageal SCC incidence is in decline in most developed countries, whereas adenocarcinoma of the oesophagus, linked to gastro-oesophageal reflux and obesity, is increasing (Vizcaino *et al*, 2002; Bosetti *et al*, 2008).

Alcohol drinking, aside from tobacco smoking, is a major risk factor for UADT SCC (Franceschi *et al*, 1990; Anantharanam *et al*, 2011). Relative to other alcohol-related cancers, the risk conferred by alcohol drinking is thought to be strong for UADT cancers (Bagnardi *et al*, 2001). Consuming 50 g of alcohol per day may increase the risk of oral cavity and pharyngeal cancers by approximately threefold, the risk of laryngeal cancer by twofold relative to non-drinkers (IARC, 1988) and the risk of SCC of the oesophagus by fivefold (Rota *et al*, 2010). In contrast, alcohol drinking was not strongly associated with oesophageal adenocarcinomas (Freedman *et al*, 2011).

Independent effect. The effect of alcohol drinking has been demonstrated to be independent of tobacco smoking, in studies focusing on alcohol drinking among never smokers. Individual-level data on never tobacco users were pooled for 1072 head and neck cancer cases (including oral cavity, pharynx and larynx) and 5775 controls from 14 case–control studies by the International Head and Neck Cancer Epidemiology (INHANCE) Consortium (Hashibe *et al*, 2007). Ever drinking in general was not associated with head and neck cancer risk. However, heavy drinking of <3 drinks per day was associated with an approximate twofold increase in head and neck cancer risk. Across the head and neck cancer subsites, the risks associated with higher frequency of alcohol drinking were most pronounced for pharyngeal cancers and laryngeal cancer, compared with oral cavity cancer.

There have been few studies reporting on alcohol drinking frequency among never smokers for oesophageal SCC. Kato *et al* (1992) reported a RR of 8.6 (95% CI 2.1–6.0) for drinking 30 ml or more per day compared with <30 ml day in a cohort study including 8 oesophageal cancer cases (19). ORs for never smokers from a case control in Italy including 17 cases are shown in Table 1 (20). In a large case–control study of oesophageal cancer in the Chinese population with 415 (187 male, 228 female) never-smoking cases and 1549 (824 male, 725 female) never-smoking controls, the adjusted OR was 1.4 (95% CI 1.0–2.0) for men who ever drank alcohol and 1.4 for both men and women who consumed at least 500 ml ethanol per week (*P* for trend = 0.043; 21). According to these studies, heavy alcohol drinking appeared to be a risk factor for oesophageal SCC, independent of tobacco smoking.

Dose response. Between 1988 to 2007, the IARC monograph on alcohol drinking reported that there were 5 cohort studies on oral cavity and pharyngeal cancers, 8 case–control studies on oral cavity cancer, 9 case–control studies on pharyngeal cancer, 19 case–control studies on oral cavity/pharyngeal cancers combined, 18 case–control studies on laryngeal cancer, 16 cohort studies on oesophageal cancer and 14 case–control studies on oesophageal cancer (IARC, 1988). Most of these studies showed dose–response relations between alcohol drinking frequency and the risk of UADT cancers with adjustment on tobacco smoking, consistently across various geographical regions including Europe, Asia, North America and Latin America. On the other hand, the IARC

monograph reported that there was little information on the duration of alcohol drinking and the risk of laryngeal cancer. Additionally, no dose–response relations were observed between duration of alcohol drinking and the risk of oral cavity, pharyngeal and laryngeal among never smokers by the INHANCE consortium (Hashibe *et al*, 2007). For oesophageal cancers, most of the studies had focused on the frequency of alcohol drinking. Among the studies that reported on duration of alcohol drinking and the risk of oesophageal SCC, approximately half showed dose–response relations (IARC, 1988). A recently published large-scale case–control study of 1520 cases and 3879 controls in China showed strong dose–response associations with respect to duration, frequency and ethanol concentration (Wu *et al*, 2011a).

Cessation of alcohol drinking—Quitting drinking for 20 years or more was reported to reduce the risk of oral cavity cancer (OR = 0.45, 95% CI 0.26, 0.78) and laryngeal cancer (OR = 0.69, 95% CI 0.52, 0.91) based on the INHANCE consortium pooled analysis of 9167 head and neck cancer cases and 12 593 controls (Purdue *et al*, 2009). A reduced risk of quitting drinking 20 or more years was suggestive for pharyngeal cancer (OR = 0.74, 95% CI 0.50, 1.09). Quitting drinking for 10 years or more was also reported to be beneficial in reducing oesophageal cancer risk in three separate case–control studies (IARC, 1988; Marron *et al*, 2010).

Types of alcoholic beverages—Previously, the overall consensus was that the most common type of alcoholic beverage type in a specific region conferred the greatest risk of UADT cancers. The highest UADT cancer risks were observed for beer in North America, wine in Europe and hard liquors in Latin America (IARC, 1988). However, a recent INHANCE consortium analysis examined alcoholic beverage types among individuals who reportedly drank only one type of alcoholic beverage and did not observe large risk differences (Purdue *et al*, 2009). Head and neck cancer risks were fairly consistent among individuals who drank increasing frequencies of beer only, liquor only or wine only, supporting ethanol and its metabolites as the principal carcinogen rather than other components in the each specific alcohol type.

Drinking >30 alcoholic drinks per week resulted in head and neck cancer risk increases of fourfold for liquor, fivefold for beer and sixfold for wine. In North America, the head and neck cancer risk estimates for liquor and beer appeared to be slightly higher, whereas the risk estimates for wine were higher in Europe and Latin America. For oesophageal SCC, three cohort studies and three case–control studies investigated differences in alcoholic beverage types. Although there were suggestions of higher risks of oesophageal cancer for wine in a Japanese cohort study (Sakata *et al*, 2005) and for wine and wine + spirits in an Italian study (Barra *et al*, 1990), neither of these studies showed significant risk differences in oesophageal SCC risk due to alcoholic beverage type.

Tobacco and alcohol—Numerous epidemiological studies have examined interactions between tobacco and alcohol for UADT cancers, but many reports assessed interactions only descriptively, without applying formal statistical testing (IARC, 2004a). While some studies tested for interactions on the additive scale, others tested on the multiplicative scale, and different categories were used for tobacco use and alcohol use. Overall, the majority of these studies demonstrated a joint effect between alcohol and tobacco consumption (Goldstein *et al*, 2010).

In the INHANCE Consortium pooled data analysis, multiplicative interaction parameters were estimated for tobacco and alcohol drinking (Hashibe *et al*, 2009). When the multiplicative interaction parameter is >1 and the 95% CIs do not cross the null value of 1, an interaction on the multiplicative scale is suggested. Interactions were suggested for oral cavity and pharyngeal cancers. The estimate for laryngeal cancer was not significant, although a more than additive interaction was confirmed (Hashibe *et al*, 2009). For head and neck cancer, regardless of the subgroups by gender, age or geographical region, a clear interaction on the multiplicative scale was demonstrated. Similarly, interactions on the multiplicative scale have also been reported in nine case–control studies and two cohort studies for oesophageal cancer (IARC, 1988; Wu *et al*, 2011b).

The proportion of head and neck cancer cases attributable to alcohol drinking alone appears to be fairly small, based on INHANCE consortium analysis (Hashibe *et al*, 2009). However, this does not take away from the fact that alcohol is an independent risk factor for UADT cancers among never smokers. Alcohol alone appeared to play a larger role in pharyngeal cancer than for oral cavity or laryngeal cancers. In combination with tobacco, alcohol accounted for large proportions of head and neck cancer cases, ranging from 24.3% of head and neck cancer in women to 46.5% of head and neck cancer cases in Europe. The proportion of oesophageal cancer cases attributable to alcohol have been reported as 48.6% in Japan (28), 42.5% in Western Europe (3.6% alcohol alone, 38.9% alcohol + tobacco; 29), 47% in China (15.6% alcohol along and 31.4% alcohol + tobacco; Wu *et al*, 2011b) and 72.4% in the United States (Engel *et al*, 2003).

Summary—Alcohol is clearly a major risk factor for SCC of the UADT. While tobacco smoking is the most important risk factor for SCCs of the UADT, studies focusing on never smokers have demonstrated an independent effect of alcohol. Dose–response relations between the risk of UADT SCC and alcohol frequency are very prominent, whereas the dose response with the years of alcohol drinking appeared to be important only for oesophageal cancers. Although previously it was believed that the most common type of alcoholic beverage in a particular geographical region was responsible for the greatest UADT SCC risk, an updated review of the evidence suggests that significant differences in risk by alcoholic beverage type are not present. The interaction between alcohol drinking and tobacco smoking on UADT cancer risk is substantial, with attributable fractions suggesting that alcohol mainly plays an important role in carcinogenesis together with tobacco rather than alone. While numerous epidemiological studies have contributed

to elucidating the role of alcohol in UADT SCC development, the collaborative efforts of pooling data within the INHANCE consortium for oral cavity, pharyngeal and laryngeal cancer have been highly beneficial. Similar efforts for oesophageal SCC would be invaluable in further contributing to the research.

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6.2 Tobacco smoking

Epidemiological studies from various populations have consistently shown that tobacco smoking (including filter and non-filter cigarettes, cigars and pipe tobacco) increases oral cancer risk (IARC, 1986). In sum, these results indicate that ever smokers experience an increased risk; current smokers have a higher risk than ex-smokers; those who started smoking at younger ages have a higher risk than those that started at later ages; risk increases with amount of cigarettes smoked per day, duration of smoking and lifetime pack-years of smoking; smokers of filter cigarettes have lower risk than smokers of unfiltered cigarettes. Epidemiological studies have also shown that other factors may also contribute to the effect of smoking on oral cancer risk. For example, alcohol consumption dra-

matically increases the effect of tobacco smoking on the risk of oral cancer. Similarly, xenobiotic-metabolizing enzymes, involved in the metabolism of tobacco carcinogens, have a significant impact on the relationship between tobacco smoking and oral cancer risk. Overall, based on very conservative estimates, about 46% of the cancers of the oral cavity and pharynx in men and 11% in women are attributable to smoking worldwide, with considerable variation by location (Parkin *et al*, 2000).

In this review, the results from major epidemiological studies investigating the association between oral cancer and tobacco product use, including cigarette smoking, pipe tobacco and cigar smoking, and smokeless tobacco use (snuff dipping and chewing tobacco), will be summarized. In most of the epidemiological studies, *oral cancer* includes cancer of the tongue, mouth and pharynx, with a few including larynx. Cancers of the lip and nasopharynx were not included in most of the epidemiological studies of oral cancer, and therefore, cancers of these sites will not be discussed in this review. These cancer sites also appear to have quite different aetiological profiles and very distinct natural histories as reviewed by Boyle *et al* (1995).

Cancers of the oral cavity and pharynx combined are the sixth most common cancer site for both sexes (reviewed in Boyle *et al*, 1990a; Boyle *et al*, 1995; La Vecchia *et al*, 1997; Franceschi *et al*, 2000). Combined, these cancers account for approximately 220 000 new cases per year in men and 90 000 in women worldwide. Their incidence rates vary approximately 20-fold in both sexes across the world. At present, high incidence rates are found in southern India, Pakistan, northern France and a few areas of central and eastern Europe, with the highest rate recorded in Bas Rhin, France (49.4/100 000 men; Franceschi *et al*, 2000).

Men are more likely to be diagnosed with oral cancer than women. Male-to-female ratios for oral and pharyngeal cancers ranged between 4 and 20 in southern, central, and eastern Europe (Franceschi *et al*, 2000). In the United States, the rates for men are more than double those for women (Weller *et al*, 1993). Based on the SEER programme, in the United States, African Americans have an overall incidence of oral cancer 13.2/100 000, 65% higher than Whites of 8.0/100 000 (Weller *et al*, 1993). The majority of these racial and gender differences in the United States is attributable to the effects of tobacco and alcohol (Day, 1993).

In many parts of the world (such as India, Puerto Rico and Colombia), a steady decline in oral cancer incidence in both sexes has been observed, while a stable incidence rate was observed in the USA (Franceschi *et al*, 2000). There are, however, reports that oral cancer is increasing, particularly among younger persons in the Nordic countries and Europe with the reasons unknown (Boyle *et al*, 1990b; Macfarlane *et al*, 1994).

Oral cancer and smoking have been investigated for many years. The early studies were restricted by the methodologies of their time and have been the subject of numerous other reviews (Boyle *et al*, 1995). Accordingly, this review will focus on more recent studies using newer epidemiological methods. In fact, a large number of studies have explored the relationship of cigarette smoking and oral cancer risk over the past two decades. The methods have varied but largely consist of hospital-based case–control studies, population-based case–control studies and prospective follow-up studies.

A number of hospital-based case–control studies have been conducted and have clearly shown a strong relationship between oral cancer and cigarette smoking. In interpreting the results from these hospital-based case–control studies, a major concern is whether the control group selected represents the population that produced the cases. Indeed, in some of the hospital-based case–control studies, the control group considered included cancers thought to be unrelated to smoking and drinking and some with benign neoplastic or non-neoplastic lesions, which may be smoking related. If it turns out that these diseases were actually associated with tobacco smoking, then the true relationship between tobacco smoking and oral cancer risk would be underestimated.

As most of these studies showed a strong association between cigarette smoking and oral cancer risk, underestimation is less of a concern. But the variation of disease in controls in different studies is still important to note as when considered together with the relatively small sample size in some of the studies, it may explain in large part the significant variation in the magnitude of the reported association between tobacco smoking and oral cancer risk.

In 1984, Elwood *et al* reported an alcohol-adjusted OR of 2.8 (95% CI 1.3, 6.0) for smoking 50 or more cigarettes per day when compared with never smokers. Additional adjustment for 4 other factors reduced the OR to 2.1 (95% CI 0.9, 4.8). A weak association observed in this study could be due to the fact that controls were composed of various cancer patients (including cancer of the prostate, colorectum, skin, breast, etc.).

In a case–control study of oral cancer in Brazil, Franco *et al* (1989) reported that tobacco smoking was, by any measure, the strongest risk factor for oral cancer in this population. The adjusted ORs for ever *vs* never smokers were 6.3 (95% CI 2.4, 16.3), 5.5 (95% CI 1.2, 24.8), 13.9 (95% CI 4.4, 44.2) and 7.0 (95% CI 2.7, 18.7) for industrial brand cigarettes, cigars, pipe and hand-rolled cigarettes, respectively. The OR for the heaviest *vs* the lowest consumption categories (>100 *vs* <1 pack-years) was 14.8 (95% CI 4.7–47.3). Smoking cessation also resulted in a significant risk reduction, with levels close to those of never smokers after 10 years stopping smoking.

A study by Spitz *et al* (1988) in Texas, United States, also showed a linear increase in risk with increasing pack-years of cigarette smoking ($P_{trend} < 0.01$ for both males and females). In males, the OR rose from 1.8 (95% CI 0.7, 4.4) for those who smoked 1.24 pack-years to 7.5 (95% CI 3.7, 15.3) for those who smoked >49 pack-years when compared with non-smokers. In females, the corresponding values were 1.5 (95% CI 0.4, 5.1) and 12.0 (95% CI 3.8, 38.0), respectively. Site-specific analysis for males also showed a significant increase with increasing pack-years of cigarette smoking for cancer of the larynx ($P_{trend} < 0.01$), tongue ($P_{trend} < 0.01$) and floor of mouth ($P_{trend} = 0.02$), but not for orohypopharynx ($P_{trend} = 0.13$), and other oral cavity ($P_{trend} = 0.15$). A

significant risk reduction was observed after 15 or more years of smoking cessation.

Zheng *et al* (1990) conducted a case–control study of oral cancer in Beijing, China, including 404 histologically confirmed incident cases and an equal number of hospital lbased controls. The study reported an alcohol-adjusted OR of 2.4 (95% CI 1.5, 4.0) for male smokers when compared with never smokers. Three measures of level of exposure, cigarette equivalents smoked per day, years smoked and lifetime pack-years of smoking, all showed highly significant exposure response relationships ($P_{trend} < 0.001$). Among females, while the numbers of smokers is small, the same trends as were seen among males are evident. An OR of 2.1 (95% CI 1.1, 4.2) for male cigarette smokers was also reported from a case–control study in India by Nandakumar *et al* (1990). The ORs were 2.2 (95% CI 1.1, 4.3) for those who smoked for more than 25 years and 2.1 (95% CI 1.0, 4.4) for those who smoked more than 20 cigarettes per day. A small study from the United States by Kabat *et al* (1989) also reported an alcohol-adjusted OR of 2.0 (95% CI 1.0, 4.0) for current smokers. Being an ex-smoker was not found to be associated with an increased risk in this study (OR = 1.0, 95% CI 0.5, 2.1).

Using the data from the northern Italy study, Talamini *et al* (1990) further examined the role of tobacco in non-drinkers for oral and pharyngeal cancer. They found that, among non-drinkers, ex-smokers had a risk four times that of never smokers (OR = 4.1, 95% CI 0.5, 93.6). For current smokers, ORs for smokers of <15 and 15 cigarettes per day were 3.8 (95% CI 0.2, 58.2) and 12.9 (95% CI 2.3, 106.3), respectively. The test for trend in risk was highly significant ($P_{trend} < 0.001$). La Vecchia *et al* (1990) further examined the risk by low/medium or high-tar contents of the cigarettes smoked, using only the male subjects of the study. They found that, among ever smokers, the ORs for oral and pharyngeal cancer were 8.5 (95% CI 3.7, 19.4) for low to medium tar and 16.4 (95% CI 7.1, 38.2) for high tar. The corresponding estimates were 4.8 (95% CI 2.3, 10.1) and 7.1 (95% CI 3.2, 15.6) for laryngeal cancers. A direct comparison between high *vs* low tar cigarettes showed an OR = 2.3 for oral cavity and pharyngeal cancers and 3.8 for laryngeal cancer, and all these estimates were statistically significant.

Franceschi *et al* (1990) reported the results from a case–control study involving cancer of the tongue and oral cavity, pharynx and oesophagus. The study found that the alcohol-adjusted ORs for current smokers of cigarettes were 11.1 for oral cavity, 12.9 for pharynx and 4.6 for larynx. The risks increased significantly with increasing the number of cigarettes smoked per day, duration of smoking and decreasing age they started smoking and, however, decreased with smoking cessation.

Using the data from a case–control study in northern Italy, Negri *et al* (1993) reported that, compared with non-smokers, the alcohol-adjusted ORs were 3.6 for moderate smokers and 9.4 for heavy smokers. In another report from northern Italy, Franceschi *et al* (1992) reported that, among current smokers, the risk associated with cigarette smoking was similar for cancer of the tongue (OR = 10.5, 95% CI 3.2, 34.1) and for cancer of the mouth (OR = 11.8, 95% CI 3.6, 38.4). The risks also increased significantly with increasing number of cigarette smoking and duration of smoking for both cancer sites. An early age at starting smoking led to an OR of 7.6 (95% CI 2.3, 25.0) for cancer of the tongue and 11.0 (95% CI 3.3, 36.4) for cancer of the mouth. Smokers smoking high-tar cigarettes had a 10-fold increased risk of cancer of the tongue (95% CI 2.9, 33.1) and a 14-fold increased risk (95% CI 4.2, 49.5) of cancer of the mouth compared with non-smokers. Ex-smokers, who had quit smoking for more than 10 years, had an OR close to unity in this study.

Mashberg *et al* (1993), in a study of US veterans in New Jersey, reported that smokers of filter cigarettes had a lower risk of oral cancer than that of smokers of unfiltered cigarettes. For smokers of unfiltered cigarettes, the ORs were 7.8 (95% CI 2.4, 19.0), 7.7 (3.6, 16.5), 12.3 (5.3, 28.6) and 7.6 (3.5, 16.8) for consumption of 6–15, 16–25, 26–35 and 36 or more cigarettes per day, respectively. The corresponding ORs for smokers of filtered cigarettes were 1.5 (0.5, 4.2), 3.6 (1.6, 7.7), 1.9 (0.7, 5.0) and 2.3 (1.0, 5.2), respectively. Using the same data set, Boffetta *et al* (1992) showed that soft palate had the highest ORs associated with tobacco smoking (OR = 4.9, 95% CI 1.1, 21.5) for those smoking more than 35 cigarettes per day. A similar susceptibility to tobacco was shown for floor of the mouth (OR = 4.0, 95% CI 1.5, 10.3), $P_{trend} < 0.01$ for those smoking more than 35 cigarettes per day). A stronger effect of tobacco on posterior sites of the oral cavity, such as soft palate, is consistent with the earlier studies by Hirayama (1966) and by Jussawalla and Deshpande (1971).

Kabat *et al* (1994) reported a large hospital-based case–control study in eight US cities, involving 1560 histologically incident cases of oral and pharyngeal cancer and 2948 controls (including both cancerous and non-cancerous controls). The study found that the OR for oral cancer was significantly increased in current smokers for both males (OR = 3.3, 95% CI 2.4, 4.3) and females (OR = 4.3, 95% CI 3.2, 5.9). Among current smokers of both sexes, the OR increased with amount of smoking, and among ever smokers, the risk increased with duration of smoking. Compared with lifetime non-filter smokers, lifetime filter smokers or those who switched to filter cigarettes had a reduced risk of oral cancer. Quitting smoking was associated with a substantial reduction in cancer risk which was evident even in the first few years following cessation.

Macfarlane *et al* (1995) also reported a higher risk of female smokers from tobacco smoking in a combined analysis of three case–control studies from China, United States and Italy. They found that, among men, the ORs were 1.7 (95% CI 1.2, 2.5) for those who smoked 33 pack-years or less and 3.8 (95% CI 2.5, 5.8) for those who smoked more than 33 pack-years. Among women, the corresponding ORs were 2.7 (95% CI 1.6, 4.7) and 6.2 (95% CI 3.4, 11.2), respectively. The large sample size of the combined analysis allowed the authors to examine the risk associated with smoking among never alcohol drinkers. They reported that, among those who never consumed alcohol, the risk of oral cancer increased

with increasing consumption of tobacco and the risk again was found to be higher for females among whom the increases were statistically significant. Smoking cessation resulted in a significant risk reduction, those who had stopped smoking for more than 9 years had a risk half that of current smokers (OR = 0.5, 95% CI 0.3, 0.7).

In a case–control study of 1009 oral cancer patients and 923 age-matched controls in the United States, Muscat *et al* (1996) not only found a significant dose–response relationship between oral cancer risk and lifetime cumulative tar intake ($P_{trend} < 0.01$) or lifetime pack-years of smoking ($P_{trend} < 0.01$), they also reported a significant gender difference in the smoking-related risks for oral cancer. For example, the adjusted OR for men, according to increasing quartile of cumulative lifetime tar consumption and relative to never smokers, was 1.0, 0.9, 1.6 and 2.1. Among women, the corresponding ORs were 1.8, 2.8, 3.2, and 4.6.

Zheng *et al* (1997) reported a case–control study of tongue cancer in Beijing, China. They found a significantly increased risk of tongue cancer among ex-smokers (OR = 2.2, 95% CI 1.1, 4.6) and among current smokers (OR = 2.7, 95% CI 1.3, 5.9). The risk also increased with increasing tobacco smoking, as reflected by both cigarette equivalents smoked per day and lifetime pack-years of tobacco smoking. Quitting smoking was associated with a significant risk reduction for tongue cancer.

In a case–control study in Uruguay, De Stefani *et al* (1998) reported an increased risk of squamous cell carcinoma of the oral cavity and pharynx for ever smokers (OR = 7.4, 95% CI 3.7, 14.8), current smokers (OR = 10.5, 95% CI 5.2, 21.3) and former smokers (OR = 4.5, 95% CI 2.2, 9.2). The risk increases with the increasing intensity of smoking, smoking duration and pack-years of smoking. An OR of 13.4 (95% CI 6.5, 27.9) was observed for heavy smokers (more than 62 pack-years). Analyses by type of tobacco products showed that risk was higher for black tobacco smokers (OR = 10.2, 95% CI 5.0, 20.5) than for blond tobacco smokers (OR = 4.5, 95% CI 2.2, 9.2).

A study from Brazil by Schlecht *et al* (1999) investigated the relationship between different types of tobacco smoking and oral cancer risk. The study found that smokers of non-filter cigarettes had an OR of 6.9 (95% CI 4.1, 11.8), and smokers of filter cigarettes had an OR of 6.2 (3.9, 10.0). Smokers of more than 40 pack-years of commercial cigarettes had an OR of 8.0 (95% CI 4.6, 13.8), and smokers of more than 40 pack-years of black tobacco had an OR of 7.0 (95% CI 4.2, 11.5). Current smokers were found to have an alcohol-adjusted RR of 8.1 (95% CI 4.9, 13.4) compared with never smokers. As observed in other studies, smoking cessation resulted in a significant risk reduction, decreasing nearly to the levels of never smokers after 20 years of abstention.

Franceschi *et al* (1999) also reported a dose-dependent relationship between cigarette smoking per day and oral cancer risk. The alcohol-adjusted OR was 3.3 (95% CI 1.5, 7.2) for smoking 1.14 cigarettes daily, 7.7 (95% CI 3.8, 15.4) for smoking 15–24 cigarettes daily and 10.7 (95% CI 5.0, 22.8) for smoking 25 or more cigarettes daily.

Similarly, a dose–response relationship was observed for daily cigarette smoking by Moreno-Lopez *et al* (2000) in Spain. The study reported that the alcohol-adjusted OR was 3.1 (95% CI 1.4, 6.7) for smoking 1.20 cigarettes per day and 8.3 (95% CI 3.4, 20.4) for smoking more than 20 cigarettes daily.

A study in the south of Greece by Zavras *et al* (2001) also reported a significantly increased risk of oral cancer among current smokers (OR = 3.0, 95% CI 1.4, 6.6). No increased risk, however, was observed for former smokers (OR = 0.9, 95% CI 0.4, 2.1).

A strong dose–response relationship was observed for pack-years of tobacco smoking ($P_{trend} = 0.01$). For those who had more than 50 pack-years of tobacco smoking, the alcohol-adjusted OR was 3.3 (95% CI 1.3, 8.5) when compared with never smokers.

In a study from north-eastern Italy, Talamini *et al* (2000) reported an OR of 2.4 (95% CI 1.0, 5.8) for former smokers compared with never smokers. Among current smokers, risk increased with increasing number of cigarettes smoked per day ($P_{trend} < 0.001$). An alcohol-adjusted OR of 14.8 (95% CI 3.1, 70.4) was observed for those who smoked 25 or more cigarettes per day.

In a combined analysis of two hospital-based case–control studies from Italy and Switzerland involving 1280 oral and pharyngeal cases and 4179 controls, La Vecchia *et al* (1999) reported an OR of 8.4 (95% CI 6.6, 10.6) for current smokers. A significantly reduced risk was observed following smoking cessation: the ORs were 6.2 for those who had stopped smoking for <2 years, 4.5 for those who had stopped for 3.5 years, 3.5 for those who had stopped for 6.9 years, 1.6 for those who had stopped for 10.14 years and 1.4 for those who had stopped for 15 or more years.

Garrote *et al* (2001) reported the results from a case–control study of tobacco smoking and risk of oral and oropharyngeal cancers in Cuba. A strong dose response was reported between cigarette smoking per day and risk of oral cancer among current smokers ($P_{trend} < 0.01$). The alcohol-adjusted OR for smoking 30 cigarettes or more per day, compared with never smokers, was 20.8 (95% CI 8.9, 48.3) among current smokers. Former smokers also had an OR of 6.3 (95% CI 3.0, 13.4), but risk was significantly reduced after 10 or more years smoking cessation.

A potential advantage of population-based case–control study design is that controls are randomly selected from the population which produced the cases and therefore more likely to represent the population with regard to the major risk factors. However, the relatively higher refusal rate from potential study subjects may still hamper the interpretation of the study.

In a multicenter study in the four areas of the United States, Blot *et al* (1988) found that, compared with never smokers, cigarette smokers had twice the risk in males (OR = 1.9, 95% CI 1.3, 2.9) and three times the risk in females (OR = 3.0, 95% CI 2.0, 4.5). The risks of oral cancer rose with the number of cigarettes smoked per day and with the duration of cigarette smoking. Those who smoked only filter cigarettes had a 50% (95% CI 30, 80) of the risk of those who smoked only non-filter cigarettes, and smoking cessation resulted in a rapid decline in risk.

In a multicentre case–control study in four European countries, Tuyns *et al* (1988) reported a clear dose–response relationship between cigarette smoking and risk of cancer of the larynx and hypopharynx. For those who smoked more than 26 cigarettes per day, the ORs were 24.0 (95% CI 11.8, 48.7) for cancer of the supraglottic, 10.2 (95% CI 5.4, 19.3) for cancer of the glottic and subglottic, 9.4 (95% CI 3.2, 28.0) for cancer of the epilarynx and 20.0 (95% CI 7.9, 51.0) for cancer of the hypopharynx.

The study also found that earlier age started smoking carried a higher risk. Unlike the study by Merletti *et al* (1989), the smokers of exclusively filter cigarettes in this study were found to have only half the risk of laryngeal or hypopharynx/epilarynx cancer as compared with smokers of only plain cigarettes. As reported by Schlecht *et al* (1999) and De Stefani *et al* (1998), smokers of black tobacco cigarettes had higher risk than smokers of blond tobacco. Smoking cessation resulted in a decrease in risk after 5 years of abstention.

Merletti *et al* (1989) from Italy reported a four- to six-fold increased risk among subjects with medium or high tobacco consumption in both males and females. A trend in increasing risk with duration of smoking was observed in men, but not in women. As reported by Franceschi *et al* (1990), younger age at start of smoking was found to be associated with a higher risk in this study, and smoking cessation is associated with a sharp risk reduction. Subjects smoking black cigarettes had a higher risk, while use of filter cigarettes showed no clear risk difference.

Marshall *et al* (1992) reported the results from a case–control study in western New York, and they found that while the risk associated with cigarette smoking did not increase in strict dose–response fashion, it was sizably and significantly elevated from those who had 21.30 pack-years of smoking (OR = 2.7, 95% CI 1.2, 6.0) to those who had more than 70 pack-years of smoking (OR = 5.7, 95% CI 2.7, 12.1).

Using data from a multicenter population-based case–control study of oral cancer risk factors in the United States (1065 cases and 1182 controls), Day *et al* (1993) examined the black–white differences in the risk of oral cancer associated with tobacco smoking. The study found that the patterns of risk among smokers were generally similar among blacks and whites. After controlling for alcohol consumption, the risk was almost doubled for those who smoked 20.39 cigarettes per day and tripled for those who smoked 40 or more cigarettes per day. The alcohol-adjusted OR for current smokers was higher among Whites (OR = 3.6, 95% CI 2.6, 4.8) than among African Americans (OR = 2.3, 95% CI 1.1, 4.7), but this difference was not statistically significant. The risk declined sharply with cessation of smoking for both racial groups, with little elevation in risk even for those who had quit smoking 1.9 years earlier.

Tobacco smoking was also found to be significantly associated with the risk of second cancers of the oral cavity and pharynx in a nested case–control study by Day *et al* (1994). The effects of smoking were found to be more pronounced than those of alcohol in this study. Current smokers relative to never and former smokers had an OR of 4.3 (95% CI 1.6, 12). The alcohol-adjusted ORs for smoking rose with duration and intensity of smoking. Risk, however, was significantly reduced 5 years after smoking cessation.

Bundgaard *et al* (1995) in Denmark also reported an increased risk of oral cancer associated with increasing lifetime kilogram cigarette smoking ($P_{trend} < 0.001$) and with current daily amount of smoking ($P_{trend} < 0.001$). For those who had a lifetime consumption of cigarettes >235 kg, the OR was 6.3 (95% CI 3.1, 12.9). The OR was 5.8 (95% CI 3.1, 10.9) for those with current consumption of more than 20 cigarettes per day.

A dose–response relationship was observed for daily grams of cigarette smoking in a case–control study by Andre *et al* (1995) in France. The study found that those who smoked more than one packet of cigarettes a day had a risk that was 13 times higher than that of non-smokers. Subjects who smoked only non-filter cigarettes had a higher risk (OR = 2.0) than those who smoked filter cigarettes, and risk decreased after stopping smoking.

A small study by Hung *et al* (1997) in Taiwan found that, compared with non-smokers, cigarette smoking had an increased risk of oral cancer (OR = 5.0, 95% CI 1.7, 15.1). The risk increased with increasing lifetime pack-years of smoking: the ORs were 4.0 (95% CI 1.2, 13.5) for those with <22.5 pack-years of smoking and 5.9 (95% CI 1.9, 18.5) for those with more than 22.5 pack-years of smoking.

In a case–control study in Sweden, Lewin *et al* (1997) reported that men who smoked only cigarettes had an OR of 3.7 (95% CI 2.5, 5.5). The risk was considerably lower for ex-smokers (OR = 1.9, 95% CI 1.3, 2.8) than for current smokers (OR = 6.5, 95% CI 4.4, 9.5). No increased risk was found for men who had stopped smoking for more than 20 years. There was dose-dependent excess risk associated with duration of smoking, total lifetime kilograms of tobacco smoking, and daily grams of tobacco smoking. Analysis by cancer site showed that, for men who smoked 45 years or longer, the ORs were 6.3 (95% CI 3.2, 12.4) for cancer of the oral cavity, 10.1 (95% CI 4.6, 22.1) for pharynx, 7.6 (95% CI 3.9, 14.7) for larynx and 5.4 (95% CI 2.7, 11.0) for oesophagus.

In a case–control study of oral cancer in western Washington state, USA, Schwartz *et al* (1998) reported a significantly increased risk of oral cancer for both current and past cigarette smokers. For those who smoked ≥20 pack-years of cigarettes, the OR was 2.5 (95% CI 1.5, 4.3) for past smokers and 5.5 (95% CI 3.5, 8.6) for current smokers.

In a case–control study of oral cancer in Sweden, Schildt *et al* (1998) found a significantly increased risk of oral cancer among the current smokers (OR = 1.8, 95% CI 1.1, 2.7), while found no increased risk for ex-smokers (OR = 1.0, 95% CI 0.6, 1.6). They found, however, that OR was 1.8 (95% CI 1.2, 2.8) for current smokers with more than 124.8 kg cigarette consumption. Analysis by anatomical site showed that the risk appeared to be the highest for cancer of the floor of the mouth (OR = 8.0, 95% CI 1.0, 64.0).

Hayes *et al* (1999) conducted a case–control study in Puerto Rico and found that any cigarette use was associated with an increased risk of oral cancer among men (OR = 3.9, 95% CI 2.1, 7.1) and women (OR = 4.9, 95% CI 2.0, 11.6). Risks increased with increasing cigarette

use, whether estimated by usual daily amount ($P_{trend} < 0.0001$) or by cumulative lifetime consumption (P_{trend}) < 0.0001. As reported by Macfarlane *et al* (1995) and Muscat *et al* (1996), this study also reported that women seemed to have greater risk at a given amount of cigarette consumption. Unlike the study of Mashberg *et al* (1993), this study did not find a reduced risk associated with smoking filter cigarettes compared with smoking non-filter cigarettes. Smoking cessation was shown to reduce the risk gradually, with the risk remaining elevated up to 19 years after smoking cessation.

A prospective follow-up study is conducted based on the presence or absence of exposure of investigation without the information regarding disease status. Therefore, a follow-up study is less prone to selection bias at the start of the study. However, the losses to follow-up may still pose an issue for the interpretation of the study results, especially for diseases, such as cancers, which have long induction and latency periods.

Hammond and Seidman (1980) reported a prospective mortality study of over 1 million Americans in 1721 counties in 25 states in the United States. The study reported that, among men, oral cancer mortality rates were 2.3/100 000 for those who never smoked regularly, 11.7/100 000 for pipe and cigar smokers and 15.0/100 000 for cigarette smokers. Among women, the oral cancer mortality rates were 2.0/100 000 for those who never smoked regularly and 6.5/100 000 for cigarette smokers.

Another prospective cohort mortality study from Japan by Akiba and Hirayama (1990) examined the site-specific cancer risk associated with cigarette smoking, using the data from 265 000 residents of 29 public health districts in six prefectures throughout Japan. The study reported a statistically significant dose–response relationship between cigarette smoking and mortality rate for cancer of the oral cavity, larynx, oesophagus, bladder and stomach in men. Compared with never smokers, the RRs were 2.5 (95% CI 1.3, 5.7) for cancer of the oral cavity and 23.8 (95% CI 5.3, 420.0) for cancer of the larynx among males. Very few women smoked cigarettes in this population.

Chyou *et al* (1995) reported a cohort study of upper aerodigestive tract cancer among 7995 Japanese American men in Hawaii in which they examined the potential impact of smoking and other risk factors on the incidence of upper aerodigestive tract cancer (30 men with oral/pharyngeal cancer, 27 men with laryngeal cancer and 35 men with oesophageal cancer). The study found that current cigarette smokers at time of examination had a threefold risk of upper aerodigestive tract cancer compared with never smokers (RR = 3.2, 95% CI 1.7, 5.9). A significant positive linear trend in relative risk was observed in number of cigarettes smoked per day ($P_{trend} = 0.002$) and number of years of smoking ($P_{trend} = 0.0006$).

Another major source of exposure to tobacco is through cigar and pipe smoking. Similar to the observations for cigarettes smoking and oral cancer risk, the vast majority of studies have identified a strong association between cigar and pipe smoking and oral cancer risk (Blot *et al*, 1988; Spitz *et al*, 1988; Franco *et al*, 1989; Merletti *et al*, 1989; Franceschi *et al,* 1990; La Vecchia *et al*, 1990, 1998; Zheng *et al,* 1990; Mashberg *et al*, 1993; Schildt *et al*, 1998; Hayes *et al*, 1999; Schlecht *et al*, 1999; Shapiro *et al*, 2000; Garrote *et al*, 2001), with only a few studies, such as the study by Marshall *et al* (1992), reporting little or no association. In some studies, the risk of oral cancer was actually found to be higher for pipe and cigar smokers than for cigarette smokers. For example, a case–control study by Franceschi *et al* (1990) reported an OR of 20.7 (95% CI 5.6, 76.3) for pipe and cigar smokers compared with an OR of 11.1 (95% CI 3.4, 34.8) for cigarette smokers.

Zheng *et al* (1990) reported an OR of 5.7 (95% CI 2.4, 13.3) for male pipe smokers compared with 1.6 (95% CI 1.0, 2.6) for male cigarette smokers in their case–control study. Franco *et al* (1989) reported a higher risk of oral cancer for pipe smoking compared with other smoking behaviours, particularly for cancer of other parts of the mouth (ICD9 143.145).

Pipe smoking also showed a strong dose-dependent relationship with oral cancer risk in several studies (Blot *et al*, 1988; Mashberg *et al*, 1993; Schlecht *et al*, 1999). For example, the study by Schlecht *et al* (1999) reported an OR of 6.7 (95% CI 3.1, 14.8) for those who smoked 1–20 pack-years of commercial-cigarette equivalents of pipe and 8.2 (95% CI 3.7, 17.8) for those with more than 20 pack-years of commercial-cigarette equivalents of pipe smoking. Similarly, in a large case–control study, Blot *et al* (1988) reported an OR of 1.9 (95% CI 1.1, 3.4) for those exclusively smoking cigars and/or pipes, with a positive trend associated with increasing numbers of cigars/pipes smoked. The OR rose to 16.7 (95% CI 3.7, 76.7) for men who smoked 40 or more cigars per week and to 3.1 (95% CI 1.1, 8.7) for those consuming 40+ pipefuls per week.

However, other studies, such as Merletti *et al* (1989), reported a higher risk of oral cancer for cigar smokers (OR = 14.6, 95% CI 4.7, 45.6) than pipe smokers (OR = 3.8, 95% CI 1.1, 12.6) and than cigarette smokers (OR = 3.9, 95% CI 1.6, 9.4) among male smokers. A strong dose–response relationship has been shown between cigar smoking and risk of oral cancer. In particular, the study by Garrote *et al* (2001) showed a strong dose–response relationship between cigar smoking and oral cancer risk $P_{trend} < 0.01$). They found that, compared with never smokers, those who smoked <4 cigars or equivalents per day had an OR of 4.3 (95% CI 1.1, 16.4) and those who smoked. Four cigars or equivalents per day had an OR of 20.5 (95% CI 4.7, 89.7).

Shanks and Burns (1998) reported a RR of 7.9 for ever cigar smokers and a RR of 15.9 for heavy cigar smokers (>5 cigars per day) for oral and pharyngeal cancer risk. In a case–control study of oral and oesophageal cancers involving only those who never smoked pipe tobacco or cigarettes, La Vecchia *et al* (1998) reported an OR of 6.8 (95% CI 2.5, 18.5) for ever smokers and 8.9 for smokers of more than three cigars per day and 14.9 (95% CI 4.0, 55.9) for current cigar smokers when compared with never cigar smokers.

In either case, pipe and cigar smoking clearly increases the risk of oral cancer. This risk seems to vary by anatomical subsite. Shapiro *et al* (2000) found that, compared with never smokers, current cigar smokers had an RR of

4.0 (95% CI 1.5, 10.3) for cancer of the oral cavity/pharynx compared with 10.3 (95% CI 2.6, 41.0) for cancer of the larynx. Former smokers had an OR of 2.4 (95% CI 0.8, 7.3) for cancer of the oral cavity/pharynx, but 6.7 (95% CI 1.5, 30.0) for cancer of the larynx. In their case–control study of oral and oesophageal cancers, La Vecchia *et al* (1998) reported an OR 9.0 (95% CI 2.7, 30.0) for oral and pharyngeal cancers, compared with 4.1 (95% CI 0.7, 23.0) for oesophageal cancer among ever cigar smokers. Boffetta *et al* (1992) showed that soft palate seems to be more susceptible to cigar and pipe smoking than other sites.

People are also exposed to smokeless tobacco including snuff and chewing tobacco. Snuff consists of a tobacco that has been cured and grounded into dry snuff (<10% moisture) or moist snuff (up to 50% moisture). Snuff dipping consists of taking a small amount of snuff between the gingival and the lip or the buccal mucosa and leaving there from a few minutes to several hours.

Chewing tobacco includes plug tobacco, loose-leaf tobacco, twist or roll tobacco. Chewing tobacco is held in the mouth where it can be chewed intermittently for several hours (Grasso and Mann, 1998).

Since the 1980s, there has been considerable interest in the relationship between smokeless tobacco use and oral cancer risk, and several excellent reviews have summarized the major results linking smokeless tobacco use to oral cancer risk (Winn, 1988, 1997; Vigneswaran *et al*, 1995; Gupta *et al*, 1996; Grasso and Mann, 1998; Johnson, 2001). While the IARC has concluded that 'there is sufficient evidence that oral use of snuff of the types commonly used in North America and western Europe is carcinogenic to humans' (International Agency for Research on Cancer, 1985), the relationship between smokeless tobacco use and oral cancer risk is not as consistent as what was observed for tobacco smoking from different populations, ranging from no increased risk from studies in Sweden (Axell *et al*, 1978; Lewin *et al*, 1997; Schildt *et al*, 1998) to an estimated 23-fold (rural women) and 61-fold (urban women) excess in risk associated with snuff use in Atlanta (Vogler *et al*, 1962).

A number of factors may have affected the observed relationship between smokeless tobacco use and oral cancer risk. For example, few studies were designed specifically to examine the relationship. Considering the low prevalence of smokeless tobacco users in most of the populations together with the small sample sizes in many studies, few studies would have the sufficient power to address the issue. Perhaps a more important factor, which may account for the observed inconsistent association, is that smokeless tobacco products used in different countries contain very different levels of carcinogens.

For example, smokeless tobacco used in Sweden is quite different from that used in India or in the United States. In Sweden, where studies have failed to support an association between local snuff use and oral cancer risk, snuff is not fermented and contains much lower nitrosamine levels than fermented tobaccos (Johnson, 2001). In India, however, processing of smokeless tobacco is done by individual farmers and small companies with little control over fermentation and curing (Vigneswaran *et al*, 1995); fermentation produces potentially carcinogenic nitrosamines. Also, in India, smokeless tobacco is often used in combination with betel leaf, areca nut and powdered slaked lime, and these additives make the combination more genotoxic than tobacco alone.

Fermentation is also used in the United States, although recent improvements in tobacco agriculture and smokeless tobacco processing have resulted in substantial decline in the concentration of several important carcinogens (Brunnemann and Hoffmann, 1993).

As the levels of carcinogens in smokeless tobacco vary considerably from country to country, studies of different populations have reached very different conclusions. In the following, we will review the studies based on the country of origin of the study.

Approximately, 15–20% of adult males use moist snuff in Sweden (Lewin *et al,* 1997). In fact, Sweden was the world's largest per capita consumer of smokeless tobacco throughout the twentieth century (Nordgren and Ramstrom, 1990). Although an early study by Wynder *et al* (1957) suggested an increased risk of oral cancer among snuff users, more recent studies from Sweden have found no relationship between use of local snuff and oral cancer risk. The population-based case–control study by Lewin *et al* (1997) found no increased risk of head and neck cancer with ever using oral snuff. Age started using snuff, total number of years of using snuff, and total amount of snuff used in a lifetime all had little or no impact on the risk of head and neck cancer in this study.

Another recent case–control study by Schildt *et al* (1998) also showed no increased risk of oral cancer among current snuff users regardless of tobacco smoking habits. Lifetime consumption of snuff also showed no increased risk. While ex-snuff users were found to have an increased risk, but the risk was seen only among those who were also active tobacco smokers. Users of chewing tobacco in this study (5 cases and 8 controls) also did not show an increased risk of oral cancer (OR = 0.6, 95% CI 0.2, 2.2).

An early retrospective follow-up study of 200 000 male snuff users in Sweden also failed to find a significantly increased risk of oral cancer (Axell *et al*, 1978). Ecological data from Sweden do not support an association between use of local snuff and oral cancer risk in this population (Lewin *et al*, 1997). Specifically, in geographical areas where consumption of oral snuff is highest, the incidence rate of head and neck cancers is low, and, in areas with low consumption of snuff, the incidence for cancer of the head and neck is the highest.

In the United States, while the national prevalence rate of smokeless tobacco use is low (about 5% for regular use), the rates are high in some parts of the country. In North Carolina, for example, Winn *et al* (1981) reported that 46% of the oral cancer cases and 30% of the controls were snuff users. Epidemiological studies from the United States have generally indicated an increased risk of oral cancer associated with the use of oral snuff.

The most conclusive study was conducted in North Carolina by Winn *et al* (1981). The study interviewed 232 female cases and 410 female controls and found that smokeless tobacco use was a potent risk factor for oral

cancer in this population. Among white women without a smoking habit, the oral and pharyngeal cancer cases were 4.2 times (95% CI 2.6, 6.7) more likely to have used smokeless tobacco than were controls. Among women with cancer in the cheek or gums, where tissues come in direct contact with the tobacco powder, the relative risks rose from 13-fold for <25, and 25–49 year of use, to nearly 50-fold for 50 or more years of use. It is estimated that about 31% of the oral cancer in this population could be attributable to snuff dipping alone.

While the study was criticized for using hospital-based controls that may not represent the population, which produced the cases with regard to the snuff use, the underlying association would actually be underestimated if the control diseases were in fact associated with the use of smokeless tobacco.

A large population-based case–control study by Blot *et al* (1988) also reported a significantly sixfold (95% CI 1.9, 19.8) increased risk of oral cancer due to use of smokeless tobacco among non-smoking females. Kabat *et al* (1994) reported a crude OR of 34% (95% CI 8.5, 140.1) for using snuff among female never smokers. An early hospital-based case–control study by Vogler *et al* (1962) also found an increased risk of oral cancer associated with snuff use among both urban women (Crude OR = 60.8) and rural women (crude OR = 22.9). Spitz *et al* (1988) also reported a significantly increased risk of oral cancer associated with snuff dipping among males (OR = 3.4, 95% CI 1.0, 10.9). There are also several studies of US populations that did not find an increased risk of oral cancer associated with smokeless tobacco use (Mashberg *et al*, 1993; Muscat *et al*, 1996; Schwartz *et al*, 1998).

These studies, however, generally involved populations that have a very low prevalence rate for smokeless tobacco use, and none of them were designed specifically to investigate the association between smokeless tobacco use and risk of oral cancer. For example, the study by Schwartz *et al* (1998) from western Washington State found that, of 294 female cases and controls, only one female control subject used smokeless tobacco. Among males, prior smokeless tobacco use was reported by only 6.7% of the cases and 5.6% of the controls (OR = 1.0, 95% CI 0.4, 2.3). The hospital-based case–control study by Mashberg *et al* (1993) in New Jersey also found no increased risk of oral cancer for use of snuff (OR = 0.8, 95% CI 0.4, 1.9) or chewing tobacco (OR = 1.0, 95% CI 0.7, 1.4). The proportion of snuff or chewing tobacco together was found in only 14% of the cases and 11% of the controls.

Muscat *et al* (1996) also found no increased risk of oral cancer associated with snuff use or chewing tobacco. But only 1.3% of the cases and 1.6% of the controls in males used snuff. Among women, only two cases and one control reported snuff use in this study. About 5% of the cases and controls in men and none of the women reported regularly using chewing tobacco. Sterling *et al* (1992) evaluated the relationship between smokeless tobacco use and cancer risk based on data from the national mortality follow-back survey and found no increased risk of oral or other digestive cancers associated with smokeless tobacco use, either as snuff or chewing tobacco.

However, as pointed out by Johnson (2001), a number of limitations have limited the interpretation of the study results, including small number of subjects used the products, and issues related to data collection and presentation.

Studies in India—Oral cancer is the most common cancer in India, where large quantities of smokeless tobacco are used (Jayant and Deo, 1986). Smokeless tobacco use is considered to be a major risk factor for the high incidence rate of oral cancer in this country. The study by Nandakumar *et al* (1990) reported an increased risk of oral cancer among pan tobacco chewers in both males and females and no increased risk among pan chewing without tobacco. A dose response was observed for years of chewing, number of times of chewing per day and period of retaining the pan in the mouth. A linear test for trend was statistically significant ($P < 0.001$) in all three instances. Compared with those with no history of chewing tobacco, the ORs were 8.5 (95% CI 4.7, 15.2) for those who did not chew during sleep and 17.7 (95% CI 8.7, 36.1) for those with a history of chewing during sleep.

Rao *et al* (1994) also reported a significant association between tobacco chewing and risk of oral cancer (OR = 3.0, 95% CI 2.3, 3.7), and the risk increased with increasing frequency ($P_{trend} < 0.001$). For chewers who chewed tobacco 21–30 times per day, the OR was 10.7 times higher than that for non-chewers. Several other earlier case–control studies (Sanghvi *et al*, 1955; Wahi *et al*, 1965; Jussawalla and Deshpande, 1971; Notani, 1988) and follow-up studies (Bhargava *et al*, 1975; Gupta *et al*, 1980) from different parts of India have also provided unequivocal evidence between chewing tobacco and oral cancer risk, and this risk appeared to be even higher among those who began the habit at a younger age (Jayant *et al*, 1971).

Franco *et al* (1989) reported no association between use of smokeless tobacco, either as snuff or tobacco chewing, and risk of oral cancer in Brazil. However, the number of subjects who used tobacco in this form was small (9 cases and 13 controls). In Sudan, however, an increased risk of oral cancer was reported among those who used *toombak*, a coarse powder made of dried tobacco leaves, and the risk was found to be higher for anatomical sites (buccal cavity, floor of mouth and lip) where tissues come in direct contact with the product (Idris *et al*, 1995).

6.3 Tobacco and alcohol interaction

A number of studies from different populations or racial groups have investigated the interaction between tobacco and alcohol on the risk of oral cancer, and most of them have concluded that the effects of tobacco and alcohol are certainly more than additive and seem to be consistent with multiplicative, with some suggesting a supramultiplicative effect (Negri *et al*, 1993; De Stefani *et al*, 1998; Garrote *et al*, 2001).

Studies, which presented the joint distribution of cases and controls for each combination of smoking and alcohol consumption with the corresponding ORs, have shown a sharp increase in the risk for those with the heaviest levels

of consumption of both products compared with the lowest levels of consumption of both products. The OR for the highest levels of consumption of both products reached as high as 305 (Baron *et al*, 1993).

A strong interaction between tobacco and alcohol in the risk of oral cancer was observed no matter if the relationship is expressed by lifetime consumption of the products (Franco *et al*, 1989; Zheng *et al,* 1990; Bundgaard *et al*, 1995; Schildt *et al*, 1998), or daily or weekly or monthly consumption of the products (Rothman and Keller, 1972; Wynder *et al*, 1976; Elwood *et al*, 1984; Blot *et al*, 1988; Tuyns *et al*, 1988; Merletti *et al*, 1989; Franceschi *et al,* 1990, 1999; Day *et al*, 1993; Mashberg *et al*, 1993; Kabat *et al*, 1994; Andre *et al*, 1995; Chyou *et al*, 1995; Hayes *et al*, 1999; Garrote *et al*, 2001), or by cumulative tar/amount of alcohol day (Muscat *et al*, 1996), or expressed by other means (Baron *et al*, 1993; Lewin *et al*, 1997; De Stefani *et al*, 1998; Zavras *et al*, 2001).

A strong interaction between smoking and alcohol use on the risk of oral cancer is exemplified from the study by Franceschi *et al* (1999) in Italy. In this study, the highest level of risk of oral cancer (OR = 227.8, 95% CI 54.6, 950.7) was observed among those most heavily consuming both tobacco and alcohol.

In conclusion, in 1986, an IARC Working Party concluded that there was sufficient evidence that tobacco was carcinogenic to humans and that the occurrence of malignant tumours of the upper digestive tract was causally related to the smoking of different forms of tobacco. IARC has also concluded that there is sufficient evidence that oral use of snuff of the types commonly used in North America and western Europe is carcinogenic to humans, and there was sufficient evidence that the habit of chewing betel quid containing tobacco was carcinogenic in humans (International Agency for Research on Cancer, 1985). More recent epidemiological studies and experimental studies further support these conclusions. There is convincing evidence that a large attributable risk can be ascribed to the joint habits of cigarette smoking and alcohol consumption.

Attributable risk—The International Agency for Research on Cancer has classified cancer of the oral cavity, pharynx and larynx as tobacco-related cancers. A number of case–control studies have estimated the proportion of oral cancer cases attributable to tobacco smoking, but the estimated proportion depends on the validity of the estimation of the prevalence of smoking in the population and the relative risk from the exposure. A number of factors may affect the estimation: for example, hospital-based studies with patients as controls, population-based studies with high refusal rate or lack of adequate control for major confounding factors (such as alcohol consumption). As smoking and drinking are highly correlated, some studies calculated estimates of the population attributable risk (PAR) of oral cancer due to smoking and/or drinking rather than due to smoking alone. In most of the studies, the reported PAR from smoking did not include the impact from smokeless tobacco use or even pipe and cigar smoking.

Parkin *et al* (2000) have estimated that about 46% of the cancer of the oral cavity and pharynx in men and 11% of these diseases in women are attributable to smoking worldwide. Estimates vary for specific countries and are presented below.

In Italy, Merletti *et al* (1989) estimated that 72.4% of oral cancer cases in men and 53.9% of the cases in women are attributable to smoke of more than 7 g of tobacco per day. The study by Negri *et al* (1993) reported that, for both sexes, the single factor with the highest attributable risk was smoking, which in males accounted for 81.87% of oral cancer cases and in females for 42.47%.

In the United States, Mashberg *et al* (1993) estimated that 74% of oral cancer in this population was attributable to smoking six or more cigarette equivalents per day, and 97% of the disease was attributable to the combination of smoking and drinking. In a population-based case–control study of oral cancer involving four states in the United States, Blot *et al* (1988) estimated that 80% of the oral and pharyngeal cancer cases in men and 61% in women were attributable to smoking and alcohol drinking. Using the data from this population-based case–control study, Day *et al* (1993) estimated that 83% of blacks and 73% of whites developed oral cancer as a result of alcohol and/or tobacco consumption, with most tumours arising from the combined effect of drinking and smoking.

Almost half of all oral cancer (48%) among black men was attributed to smoking one pack or more daily in combination with heavy drinking (30 drinks per week). For white men, 36% of oral cancers were accounted for by this level of smoking and drinking. Tobacco and alcohol consumption account for bulk of the racial and gender differences in oral cancer in the United States. In Beijing, China, Zheng *et al* (1990) found that tobacco smoking accounts for about 34% of all cases of oral cancer in the Chinese population (45% among males and 21% among females) and 44% of all oral squamous cell carcinoma. In Bombay, it is estimated that 70% of oral cancer cases were attributable to smoking and chewing tobacco.

A most recent large, multicentric study from Europe concluded that tobacco and alcohol are major risk factors for upper aerodigestive tract (UADT) cancer, and significant variation is observed in UADT cancer rates across Europe. Anantharaman *et al* (2011) have estimated the proportion of UADT cancer burden explained by tobacco and alcohol and how this varies with the incidence rates across Europe, cancer subsite, gender and age. This should help estimate the minimum residual burden of other risk factors to UADT cancer, including human papillomavirus. They analysed 1981 UADT cancer cases and 1993 controls from the ARCAGE multicentre study. They estimated the population attributable risk (PAR) of tobacco alone, alcohol alone and their joint effect.

Tobacco and alcohol together explained 73% of UADT cancer burden of which nearly 29% was explained by smoking alone, <1% due to alcohol on its own and 44% by the joint effect of tobacco and alcohol. Tobacco and alcohol

together explained a larger proportion of hypopharyngeal/laryngeal cancer (PAR = 85%) than oropharyngeal (PAR = 74%), oesophageal (PAR = 67%) and oral cancer (PAR = 61%). Tobacco and alcohol together explain only about half of the total UADT cancer burden among women.

Geographically, tobacco and alcohol explained a larger proportion of UADT cancer in central (PAR = 84%) than southern (PAR = 72%) and western Europe (PAR = 67%). While the majority of the UADT cancers in Europe are due to tobacco or the joint effect of tobacco and alcohol, these results support a significant role for other risk factors in particular, for oral and oropharyngeal cancers and also for UADT cancers in southern and western Europe (Anantharaman *et al*, 2011).

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6.4 Oral cancer and mouthwash

There are an estimated half a million of cases of cancer of the oral cavity and pharynx occurring annually and a quarter of a million deaths (Boyle and Levin, 2008). The higher rates (incidence and mortality) are in Central Europe and France (Macfarlane *et al*, 1994; Boyle and Smans, 2008) and on the Indian subcontinent (Curado *et al*, 2009).

The majority of oral cancers are squamous cell carcinoma (SCC), and the main risk factors for these cancers are tobacco and alcohol consumption. Tobacco smoking is the most important risk factor for head and neck cancer, and the risk is higher for heavy smokers, long-term smokers and smokers of black tobacco or high-tar cigarettes. Cigar smoking and pipe smoking also pose a risk, while stopping smoking is followed by a decrease in risk (IARC, 2007). Smoking of bidis (small cigarettes common in parts of Asia) also carries a substantial risk of oral cancer (Sapkota *et al*, 2007).

Consumption of alcoholic beverages increases the risk of oral cancer and other cancers of the head and neck. Relative to abstainers and very light drinkers, the risk in heavy drinkers is in the order of tenfold. Although the effect of alcohol and tobacco may vary slightly according to the different subsites, the combined effect of both exposures accounts for the majority of all head and neck cancers that occur globally.

A recent pooled analysis from the INHANCE consortium based on over 10 000 cases and 15 000 controls shows that approximately 70% of such cancers can be explained by these two exposures, ranging from 65% for oral cavity cancer (51% for women and 65% for men) to 86% for larynx cancer (79% for women and 86% for men). The proportion of those cancers caused by alcohol and tobacco was reduced with decreasing age, being just 32% for cancers diagnosed prior to age 45. Strong interaction between the two exposures is also apparent.

In addition to the dominant roles of tobacco smoking and alcohol drinking in the causation of oral cancer, other established risk factors specifically for oral cavity cancer are betel quid and areca nut in India and Taiwan (IARC, 2006; Secretan *et al*, 2009) and poor oral health (Zheng *et al*, 1990). Chronic infection with human papillomavirus (HPV) is emerging as an important risk factor particularly for tongue cancer and oropharyngeal cancer (Kreimer *et al*, 2005).

In Europe, the distribution pattern of oral cancers follows that of alcohol consumption. While the incidence is decreasing in many countries, it is still on the rise in Central Europe. Mortality is also declining in many countries but is still very high in Central Europe and still increasing among younger birth cohorts.

Alcohol drinking, ethanol and acetaldehyde associated with alcohol drinking have been identified as human carcinogens (Baan *et al*, 2007). Acetaldehyde from drinking alcohol was considered a human carcinogen based on studies from Japan regarding risk of oesophageal cancer.

In recent studies, it has been shown that the relative risk of oral cancer increases with the average daily amount of alcohol consumed. The total ethanol content of alcohol drunk has been consistently demonstrated to be the main factor in determining cancer risk.

Ethanol is contained in a number of ready-to-use mouthwashes in a concentration typically between 5% and 27% volume. There are two main questions to be resolved. First of all, the question remains as to whether there is a threshold for alcohol consumption in increasing oral cancer risk and, secondly, is there any risk associated with rinsing the mouth with an alcohol-containing mouthwash which is not consumed?

The potential association between use of mouthwash and an increased risk of oral cancer has been a source of controversy for decades. In recent times, attention has focused on a role for those mouthwashes containing alcohol. There have been reports in the scientific literature, spread over the past 30 years, investigating the potential association between mouthwash use and its impact on the risk of oral cancer. Epidemiological studies have been relatively few and frequently contradictory.

Mouthwash contains a variety of active and inactive ingredients. Ingredients of a mouthwash include antibacterial agents, at least 50% water, stabilizers for non-water-soluble ingredients, substances to improve palatability and stability and preservatives to increase shelf-life. Ethanol is used in some mouthwash formulations as a solubilizer, stabilizer, preservative, sensory cue with a distinctive taste and as an antiplaque efficacy enhancer (adjuvant effect).

Ethanol at 18–27% concentration enhances the effect of essential oils (high penetration achieved in 30 s).

To shed clarification on the issue of mouthwash use and oral cancer risk, a comprehensive literature review and formal meta-analysis was carried out on Mouthwash use and oral, oropharyngeal and laryngeal cancers.

Materials and methods—A systematic literature search and quantitative analysis were planned, conducted and are reported following MOOSE guidelines regarding meta-analysis of observational studies (Stroup *et al*, 2000).

Definition of exposures and outcome—The definition used for the exposure variable is 'regular use of mouthwash' which was classified as regular use in average 'once or twice a day'. When risk estimates for more than one definition were presented, definitions like 'daily use' and 'ever use' were preferred to higher 'doses' (e.g. more than twice a day). Whenever possible, the estimates for mouthwash with specified content of alcohol >25% were chosen.

The outcome variable was 'oral cancer' but estimates for oral and pharyngeal cancers together were also included, relying on the definition as published in each report.

Data sources and search strategy—Published reports were obtained from the following databases using validated search strategies: Ovid MEDLINE database; ISI Web of Science Science Citation Index Expanded (SCI Expanded); and PUBMED (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi). Other sources were found in the reference lists of the retrieved articles and preceding reviews on the topic.

The following search terms (both as MeSH terms and as keywords) were used to identify potentially relevant studies in the three databases mentioned above: oral, oral-pharyngeal, oral-pharyngeal-laryngeal cancer, leukoplakia or oral epithelial dysplasia and mouthwash, oral rinse or Listerine. The search was limited to human studies but no language or time restrictions were applied.

Selection of articles—All searches were made independently by two abstractors (S. Gandini and E. Negri); in case of disagreement or uncertainty, a third reviewer (C. La Vecchia) was consulted.

Usual inclusion criteria were used for the selection of all relevant articles (i.e. case–control, cohort or cross-sectional studies) published as an original article. These criteria included that studies should have sufficient information to allow adequate estimation of the relative risk (RR) and 95% confidence intervals (95% CI): that is, the authors should report either adjusted odds ratios or RRs or crude data and SEs, variance, CIs or *P* values of the significance of the estimates and that the studies should be independent to avoid giving double weight to some estimates.

Extraction and classification of the data—For each study, the following data were retrieved:

Study: publication year, study design, study location, mean age of study population, gender;

Exposure: definition of the types of use of mouthwash and time of ascertainment of mouthwash (how long before cancer diagnosis?);

Cases: number and source of cases, accrual period, histological confirmation, type of registration: incident *vs* prevalent cases;

Controls: number and source of controls, matching design, inclusion/exclusion of specific types of diseases/cancers;

Statistics: statistical methods used and adjustment for confounding variables (e.g. smoking and/or alcohol consumption), restriction of analysis on specific subgroup (smokers, non-smokers nor drinkers…).

Fully adjusted RRs, when available, were retrieved for each dose of mouthwash use, for all the population under study and for smokers, non-smokers/non-drinkers, by cancer subsites and by gender.

Statistical methods—The various estimates of RR and their CIs were transformed into log RR, and the corresponding variance was calculated using the formula proposed by Greenland, 1987. When estimates were not given, they were calculated from tabular data and using Woolf's formula to evaluate the s.e. of the log odds ratio (Greenland, 1987).

The homogeneity of the effect across studies was assessed by using the large sample test based on the chi-square statistic. Because this test has limited power, statistically significant heterogeneity was considered to be at the $P = 0.10$ level of association. Heterogeneity across studies was also evaluated by I^2, which represents the percentage of total variation across studies that is attributable to heterogeneity rather than to chance (Higgins and Thompson, 2002).

Random-effects models were used including the two sources of variation (within and between studies), to take into account correlation within study when more than one estimate was extracted from a single study. Summary estimates were obtained with maximum likelihood estimates from random-effects models (REF Proc Mixed in SAS software, version 8.02; SAS Institute, Cary, NC, USA; van Houwelingen *et al*, 2002).

Subgroup analyses and meta-regression were carried out to investigate between-study heterogeneity and to evaluate the effect on the summary estimates of study features, types of population, types of mouthwash definitions and use. Sensitivity analyses were carried out to evaluate the influence of various inclusions/exclusion criteria and specific studies.

For dose–response estimates, RRs, 95% CIs and number of cases and controls were retrieved by each category of exposure. Within each study, a linear model was employed to estimate the RRs associated with an increase in mouthwash use of 1 time per day.

Each category of mouthwash use was assigned the value corresponding to the midpoint of the range. Summary RRs were obtained by pooling the study-specific estimates by the random-effects models proposed by Greenland and Longnecker (1992), which adjust the estimates for within-study covariance and account for the

correlation between estimates. Estimates for 2 and 3 times a day were estimated from linear dose–response model (Greenland and Longnecker, 1992).

The impact of whether publication bias might affect the validity of the estimates was investigated using a funnel-plot-based approach: the regression of ln(RR) on the sample size, weighted by the inverse of the variance (Macaskill *et al*, 2001).

Results—Literature search and data extraction—The flow chart of included and excluded studied is presented in Figure 6.1. Through the literature searches, 18 full-text articles were found that were considered for inclusion in the meta-analysis. Two studies were excluded because they were not independent (Fernandez Garrote *et al*, 2001; Marques *et al*, 2008).

Features of the 16 studies included in the main analysis and in the sensitivity analyses are presented in Table 6.1, the estimates reported by the authors are presented in Table 6.2.

Twelve studies published from 1983 to 2010 were available for the main analysis (Table 6.1). All of them were case–control studies, four were population based (Winn *et al*, 1991; Winn *et al*, 2001; Divaris *et al*, 2010; Macfarlane *et al*, 2010), one was conducted in Italy (La Vecchia *et al*, 1997), one was a mixture (Latin American and Europe; Guha *et al*, 2007) and one a mixture of European countries (Macfarlane *et al*, 2010), all the others were from United States (Blot *et al*, 1983; Mashberg *et al*, 1985; Young *et al*, 1986; Kabat *et al*, 1989; Winn *et al*, 1991). Two publications presented data on women (Blot *et al*, 1983; Kabat *et al*, 1989), three were on oral cancer (Young *et al*, 1986; La Vecchia *et al*, 1997; Divaris *et al*, 2010) and all the others on oral and pharyngeal cancers. One study (23) included also 5% of oesophagus cancers among cases (Macfarlane *et al*, 2010).

For one study (Kabat *et al*, 1989), the estimate that refers to the use 10 years before the diagnosis was chosen instead of the most recent one, because of the lag time of exposure for cancer and also because clinical manifestations of early oral cancer could modify the subject's use of an agent such mouthwash (e.g. it might be used to treat symptoms).

Statistical analysis—No significant association was found between mouthwash use and oral cancer: SRR = 1.13 (95% CI 0.95, 1.35). The forest plot is presented in Figure 6.2.

Nine studies were available for the dose–response analysis (Blot *et al*, 1983; Winn *et al*, 1991; La Vecchia *et al*, 1997; Winn *et al*, 2001; Guha *et al*, 2007; Macfarlane *et al*, 2010). RRs for three 'doses' were abstracted: two or more times daily, once a day and no exposure to mouthwash. The summary relative risks estimates for 1–3 times a day of mouthwash showed no statistically significant increase risk of oral cancer compared with no exposure: 1.19 (95% CI 0.95, 1.5), 1.42 (95% CI 0.91, 2.24) and 1.7 (95% CI 0.86, 3.35), respectively, with $I^2 = 76\%$ and chi-square $P < 0.001$. The forest plot of dose–response estimates is presented in Figure 6.3.

Subgroup and sensitivity analyses were carried out including risk estimates for: oral cancer only (excluding oral-pharyngeal cancers), only non-smokers (and non-drinkers when possible), only smokers, mouthwash with specified 25% of alcohol content, high dose of mouthwash use (2+ times a day were chosen when possible), population-based studies publishing estimates adjusted for smoking and preferably alcohol consumption and all possible studies, including the four studies excluded from the main analysis (Weaver *et al*, 1979; Marshall *et al*, 1992; Morse *et al*, 1997; Mascarenhas *et al*, 2002).

Weaver (1979) was the first study published on mouthwash and oral cancer and involved a case series of 11 women with oral cancer (10 of them were heavy users of mouthwash) and compared them with 50 men. For this study, an estimate of cancer risk was obtained from the percentages of mouthwash use presented in the text. Mascarenhas *et al* (2002) evaluated the effect of mouthwash use on leukoplakia. Morse *et al* (1997) evaluated the effect of mouthwash use on oral epithelial dysplasia; and Marshall *et al* (1992) did not publish an estimate for mouthwash use and oral cancer but in the text of Results section described a significant effect for regular use. To be conservative, the estimate of this study was imputed considering the highest estimate published by the other authors.

Summary risk estimates for subgroup analyses and sensitivity analyses are presented in Table 6.3. None of the factors evaluated through meta-regression significantly explained between-study heterogeneity (Publication year $P = 0.66$, gender $P = 0.36$), and no evidence of publication bias was found ($P = 0.31$).

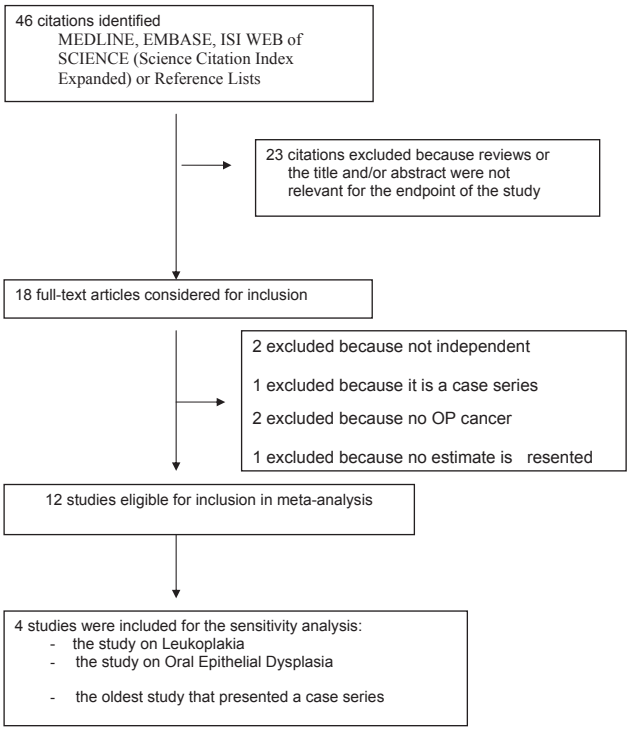

Figure 6.1 Flow chart of selection of studies for inclusion in meta-analysis

Table 6.1 Study characteristics of article evaluated in the meta-analysis

FA	*PY*	*Study period*	*Gender*	*Design*	*Country*	*Cancer type*	*Info Source*	*No. cases*	*No. controls*	*% Regular use cases*	*% Regular use contr.*
Blot	1983	1975–1978	W	CC	USA	OP	Hospital	206	352	44	42
Wynder	1983	1977–1980	M + W	CC	USA	OP	Hospital	571	568	47	56
Mashberg	1985	1981–1983	M + W	CC	USA	OP	Hospital	95	913	43	48
Young	1986	NA	M + W	CC	USA	O	Hospital	202	306	NA	NA
Kabat	1989	1983–1987	W	CC	USA	OP	Hospital	125	107	29	33
Winn	1991	1984–1985	M + W	CC	USA	OP	Populat.	866	1249	54	44
Talamini[a]	2000	1996–1999	M + W	CC	Italy	O	Hospital	121	137	9	9
Winn	2001	1992–1995	M + W	CC	USA	OP	Populat.	328	496	33	38
D'Souza	2007	2000–2005	M + W	CC	USA	OP	Hospital	100	200	40	36
Guha[b]	2007	1998–2003	M + W	2CC	Mixed	OP	Hospital	918	2752	5	3
Divaris	2010	2002–2006	M + W	CC	USA	O	Populat.	692	1361	NA	NA
Macfarlane[c]	2010	NA	M + W	CC	Europe	OP	Populat.	260	340	11	10
Included only in the sensitivity analysis											
Weaver[d]	1979	NA	M + W	–	USA	O	Hospital	11	50	91	80
Marshall	1992	1975–1983	M + W	CC	USA	OP	Hospital	290	290	NA	NA
Morse	1997	1990–1993	M + W	CC	USA	OED	Hospital	127	127	41	47
Mascarenhas	2002	1997–1998	M + W	CC	USA						
Leukoplakia		Hospital	58	58	10	10					

O, oral cancer; OP, oral-pharyngeal cancer; OED, oral epithelial dysplasia; M, men; W, women; CC, case–control study.
[a]Cases evaluated include oral cavity and pharynx; controls used for pharynx are 1378 and 1225 for oral cavity.
[b]Frequency of use of cases is in average 2 or more a day, in controls is 'at least occasionally'.
[c]The 5% of cases are oesophagus cancers.
[d]Frequency of regular use is >2 a week, cancer, regular use: 1 or more times a day.

Discussion: awaiting FDA evaluations—The potential association between use of mouthwash and an increased risk of oral cancer has been a source of controversy for several decades since the initial observation of Weaver (1979). Evaluation of the available published epidemiological information in the 1990s concluded that there was no association (Elmore and Horwitz, 1995; Shapiro *et al*, 1996; Cole *et al*, 2003).

In recent times, attention has focused on a role for those mouthwashes containing alcohol on impacting the risk of oral cancer. This study set out to examine the potential effect of mouthwash use, and particularly use of mouthwash containing a high alcohol content, on the risk of oral cancer in a quantitative manner. All published studies were identified using a thorough literature review and examination of reference lists in published articles.

Standard criteria were used to determine which of the identified studies should be included in the analysis. Studies were required to have sufficient information to allow adequate estimation of the relative risk (RR) and 95% confidence intervals (95% CI). Following this strategy, 18 full-text articles able were included in the main analyses and some in selected sensitivity analyses. This ensured that the maximum amount of information on the subject could be employed. However, the quality of many of the available studies from the epidemiological viewpoint is relatively poor, and mouthwash use has rarely been the principal hypothesis investigated in these studies.

There was no statistically significant association found between regular use of mouthwash and risk of oral cancer (RR = 1.13; 95% CI 0.95–1.35). There was no significant trend in risk of oral cancer associated with increased daily usage of mouthwash ($P = 0.11$). In sensitivity analyses, there was no association found when analysis was restricted to a number of factors including oral cancer only, smokers, non-smokers and when all possible studies were included. There was no association between reported use of mouthwash specifically containing alcohol and risk of oral cancer (RR = 1.0; 95% CI 0.39, 2.60).

Overall, alcoholic beverage consumption has been held to be carcinogenic to humans (Group 1) and ethanol in alcoholic beverages carcinogenic to humans (Group 1; IARC, 1988). In October 2009, the IARC Working Group for Monograph Volume 100E confirmed 'alcohol drinking' as a Group 1 agent. This Working Group considered that acetaldehyde is a genotoxic compound that is detoxified by aldehyde dehydrogenases (ALDH). The ALDH2*2 variant allele, which encodes an inactive enzyme, is prevalent in up to 30% of east-Asian populations and that heterozygous carriers accumulate acetaldehyde and have considerably higher relative risks for alcohol-related oesophageal and head and neck cancers compared with individuals with the common alleles.

In 2008, an IARC Working Group acknowledged the important role of acetaldehyde in the development of alcohol-related cancer, especially of the oesophagus, but refrained from making a formal evaluation. However in October, 2009, for Volume 100E, the Working Group concluded that 'acetaldehyde associated with alcoholic beverages' is carcinogenic to humans (Group 1).

Alcohol-containing mouthwashes/rinses pose little threat to systemic human exposure to alcohol itself or products of its metabolism if used as directed. Application of alcohol-containing mouthwash/rinse solutions has been shown to briefly elevate acetaldehyde concentration in the saliva of human volunteers (Lachenmeier *et al,* 2009). The latter study was severely limited in the number of subjects

Table 6.2 Risk estimates of the studies evaluated in the meta-analysis

FA	*Gender*	*cancer*	*No. cases*	*No. controls*	*% Regular use Cases*	*% Regular use Controls*	*OR*	*Smk or alc. Adj.*	*Exposure definition*	*Smokers or alcohol drinkers*	*% Of alcohol*	*Controls*
Weaver (1979)[a]	3	O	11	50	91	80	2.5 (0.29, 21.88)	0	2+ daily for 20 years	Neither*	Majority 27% alcohol	Male surgery pts
Blot (1983)	2	OP	206	352	44	42	1.15 (0.8, 1.7)	1	Ever use on regular basis			
	2	OP	31	138			1.94 (0.8, 4.7)	1		No Tobacco		
Wynder *et al* (1983)	2	OP	157	157	57	46	1.54 (0.82, 2.89)	0	<1 a day			
	1	OP	414	411			0.79 (0.55, 1.15)	0	<1 a day			
	2	OP					2.79 (1.67, 4.66)	0	1 or more times a day			
	1	OP					1.13 (0.83, 1.54)	0	1 or more a day			
	2	OP	36	88			3.63 (1.48, 8.92)	0	Daily use	Neither*		
	1	OP	9	105			0.23 (0.03, 1.79)	0	Daily use	Neither*		
Mashberg (1985)[b]	3	OP	95	913	43	48	0.94 (0.61, 1.47)	1	4 times weekly			Non-tobacco-related cancers
	3	OP	10	396			2.01 (0.52, 7.66)	0		No Tobacco		
	3	OP	28	508			0.83 (0.39, 1.77)	0		No alcohol		
	3	OP	41	438			0.57 (0.29, 1.13)	0			≥25	
Young (1986)	2	O	52	155			0.52 (0.25, 1.1)	0	MW users			Cancer pts (including larynx)
	1	O	150	468			1.02 (0.67, 1.56)	0				
	2	Oroph. + Hyp.	27	155			0.55 (0.22, 1.4)	0				
	1	Oroph. + Hyp.	88	468			0.96 (0.52, 1.5)	0				
	2	O					0.41 (0.12, 1.43)	0		No Tobacco		
	1	O					2.63 (0.5, 13.73)	0		No Tobacco		

FA	*Gender*	*Cancer*	*No. cases*	*No. controls*	*% Regular use cases*	*% Regular use controls*	*OR*	*Adj.*	*Exposure definition*	*Smokers or alcohol drinkers*	*% Of alcohol*	*Controls*
Kabat (1989)	2	OP	124	107	29	33	0.74 (0.40, 1.49)	1	Regular use for at least 1 year, 10 years before diagn.			Cancer pts (including larynx)
	2	OP					0.94 (0.39, 2.28)	1	Occasional use, 10 ys before diag.			
	2	OP					1.38 (0.42, 4.55)	1	Daily	Neither*		
	2	OP					0.74 (0.4, 1.4)	1	Regular use for at least 1 year, 10 years ago			
Winn (1991)	2	OP	293	428	58	45	1.9 (1.1, 3.3)	1	≥1 a week for 6 months		≥25	
	1	OP	573	821	49	44	1.6 (1.1, 2.3)	1			≥25	
	2	OP					1.6 (1.1, 2.3)	1				
	1	OP					1.4 (1, 1.8)	1				
	2	OP	30	108			1.1 (0.5, 2.6)	1		Neither*		
	1	OP	11	63			1.3 (0.3, 4.6)	1		Neither*		
Marshall (1992)	3	OP	290	290			Significant risk elevation	NA	Recent users			

(continued)

Table 6.2 (continued)

FA	*Gender*	*Cancer*	*No. cases*	*No. controls*	*% Regular use cases*	*% Regular use controls*	*OR*	*Adj.*	*Exposure definition*	*Smokers or alcohol drinkers*	*% Of alcohol*	*Controls*
Morse (1997)	3	OED	127	127	41	47	0.8 (0.4, 1.5)	1	1+ uses per week for 6 months			
	2	OED	51	51			0.5 (0.2, 1.4)	1				
	1	OED	76	76			1.3 (0.5, 3.4)	1				
	3	OED					1 (0.3, 3.4)	1		No Tobacco		
	3	OED					0.5 (0.2, 1.4)	1			≥25	
Talamini (2000)[c]	3	OP	121	137	9	9	1.5 (0.5, 3.8)	1	1–2 times a week			
							1.2 (0.4, 3.5)	1	>2 a week			
Winn (2001)	3	OP	328	496	36	41	1 (0.7, 1.4)	1	1+ a week for >6 moths, 1 year ago		≥25	
	2	OP					2.1 (0.9, 5)	1			≥25	
	1	OP					0.8 (0.5, 1.2)	1			≥25	
	3	OP					1.1 (0.7, 1.8)	1	<2 a day		≥25	
	2	OP					2.9 (1.0, 8.5)	1	<2 a day		≥25	
	1	OP					0.8 (0.4, 1.4)	1	<2 a day		≥25	
	3	OP					0.9 (0.5, 1.4)	1	≥2 a day		≥25	
	2	OP					1.5 (0.5, 4.5)	1	≥2 a day		≥25	
	1	OP					0.7 (0.4, 1.3)	1	≥2 a day		≥25	
	3	O					1.1 (0.7, 1.8)	1				
Mascarenhas (2002)	3	Leukoplakia	58	58	10	10	2.3 (0.4, 12)		Viadent rinse			
D'Souza *et al* (2007)	3	OP	100	200	40	36	1.3 (0.8, 2.1)	1	1–2 times a day			
	3	OP					3.8 (0.9, 16.5)	1	3–4 times a day			
Guha (2007)[d]	3	O	316	1225	5	3	1.13 (0.68, 1.85)	1	<1 a day			
	3	O					1.57 (0.8, 3.1)	1	1 a day			
	3	O					5.86 (2.91, 11.77)	1	≥2 a day			
	3	OP	81	413			1.54 (0.71, 3.37)	1	<1 a day	No Tobacco		
	3	OP					1.89 (0.45, 7.84)	1	1 a day	No Tobacco		
	3	OP					2.71 (0.74, 9.97)	1	≥2 a day	No Tobacco		
	3	OP	137	401			0.56 (0.21, 1.50)	1	<1 a day	Never drinkers		
	3	OP					4.27 (1.14, 16)	1	1 a day	Never drinkers		
	3	OP					4.96 (1.85, 13.31)	1	≥2 a day	Never drinkers		
Divaris (2010)	3	O	692	1361			0.97 (0.78, 1.22)	1	Regular use			
	3	OPL					0.95 (0.78, 1.15)	1	Regular use			
	3	OPL					0.96 (0.44, 2.12)	1		Neither*		
Macfarlane (2010)[e]	3	OPL	260	340	11	10	1.02 (0.66, 1.60)	1	<Once a day			
	3	OPL					1.22 (0.65, 2.30)	1	Once a day			
	3	OPL					1.70 (0.73, 3.95)	1	2+ times a day			

[a]Frequency of use of cases is in average 2 or more a day, in controls is 'at least occasionally'.
[b]% Refers to 'users', we do not know the frequency of use per day.
[c]No. of cases and controls with information on mouthwash use.
[d]No. of oral cancer cases with information on mouthwash use; % of regular use (once a day) on OP.
[e]About 5% of cases are oesophagus.

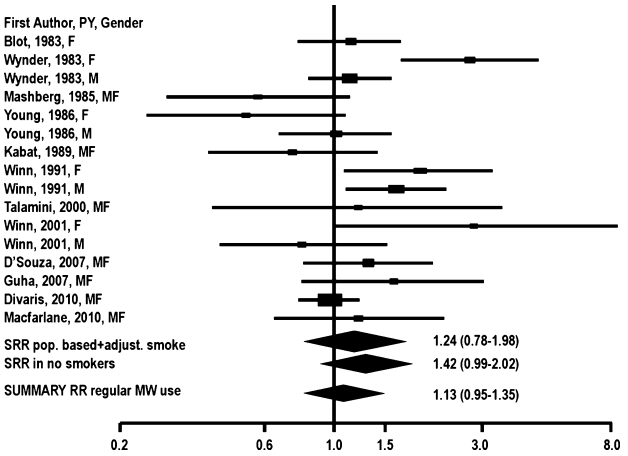

Figure 6.2 Forest plot for regular mouthwash use and oral cancer

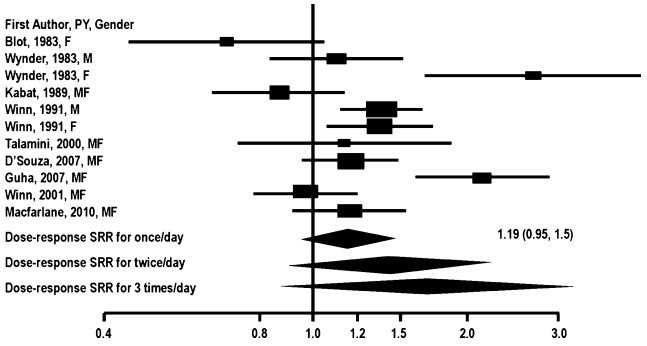

Figure 6.3 Forest plot from dose–response models on number of times per day of mouthwash use and oral cancer

Table 6.3 Summary relative risk estimates for mouthwash use

No. studies	*RR 95% CI*	I^2 %	P – χ^2	*Definitions*
Main analysis on Oral-Pharyngeal cancer				
12	1.13 (0.95; 1.35)	58	0.002	Ever use
9	1.19 (0.95, 1.5)	76	<0.001	Once a day
Sensitivity analyses				
4	0.99 (0.75; 1.31)	19	0.30	Only oral cancer
10	1.42 (0.99; 2.02)	21	0.23	In no smokers
6	0.89 (0.74; 1.07)	97	<0.001	In smokers
3	1.16 (0.44; 3.08)	72	0.01	With alcohol content at 25%
12	1.31 (0.91; 1.88)	74	<0.001	OP cancer with high use
4	1.24 (0.78; 1.98)	94	<0.001	Pop based and adjusted for smoking
16	1.19 (0.98; 1.44)	70	<0.001	Including all possible studies

evaluated ($n = 4$) and has other important limitations in experimental design.

Lachenmeier *et al* (2009) demonstrated that concentrations of acetaldehyde in the saliva of subjects who applied a mouthwash/rinse increase rapidly from nearly 0 (it was not possible to estimate precise background concentrations of acetaldehyde in human saliva from the data presented in the article) to a maximum 65 μM after 2 min. Acetaldehyde levels returned rapidly to those at baseline.

Moazzez *et al* (2011) measured acetaldehyde in products and in saliva before and up to 60 min after single rinse with alcohol-containing mouthwashes and ethanol solution compared with water. This study employed a single-use, controlled, randomized, crossover clinical trial comprising 16 healthy volunteers. A number of mouthwash products were tested: Listerine Coolmint with 21.6% ethanol, 21.6% ethanol in water, Tesco Daily Care with 8.6% ethanol and CPC, and a water control. Outcome measures included acetaldehyde level in products, acetaldehyde in saliva before rinsing and at 0.5, 2, 5, 10, 30, 60 min after rinsing, plaque levels before rinsing and bacterial levels and microbiological typing of saliva sample before rinsing.

Moazzez *et al* (2011) found a rapid and transient increase in acetaldehyde after rinsing with ethanol solutions or alcohol-containing mouthwashes. The levels of acetaldehyde in saliva were at the lower end of the range of those measured by Lachenmeier *et al* (2009), who evaluated 13 mouthrinses in 4 subjects. The levels of acetaldehyde were very transient, decreasing rapidly to undetectable levels within 10 min (compare to the 3–4 h after moderate alcohol drinking).

The peak level found for *Listerine* (a mouthwash containg 27% alcohol) was 44.3 μmole at 30 s, a concentration which is more than 1000 times lower than the levels required to demonstrate formation of DNA damage in cultured buccal epithelial cells (Vaca *et al*, 1998).

The authors put into context the relative levels of salivary acetaldehyde from ACMs by explaining that acetaldehyde is found in human body as well as in fruits and vegetables and is a metabolite produced from ingesting them. Therefore, they state it is not practical to eliminate human exposure to acetaldehyde, and a balanced risk assessment should take into consideration the exposure to endogenous acetaldehyde produced as a function of normal metabolic activity and exposure to common foods and non-alcoholic beverages. The authors stated that particular risk assessment of acetaldehyde production from ethanol-containing mouthrinse should take into account the oral and systemic benefits of the rinses (Moazzez *et al*, 2011).

The metabolism of alcohol-containing beverages to acetaldehyde in the oral cavity (without swallowing) has been demonstrated (Lachenmeier and Monakhova, 2011; Linderborg *et al*, 2011). However, studies with an *in vitro* oral buccal mucosal construct (EpiOral) have not provided evidence that alcohol is metabolized to acetaldehyde (Koschier *et al*, 2011).

Studies of alcohol drinking in humans and oral exposure of animals to alcohol-containing solutions or acetaldehyde provide little information with regard to the potential cancer risks in the oral cavity of alcohol-containing mouth wash/rinse when these are used as directed. While acetaldehyde is a mutagenic and cytotoxic compound that has been shown to cause DNA damage and mutations in a variety of test systems (Dellarco, 1988; Brooks & Theruvathu, 2005), it is also a naturally occur-

ring substance and is a product of normal metabolism. It is not clear whether acetaldehyde concentrations detected in human volunteer studies (Lachenmeier *et al*, 2009) may result in DNA damage in cells of the oral cavity, especially given the findings of Koshier *et al* (2011) which indicated no permeability of the oral mucosa acetaldehyde and the fact that commonly consumed foods can contain higher levels of acetaldehyde than were found in the saliva of subjects who rinsed with alcohol-containing mouthwash. Foods such as yoghurt would likely remain longer in the mouth than a mouthrinse.

In summary, the possibility of the alcohol in the mouthwash/rinse being converted to acetaldehyde in the oral cavity which then may cause DNA damage and lead to mutations cannot be concluded without additional studies designed to address this specific issue and to fully characterize the possibility that large interindividual variability may exist in humans with regard to acetaldehyde formation. However, such exposure is much less than that achieved by alcohol drinking which is estimated to cause <1% of oral cavity cancer in humans acting on its own.

Sources of acetaldehyde in the normal diet are taken from a variety of sources (WHO, 1998; Lachenmeier *et al*, 2009, Lachenmeier and Monakhova, 2011). It is clear that the common sources of acetaldehyde are from cigarette smoking, alcohol consumption and from certain foodstuffs such as yoghurt and peas. The contribution of acetaldehyde from mouthwash use is minimal and <1% of the daily dose. In addition, compared with acetaldehyde from alcohol drinking at different levels, the acetaldehyde is present in the saliva for minutes compared with hours as a consequence of drinking alcohol. Mouthwashes with concentrations up to 27–28% alcohol, even when used twice daily every day, would have a negligible impact on cumulative lifetime exposure to acetaldehyde derived from consuming naturally occurring (fruit and vegetables) and fermenta.

The review conducted by the German authorities (Gesundheitliche Bewertung von Acetaldehyd in alkoholischen Getränken Aktualisierte Stellungnahme Nr. 022/2010 des BfR vom 04. Mai, 2010) is of consequence. This group reviewed all the available evidence regarding acetaldehyde toxicological risk characterization and concluded that 'the expected acetaldehyde exposure from mouthwash (15 μg per day if used twice) is 0.25 μg kg^{-1} bodyweight and is minute in comparison to the amounts coming from food and alcoholic beverages'. 'The exposure lies considerably below the parameters which are possible due to the consumption of specific foods, so that the amounts obtained from mouthwash solutions are negligible compared with the total load from other areas'. 'Based on the presented toxicological profile, a carcinogenic effect or preneoplastic effect due to mouthwash solutions is not expected in the brief contact of ethanol with the mucosa if used as directed'. 'Alcohol in mouthwash solutions is not regarded as a risk to health with regard to the formation of acetaldehyde'.

In studying the association of mouthwash and oral cancer, it is important to bear in mind that there may be risk determinants as well as effect modifiers and confounders involved. Mouthwash may act as a confounder for tobacco smoking (smokers may use mouthwash to cover the tobacco smell in their mouth), and mouthwash may effect tobacco smoking and alcohol drinking acting as an effect modifier. There is very limited information available in the studies regarding why mouthwash is being used, and it would be very useful to have this information particularly about those who volunteered that they were frequent daily users. Poor oral hygiene appears to be associated with increased risk of oral cancer, independent of any effect of tobacco and alcohol consumption, and more information is needed about the tendency and use of mouthwash in person at increased risk of oral cancer due to poor oral hygiene.

The role of mouthwash use in the aetiology of oral carcinogenesis must be seen in the wider context of the biology of the mouth and the biology of oral carcinogenesis and oral cancer epidemiology. It would be valuable to conduct further evaluation of what has already been published especially to reanalyse existing studies to properly control for confounders, especially in older studies when statistical methods, such as logistic regression, were not widely available. More than anything else, there is a need to undertake studies where more attention is given to investigation of the effect of mouthwash use at different points throughout the life of subjects with a focus on the reasons for using mouthwash and the particular types of mouthwash used.

This quantitative analysis of all published epidemiological studies of mouthwash use and oral malignancy revealed (i) no statistically significant association between mouthwash use and risk of oral cancer including no significant trend in risk with increasing daily use and (ii) no association between use of mouthwash containing alcohol and oral cancer risk.

6.5 Mechanisms of carcinogenesis of alcohol relevant to oral cancer

The consumption of alcoholic beverages has been practised as a part of human culture for centuries. In addition to ethanol and water, alcoholic beverages may also contain a multitude of other compounds derived from fermentation, contamination and the use of food additives or flavours. The normal by-products of fermentation, other than ethanol are generally regarded as safe, but alcoholic beverages may contain contaminants that have been evaluated by the IARC as carcinogenic (e.g. nitrosamines and aflatoxins). However, contaminants are usually present at low concentrations, and over the past decades, these have been further reduced, at least in developed countries. For example, the concentration of nitrosamines in beer and that of lead in wine has declined significantly over the past 30 years.

Throughout the world, most alcoholic beverages are produced and consumed within the same country. Consumption has increased in developing regions, and the country that now has the highest total production is China, followed by India and Brazil. The trade in alcoholic beverages has increased over the last four decades, but its proportion has remained at approximately 0.5% of total world trade.

The consumption of alcoholic beverages can be divided into recorded consumption (estimated from sales, produc-

tion and national taxation records) and unrecorded consumption (e.g. illegal production, smuggling, home production and private importation). Overall, recorded consumption has increased slightly over the past 20 years, but more substantial increases have occurred in China and some other developing countries. In contrast, an overall decline in recorded consumption is evident in several developed countries.

More than 1.9 billion adults (1.2 billion men and 750 million women) around the world were estimated to consume alcoholic beverages in 2002 and 22% of the men and 3% of the women drank 40 g alcohol or more per day. In all regions of the world, men drink more often and in larger quantities than women, but the gender differences are largely culturally dependent; smaller differences are observed in Europe and larger differences in developing parts of the world. Consumption of alcohol is age dependent: the frequency of drinking increases until middle age and the prevalence of heavy episodic drinking decreases over the adult lifespan. Those of the lowest socio-economic class tend to drink the cheapest beverage available in their respective countries.

A large variety of substances that are not intended for human consumption are nevertheless being consumed as alcohol (surrogate alcohol such as hair spray, aftershaves, lighter fluid and medicines). They usually contain very high concentrations of ethanol and may also contain higher alcohols and toxic concentrations of methanol.

In addition to international regulations such as the Codex alimentarius, countries tend to regulate traditional local alcoholic beverages (e.g. beer, whisky and vodka), but emerging products (e.g. alcopops) are initially subject to few regulations.

A large body of evidence from epidemiological studies of different design and conducted in different populations consistently shows that consumption of alcoholic beverages is associated with a higher risk of both oral and pharyngeal cancer and that the risk increases with increasing amounts of alcohol consumed. Compared with non-drinkers, regular consumption of about 50-g alcohol (ethanol) per day is associated with an approximately threefold increase in risk of these cancers. These associations were consistently found for the types of alcoholic beverage that are commonly drunk in the areas where the studies were conducted.

Tobacco smoking is an important cause of oral and pharyngeal cancer. The association of consumption of alcoholic beverages with these cancers was evident in both smokers and non-smokers. The effects of smoking and consumption of alcoholic beverages appear to be multiplicative, such that the largest relative risks are seen in people who both smoke tobacco and drink alcoholic beverages.

Mechanisms of oral carcinogenesis: ethanol. The effect of ethanol on the development of cancer depends on a variety of factors, including doses of ethanol and time of exposure, and also on animal species, strain and sex.

Ethanol was evaluated by an IARC Working Group in 1988, and it was concluded that there was inadequate evidence for the carcinogenicity of ethanol in experimental animals (IARC, 1988). Most of the studies were criticized because of the small numbers of animals studied, the inadequate design of the experiments with uncontrolled dietary regimens, the short exposure to ethanol, low doses of ethanol and the failure to measure ethanol intake and/or concentrations in the blood. These concerns are also relevant for some of the studies that were published after 1988.

In a 2-year study, administration of ethanol to male mice in the drinking water caused a dose-related increase in the incidence of hepatocellular adenomas and hepatocellular adenomas and carcinomas. In a lifetime study, administration of ethanol in the drinking water resulted in an increase in the incidence of head and neck carcinomas in male and female rats and the incidence of forestomach carcinomas, testicular interstitial cell adenomas and osteosarcomas of the head, neck and other sites in male rats. In another lifetime study, ethanol administered in the drinking-water-induced mammary adenocarcinomas. In another study that used a genetically modified mouse model for intestinal cancer, administration of ethanol in the drinking water increased the incidence of intestinal tumours. Additional studies that encompassed oral and other routes of administration were also reviewed but were considered to be inadequate for the reasons noted above (IARC, 2012).

A number of other studies have been performed to determine whether ethanol modifies chemically induced carcinogenesis in various mouse and rat strains with a variety of carcinogens. Depending on the carcinogen and the animal model used, tumour-specific target organs included the mammary gland, oesophagus, forestomach, large intestine, liver, kidney, lung and thymus. Again, some of these studies can be criticized because of the concerns mentioned above. However, in the majority of the studies, ethanol enhanced chemically induced carcinogenesis (IARC, 2012).

Ethanol is absorbed rapidly from the upper gastrointestinal tract. Most of the ethanol is eliminated in the liver, catalysed by alcohol dehydrogenases and to a much smaller degree by cytochrome P450 enzymes and catalase. The overall rate of elimination is affected to some extent by variation in alcohol dehydrogenase isozymes. Chronic consumption of alcoholic beverages induces cytochrome P450, but variants in this enzyme have not been clearly associated with differential susceptibility to alcoholism or ethanol-related pathology.

The available data from molecular–genetic epidemiological studies suggest a positive association between the presence of alcohol dehydrogenase 1B (*1/*1) and the risk of upper aerodigestive tract cancer, but the mechanisms through which the functional polymorphism affects susceptibility to cancer have not been fully explained. The evidence for a contribution of the alcohol dehydrogenase 1C polymorphism to the development of cancer in the upper aerodigestive tract is limited (IARC, 2012).

Acetaldehyde. Acetaldehyde is formed metabolically from the oxidation of ethanol and is further metabolized, predominantly by nicotinamide-adenine-dinucleotide-dependent aldehyde dehydrogenases, to acetic acid. The importance of aldehyde dehydrogenase in the oxidative pathway of ethanol is emphasized in drinkers of alcoholic

beverages who are deficient in this enzyme: the alcoholic flush reaction that they experience correlates with the accumulation of acetaldehyde in the blood.

In the absence of alcoholic beverage consumption, acetaldehyde ingested in food or generated by microbial fermentation is rapidly reduced to acetic acid.

Laboratory animal experiments have indicated that acetaldehyde can exert toxic effects, mainly at the site of initial contact. However, it is not known what levels of acetaldehyde are required to exert toxic effects and if all tissues would be effected. Respiratory effects observed in studies in rats exposed to acetaldehyde by inhalation (for 13 weeks or 28 months) included degenerative changes in the olfactory and upper respiratory epithelium, metaplasia in the larynx and disturbances of the tracheal epithelium.

The available data from molecular–genetic epidemiological studies provide ample evidence that the heterozygous aldehyde dehydrogenase two genotype – which leads to the accumulation of acetaldehyde, for example, in the blood, saliva and liver – contributes substantially to the development of oesophageal cancers (squamous cell carcinomas) that are related to the consumption of alcoholic beverages.

While it is often difficult to differentiate clearly between the exact locations of tumours in the oropharyngolaryngeal area based on the available published data, there is strong evidence that the heterozygous aldehyde dehydrogenase two genotype contributes to the development of cancers of the oropharyngolarynx as a whole that are related to the consumption of alcoholic beverages.

Aldehyde dehydrogenase two-deficient individuals have been shown to be at higher risk of developing oesophageal cancer through alcoholic beverage consumption and also to have higher levels of acetaldehyde in the blood and saliva following alcoholic beverage drinking compared with aldehyde dehydrogenase 2-proficient individuals.

Carcinogenicity of alcohol consumption (IARC, 2012). There is sufficient evidence in humans for the carcinogenicity of alcoholic beverages. The occurrence of malignant tumours of the oral cavity, pharynx, larynx, oesophagus, liver, colorectum and female breast is causally related to the consumption of alcoholic beverages. There is evidence suggesting lack of carcinogenicity in humans for alcoholic beverages and cancer of the kidney and non-Hodgkin lymphoma.

There is substantial mechanistic evidence in humans who are deficient in aldehyde dehydrogenase that acetaldehyde derived from the metabolism of ethanol in alcoholic beverages contributes to the causation of malignant oesophageal tumours.

There is sufficient evidence in experimental animals for the carcinogenicity of ethanol, and there is also sufficient evidence in experimental animals for the carcinogenicity of acetaldehyde.

Overall, alcoholic beverages are carcinogenic to humans (Group 1), and ethanol in alcoholic beverages is carcinogenic to humans (Group 1; IARC, 1988). The latter evaluation is based on (i) the epidemiological evidence, which showed little indication that the carcinogenic effects depend on the type of alcoholic beverage, (ii) the sufficient evidence that ethanol causes cancer in experimental animals and (iii) the mechanistic evidence in humans who are deficient in aldehyde dehydrogenase that acetaldehyde derived from the metabolism of ethanol in alcoholic beverages contributes to the causation of malignant oesophageal tumours. Identification of ethanol as a known carcinogenic agent in alcoholic beverages does not rule out the possibility that other components may also contribute to their carcinogenicity.

In October 2009, the IARC Working Group for Monograph Volume 100E reviewed 'alcohol drinking' as a Group 1 agent. This Working Group considered that acetaldehyde is a genotoxic compound that is detoxified by aldehyde dehydrogenases (ALDH) and that the ALDH2*2 variant allele, which encodes an inactive enzyme, is prevalent in up to 30% of east-Asian populations; and that heterozygous carriers, who have about 10% enzyme activity, accumulate acetaldehyde and have considerably higher relative risks for alcohol-related oesophageal and head and neck cancers compared with individuals with the common alleles. In 2008, an IARC Working Group acknowledged the important role of acetaldehyde in the development of alcohol-related cancer, especially of the oesophagus, but refrained from making a formal evaluation. However, in October, 2009, for Volume 100E, the Working Group concluded that 'acetaldehyde associated with alcoholic beverages' is carcinogenic to humans (Group 1).

Alcohol-containing mouthwashes/rinses pose little threat to systemic human exposure to alcohol itself or products of its metabolism if used as directed. Application of alcohol-containing mouth wash/rinse solutions has been shown to briefly elevate acetaldehyde concentration in the saliva of human volunteers (Lachenmeier *et al*, 2009). The latter study is very severely limited in the number of subjects evaluated ($n = 4$) and has other important limitations in experimental design.

The results demonstrated that concentrations of acetaldehyde in the saliva of subjects who applied a mouthwash/rinse increase rapidly from nearly 0 (it was not possible to estimate precise background concentrations of acetaldehyde in human saliva from the data presented in the article) to a maximum 65 μM at 2 min. Acetaldehyde levels returned rapidly to those at baseline.

Moazzez *et al* (2011) reviewed the prior work by Lachenmeier (2009) and decided that there was a need for basic research to understand the levels of salivary acetaldehyde (AA) produced after rinsing with alcohol solutions and alcohol-containing mouthrinse (ACMs) and to further determine the source of the AA and the role microorganisms may play in its production. The objectives of this study were to measure acetaldehyde in products and in saliva before and up to 60 min after single rinse with ACMs and ethanol solution compared with water, to measure levels of plaque on teeth and number of microorganisms present in saliva just before rinsing and to determine whether there are any types of microorganism in saliva which are associated more frequently with higher acetaldehyde levels.

The study employed a single-use, controlled, randomized, crossover clinical trial comprising 16 healthy volunteers, orally fit, who smoked no more than five cigarettes

per day and had no more than moderate alcohol drinking habits. Subjects had to refrain from eating and drinking for 1 h and no alcohol consumption 24 h prior to sampling. Study subjects brushed teeth at home and travelled to the clinic for supervised rinsing and sampling visit. A number of mouthwash products were tested: Listerine Coolmint with 21.6% ethanol, 21.6% ethanol in water, Tesco Daily Care with 8.6% ethanol and CPC and a water control. Outcome measures included acetaldehyde level in products, acetaldehyde in saliva before rinsing and at 0.5, 2, 5, 10, 30, 60 min after rinsing, plaque levels before rinsing and bacterial levels and microbiological typing of saliva sample before rinsing.

The authors found a rapid and transient increase in acetaldehyde after rinsing with ethanol solutions or ACMs. The lowest levels of acetaldehyde were observed for Listerine Coolmint and highest for the 21.6% ethanol solution. Hence, it could be interpreted that Listerine potentially possesses acetaldehyde suppression properties (Moazzez *et al*, 2011).

The levels of acetaldehyde in saliva were at the lower end of the range of those measured by Lachenmeier (2009), who evaluated 13 mouthrinses in 4 subjects. The levels of acetaldehyde were very transient, decreasing rapidly to undetectable levels within 10 min (compare to the 3–4 h after moderate alcohol drinking). Acetaldehyde levels rose rapidly, indicating that the enzymes responsible for the break-down of ethanol to acetaldehyde are not inside bacterial cells or epithelial cells but are readily available on oral surfaces; alternatively yeasts could be the primary rapid 'digestors' of ethanol.

The peak level for Listerine was 44.3 μmole at 30 s, a concentration which is more than 1000 times lower than the levels required to demonstrate formation of DNA damage in cultured buccal epithelial cells (Vaca, 1998), and half the speculated amount (80–100 μmole) for carcinogenic risk proclaimed by Lachenmeier and Salaspuro on the basis of a test tube study which did not involve intact cells/tissues (Theruvathu, 2005).

The authors put into context the relative levels of salivary acetaldehyde from ACMs by explaining that acetaldehyde is found in human body as well as in fruits and vegetables and is a metabolite produced from ingesting them. Therefore, they state it is not practical to eliminate human exposure to acetaldehyde and a balanced risk assessment should take into consideration the exposure to endogenous AA produced as a function of normal metabolic activity and exposure to common foods and non-alcoholic beverages. The authors stated that particular risk assessment of acetaldehyde production from ethanol-containing mouthrinse should take into account the oral and systemic benefits of the rinses (Moazzez *et al*, 2011).

The metabolism of alcohol-containing beverages to acetaldehyde in the oral cavity (without swallowing) has been demonstrated (Lachenmeier and Monakhova, 2011; Linderborg *et al*, 2011). However, studies with an *in vitro* oral buccal mucosal construct (EpiOral) did not provide evidence that alcohol is metabolized to acetaldehyde (Koschier *et al*, 2011).

The human cancer risks of acetaldehyde have been considered recently by the US National Toxicology Program and the International Agency for Research on Cancer. The NTP 12th Report on Carcinogens classifies acetaldehyde as 'reasonably anticipated to be a human carcinogen' (Report on Carcinogens, 12th edn, 2010). The IARC Working Group concluded that 'acetaldehyde associated with alcoholic beverages' is 'carcinogenic to humans (Group 1)' (Secretan *et al*, 2009).

The iPRI Clinical Synthesis Working Group, established in 2011, concluded that the studies of alcohol drinking in humans and oral exposure of animals to alcohol-containing solutions or acetaldehyde provide little information with regard to the potential cancer risks in the oral cavity of alcohol-containing mouth wash/rinse when these are used as directed. Still, acetaldehyde is detected in the oral cavity of human subjects who applied these preparations under experimental conditions, and thus, the epithelial lining of the oral cavity may be exposed locally to acetaldehyde for brief periods of time. Acetaldehyde is a mutagenic and cytotoxic compound that has been shown to cause DNA damage and mutations in a variety of test systems (Dellarco, 1988; Brooks & Theruvathu, 2005). However, it is also a naturally occurring substance and is a product of normal metabolism. It is not clear whether acetaldehyde concentrations detected in human volunteer studies (Lachenmeier *et al*, 2009) may result in DNA damage in cells of the oral cavity, especially given the findings of Koshier *et al* (2011) which indicated no permeability of the oral mucosa acetaldehyde and the fact that commonly consumed foods can contain higher levels of acetaldehyde than were found in the saliva of subjects who rinsed with alcohol-containing mouthwash. Foods such as yoghurt would likely remain longer in the mouth than a mouthrinse.

In summary, the possibility of the alcohol in the mouthwash/rinse being converted to acetaldehyde in the oral cavity which then may cause DNA damage and lead to mutations cannot be concluded without additional studies designed to address this specific issue and to fully characterize the possibility that large interindividual variability may exist in humans with regard to acetaldehyde formation. However, such exposure is much less than that achieved by alcohol drinking which is estimated to cause no more than 1% of oral cavity cancer in humans.

Sources of acetaldehyde in humans. Sources of acetaldehyde in the normal diet are taken from a variety of sources (WHO, 1998; Lachenmeier, 2009, 2010) and outlined in relative terms for smokers and alcohol drinkers and in quantitative terms for smokers and drinkers and non-smokers and light drinkers. It is clear that the common sources of acetaldehyde are from cigarette smoking, alcohol consumption and from certain foodstuffs. The contribution of acetyldehyde from mouthwash use is minimal and <1% of the daily dose. In addition, compared with acetaldehyde from alcohol drinking at different levels, the acetaldehyde is present in the saliva for minutes compared with hours as a consequence of drinking alcohol.

In conclusion, mouthwashes with concentrations up to 27–28% alcohol, even when used twice daily every day, would have a negligible impact on cumulative lifetime

exposure to acetaldehyde derived from consuming naturally occurring (fruit and vegetables) and fermenta.

It is of great importance to bear in mind the recent review conducted by the German authorities (*Gesundheitliche Bewertung von Acetaldehyd in alkoholischen Getränken Aktualisierte Stellungnahme Nr. 022/2010 des BfR vom 04. Mai, 2010*). This group reviewed all the available evidence regarding acetaldehyde toxicological risk characterization and concluded that 'the expected acetaldehyde exposure from mouthwash (15 μg per day if used twice) is 0.25 μg kg^{-1} bodyweight and is minute in comparison to the amounts coming from food and alcoholic beverages'. 'The exposure lies considerably below the parameters which are possible due to the consumption of specific foods, so that the amounts obtained from mouthwash solutions are negligible compared with the total load from other areas'. 'Based on the presented toxicological profile, a carcinogenic effect or preneoplastic effect due to mouthwash solutions is not expected in the brief contact of ethanol with the mucosa if used as directed'. 'Alcohol in mouthwash solutions is not regarded as a risk to health with regard to the formation of acetaldehyde'.

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Appendix I

2008–2013 Action plan for the global strategy for the prevention and control of non-communicable diseases

The six objectives of the 2008–2013 Action Plan

1 To raise the priority accorded to non-communicable disease in development work at global and national levels and to integrate prevention and control of such diseases into policies across all government departments.
2 To establish and strengthen national policies and plans for the prevention and control of non-communicable diseases.
3 To promote interventions to reduce the main shared modifiable risk factors for non-communicable diseases: tobacco use, unhealthy diets, physical inactivity and harmful use of alcohol.
4 To promote research for the prevention and control of non-communicable diseases.
5 To promote partnerships for the prevention and control of non-communicable diseases.
6 To monitor non-communicable diseases and their determinants and evaluate progress at the national, regional and global levels.

Source

World Health Organization (2008–2013). *Action plan for the global strategy for the prevention and control of noncommunicable diseases*. WHO (2008; ISBN 978-92-4-159741-8). Also available at: http://whqlibdoc.who.int/publications/2009/9789241597418_eng.pdf.

Appendix II

Meta-analysis literature search protocol

The search process needs to be documented in enough detail throughout its entirety to ensure that it can be reported correctly in the review, to the extent that all the searches of all the databases are reproducible. It should be borne in mind at the outset that the full search strategies for each database will need to be included in an appendix of the review. The search strategies will need to be copied and pasted exactly as run and included in full, together with the search set numbers and the number of records retrieved.

Before starting the literature search, you have to

I Test and define keywords on PubMed/ISI Web of Knowledge/other databases.

When designing a search strategy, to be as comprehensive as possible, it is necessary to include a wide range of free-text terms for each of the concepts selected. For example,

- synonyms: 'colorectal' OR 'large intestine, etc;
- related terms: 'adenocarcinoma' OR 'carcinoma' OR 'cancer', etc; and
- variant spellings: 'tumour' OR 'tumor'.

In PubMed, use MeSH to define the keywords.

II Identify reviews and previous meta-analyses.

Have a quick look at them as they can be useful to find other keywords. Papers referenced in previous meta-analyses have to be kept until the final step!

III Carry out your search on PubMed/ISI and include the largest number of keywords possible.

To fulfil the 'PRISMA' table, our strategy should look like a funnel: starting from the maximum number of papers identified by the searches, then those for which we read the abstract, then those for which we read the full text, then those that we consider as eligible and finally those that are included in the meta-analysis.

From the overall list of titles identified, the selection should be done in six steps:

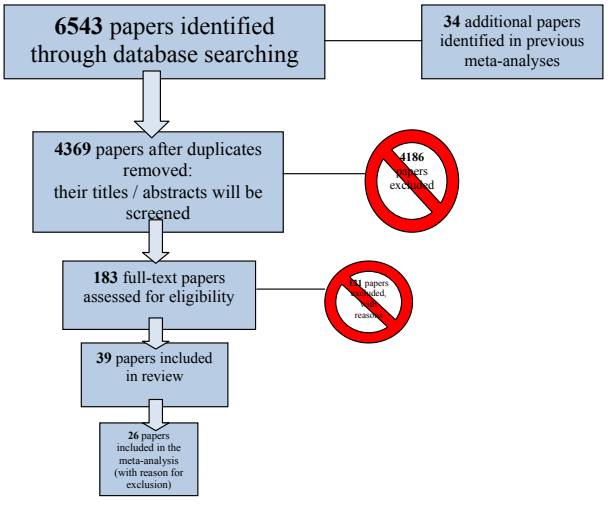
1 Overall number of paper identified by the searches (on PubMed, ISI Web of Knowledge/other databases; n = ***), among which *** are identified in the existing reviews and previous meta-analyses. Merge search results and remove duplicate records of the same paper. *** additional papers are extracted from the reviews and meta-analyses that are not in the main PubMed search.
2 Out of these, all (***) of those from reviews and previous meta-analysis are selected for abstract reading. From the PubMed search, *** are selected for abstract reading, *** are excluded after having read the title only (irrelevant reports ≥ authors should generally be over-inclusive at this stage).
3 Out of the papers with abstract selected, *** are excluded after having read the abstract and *** are included for the reading of full text (pdfs are systematically gathered at the stage).
4 From the selected full papers, we identify *** additional potential articles of interest (either reporting methods for a study published in a previous paper or reporting previous results in a publication which was not identified in the PubMed search). The overall list of publications is then ***. Out of these, *** do not meet inclusion criteria.
5 *** are eligible papers. Among these, *** don't provide point estimate, *** are duplicate with other papers and are then excluded.
6 Finally, *** are included in the meta-analysis. The data collection can begin.

At each step, the list of papers should be kept with the count updated, to present a study flow diagram (cf. PRISMA Statement).

The flow diagram should present

- number of unique records identified by the searches;
- number of records excluded after preliminary screening (e.g. of titles and abstracts);
- number of records retrieved in full text;
- number of records or studies excluded after assessment of the full text, with brief reasons;
- number of studies meeting eligibility criteria for the meta-analysis (and thus contributing to qualitative synthesis); and
- number of studies included in the meta-analysis.

Example of PRISMA chart



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Appendix III

iPRI Methods for conducting meta-analyses

iPRI have conducted a number of meta-analyses and envisage this being an important component of our ongoing and future activities. It is essential to have some guidelines for the statistical analysis of such meta-analyses to have harmonized results.

Literature search and reporting of results
A systematic literature search and quantitative analysis must be conducted and reported following MOOSE guidelines regarding meta-analysis of observational studies (Stroup *et al,* 2000). Published reports should be obtained from the following databases using validated search strategies: Ovid MEDLINE database; ISI Web of Science Science Citation Index Expanded (SCI Expanded); and PUBMED (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi). Other sources can be found in the reference lists of the retrieved articles and preceding reviews on the topic.

Statistical model

1 No distinction will be made between relative risk (RR), odds ratio (OR), hazard ratio (HR), standardized incidence ratio (SIR) and standardized mortality ratio (SMR). All will be considered equivalent to an estimation of the relative risk of the disease in question (and the disease is rare).
2 The relative risk will be transformed to logarithm before inclusion in the model.
3 For all analysis (main and all sensitivity/heterogeneity analysis) a random-effect model based on van Houwelingen *et al* (2002) method (a mixed-effect model initially made in SAS but that can be programmed in R) with summary RR obtained from maximum likelihood estimation.
4 All summary relative risks and confidence intervals will be reported using two decimal places.
5 Confidence intervals will be computed assuming an underlying t-distribution.
6 When one study did not reported a single estimate but two or more, this will be handled in SAS by adding a random effect at the study level (hierarchical model). With (computing language) R, this will be handled in two steps: by making an initial meta-analysis of the two (or more) estimates to obtain one estimate for the study before including it in the meta-analysis. (Both approaches would produce very close results.) Heterogeneity will be assessed by Higgins and Thompson's I^2 (Higgins and Thompson, 2002) and the chi-square test.

Publication bias

1 Publication bias will be evaluated with Begg and Mazumdar (1994) and Egger *et al* (1997) tests when we have 20 or more publications included. Otherwise, when <20 publications are included, a regression of ln(RR) over the sample size weighted by inverse of the variance will be used instead (Macaskill *et al,* 2001).
2 A graphical representation with a funnel plot (ln (RR) over standard error) will also be used to assess publication bias graphically.

Sensitivity analysis
A sensitivity analysis will systematically be conducted by investigating the impact on the summary RR of excluding each individual study and the impact of inclusion and exclusion criteria.

Heterogeneity analysis
Analysis will be presented for different subgroups to investigate heterogeneity. Meta-regression is useful to evaluate the impact of continuous and categorical variables that indicate study design, publication year, geographical area, study population, mean age of cases and controls, etc.

In the case of breast cancer as an example, when two or more studies are available

- Separate analysis for studies with cancer premenopause *vs* postmenopause
- Separate analysis for studies on mortality
- Separate analysis for case–control *vs* cohort studies/NCC
- Separate analysis for studies unadjusted for BMI *vs* studies adjusted for BMI
- Separate analysis when different methods of ascertaining exposure were used [laboratory techniques, definition of diabetes (self-reported *vs* medical files)…]

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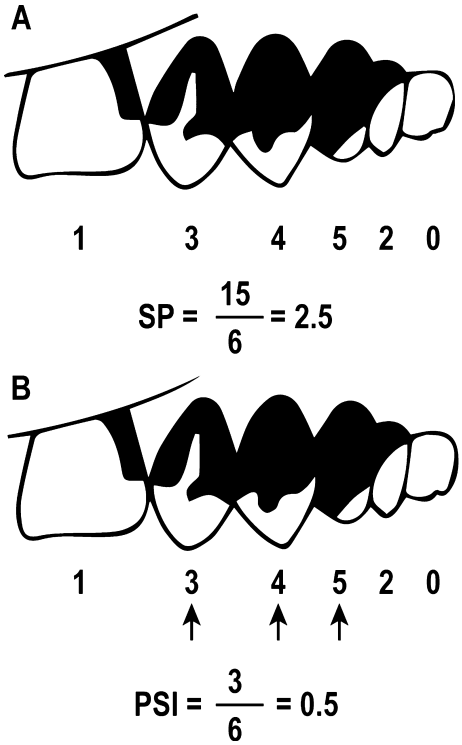
Appendix IV

Scoring methods

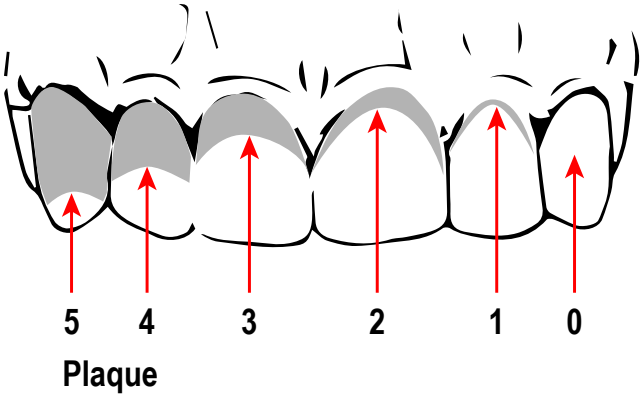
Dental plaque
Source: Triratana *et al*, *JADA*, 2002.



0 = Absence of plaque
1 = Very small amount of plaque
2 = Thin continuous band of plaque at cervical margin
3 = Plaque covering less than 1/3 of tooth surface
4 = Plaque covering more than 1/3 but less than 2/3 of tooth surface
5 = Plaque covering almost all of tooth surface

The scoring of the Quigley–Hein Plaque Index
SP: score of plaque. This is derived by adding up the scores for all teeth and dividing that total by the total number of teeth assessed. In the case shown here, a total score of 15 divided by a total of six teeth assessed yields a final score of 2.5.

The scoring of the Plaque Severity Index or PSI
This score is a percentage, derived by dividing the total number of teeth that have high scores (scores of 3 or higher) by the total number of teeth assessed. In the case shown here, three teeth had scores of three or higher; dividing that by the total number of teeth scored, six, yields a score of 50%, or 0.5.



Modified Quigley–Hein Plaque Index
Source: http://www.whocollab.od.mah.se/expl/ohituresky70.html.

Table 1 Quigley–Hein Plaque Index, modified by Turesky

Score	*Criteria*
0	No plaque
1	Separate flecks of plaque at the cervical margin of the tooth
2	A thin continuous band of plaque (up to 1 mm) at the cervical margin of the tooth
3	A band of plaque wider than 1 mm but covering less than one-third of the crown of the tooth
4	Plaque covering at least one-third but less than two-thirds of the crown of the tooth
5	Plaque covering two-thirds or more if the crown of the tooth

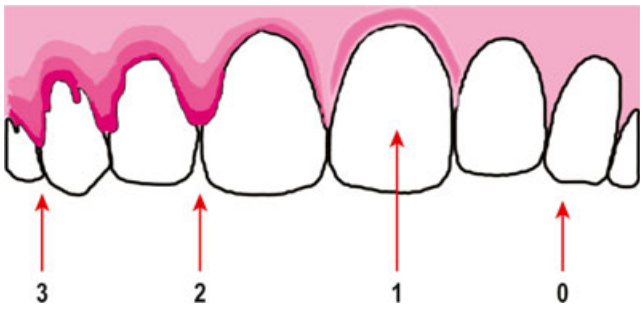
Index, total score/number of surfaces examined.

Löe–Silness Plaque Index
Source: Silness and Löe, *Acta odontologica Scandinavia*, 1964.

Plaque at six teeth, representing the six segments of the jaws, is examined:

- The maxillary right first molar (maxillary = en haut).
- The maxillary right lateral incisor (= incisive).
- The maxillary left first bicuspid (= prémolaire).
- The mandibular left first molar (mandibular = en bas).
- The mandibular left lateral incisor.
- The mandibular right first bicuspid.

Assessment of soft deposits was made according to a plaque index proposed by the authors:



0 = Absence of inflammation
1 = Mild inflammation: slight change in colour and texture; no bleeding on probing
2 = Moderate inflammation: moderate glazing, redness, edema and hypertrophy; bleeding on probing
3 = Severe inflammation: marked redness and hypertrophy; tendency toward spontaneous bleeding and ulceration

Table 2 The Plaque Index system as proposed by Loe and Silness

Scores	*Criteria*
0	No plaque
1	A film of plaque adhering to the free gingival margin and adjacent area of the tooth. The plaque may be seen *in situ* only after application of disclosing solution or by using the probe on the tooth surface
2	Moderate accumulation of soft deposits within the gingival pocket or on the tooth and gingival margin which can be seen with the naked eye
3	Abundance of soft matter within the gingival pocket and/or on the tooth and gingival margin

Each of the four surfaces of the teeth (buccal, lingual, mesial and distal) is given a score from 0 to 3, *the plaque index for the area*. The scores from the four areas are added and divided by four to give *the plaque index for the tooth*. The indices for the teeth (incisors, premolars and molars) may be grouped to designate *the index for the group of teeth*. By adding the indices for the teeth and dividing by six, *the index for the patient* is obtained. The index for the patient is thus an average score of the number of areas examined.

Gingivitis

Gingivitis Severity Index. Source: Triratana *et al*, JADA, 2002

The Gingivitis severity index score is a percentage of sites that demonstrate bleeding, derived by dividing the total number of teeth that have high scores (scores of 2 or 3) by the total number of sites scored.

Löe–Silness Gingival Index. Source: Löe and Silness, Acta Odontologica Scandinavia, 1963

Table 3 Gingival index system

Score	*Criteria*
0	Absence of inflammation
1	Mild inflammation – slight change in colour, little change in texture
2	Moderate inflammation – moderate glazing, redness, oedema and hypertrophy. Bleeding on pressure
3	Severe inflammation – marked redness and hypertrophy. Tendency to spontaneous bleeding. Ulceration

Gingiva at six teeth, representing the six segments of the jaws, is examined (same as plaque and calculus index).

Each gingival unit (buccal, lingual, mesial and distal) of the individual tooth is given a score from 0 to 3, called the *GI for the area*. The scores for the four areas of the tooth are added and divided by four to give the *GI for the tooth.* The scores for the individual teeth (incisors, premolars and molars) may be grouped to designate the *GI for the group of teeth*. Finally, by adding the indices for the teeth and dividing by six, the *GI for the patient* is obtained. The index for the patient is thus an average score for the areas examined.

Modified Gingival Index (Lobene). Source: DePaola *et al*, 1989

Table 4 Modified Gingival Index (Lobene)

Score	*Criteria*
0	Absence of inflammation
1	Mild inflammation (slight change in colour, little change in texture) of *any portion* of but not the entire gingival unit
2	Mild inflammation of the *entire* gingival unit
3	Moderate inflammation (glazing, redness, oedema and/or hypertrophy) of the entire gingival unit
4	Severe inflammation (marked redness, oedema and/or hypertrophy, spontaneous bleeding, congestion or ulceration) of the entire gingival unit

Appendix V

Method for computing the variance of the relative difference

Notations

Int_{Base} is the mean score value at baseline in the intervention group (e.g. chlorhexidine).

$Ctrl_{Base}$ is the mean score value at baseline in the control group (e.g. flavoured water).

Int_{END} is the mean score value at the end of the trial in the intervention group.

$Ctlr_{END}$ is the mean score value at the end of the trial in the control group.

Relative difference

The relative difference is defined as follows:

$$DR = \frac{Int_{END} - Ctrl_{END}}{\left(\frac{Int_{Base}+Ctrl_{Base}}{2}\right)} = 2 \times \frac{Int_{END} - Ctrl_{END}}{Int_{Base} + Ctrl_{Base}}$$

The variance of the relative difference is computed using the Delta method (Oehlert, 1992). The computations are detailed below. In a first step, partial derivatives of DR in respect with Int_{END}, $Ctrl_{END}$, Int_{Base} and $Ctrl_{Base}$ are calculated.

$$\frac{\partial DR}{\partial Int_{END}} = \frac{2}{Int_{Base} + Ctrl_{Base}}$$

$$\frac{\partial DR}{\partial Ctrl_{END}} = \frac{-2}{Int_{Base} + Ctrl_{Base}}$$

$$\frac{\partial DR}{\partial Int_{Base}} = \frac{-2(Int_{END} - Ctrl_{END})}{(Int_{Base} + Ctrl_{Base})^2}$$

$$\frac{\partial DR}{\partial Ctrl_{Base}} = \frac{2(Int_{END} - Ctrl_{END})}{(Int_{Base} + Ctrl_{Base})^2}$$

In a second step, the variance is computed:

$$\mathrm{Var}(DR) = \sum_i \left(\frac{\partial DR}{\partial B_i}\right)^2 \mathrm{Var}(B_i) = \left(\frac{2}{Int_{Base} + Ctrl_{Base}}\right)^2 \times \mathrm{var}(Int_{END}) + \left(\frac{-2}{Int_{Base} + Ctrl_{Base}}\right)^2 \times \mathrm{var}(Ctrl_{END}) + \left(\frac{-2(Int_{END} - Ctrl_{END})}{(Int_{Base} + Ctrl_{Base})^2}\right)^2 \times \mathrm{var}(Int_{Base}) + \left(\frac{2(Int_{END} - Ctrl_{END})}{(Int_{Base} + Ctrl_{Base})^2}\right)^2 \times \mathrm{var}(Ctrl_{Base})$$

Therefore:

$$\mathrm{Var}(DR) = \left(\frac{2}{Int_{Base} + Ctrl_{Base}}\right)^2 \times (\mathrm{var}(Int_{END}) + \mathrm{var}(Ctrl_{END})) + \left(\frac{2(Int_{END} - Ctrl_{END})}{(Int_{Base} + Ctrl_{Base})^2}\right)^2 \times (\mathrm{var}(Int_{Base}) + \mathrm{var}(Ctrl_{Base})).$$

If no baseline value is available

Several studies do not provide baseline scores. In this case, an alternative formula is used:

$$D = \frac{Int_{END} - Ctrl_{END}}{Ctrl_{END}}$$

Variance of D is computed using the same method. The partial derivatives of D in respect with Int_{END} and $Ctrl_{END}$ are

$$\frac{\partial D}{\partial Int_{END}} = \frac{1}{Ctrl_{END}}$$

$$\frac{\partial D}{\partial Ctrl_{END}} = \frac{-Ctrl_{END} - (Int_{END} - Ctrl_{END})}{Ctrl_{END}^2} = \frac{-Int_{END}}{Ctrl_{END}^2}$$

Therefore, the variance of D is:

$$\mathrm{var}(D) = \sum_i \left(\frac{\partial DR}{\partial B_i}\right)^2 \mathrm{Var}(B_i) = \frac{\mathrm{Var}(Int_{END})}{Ctrl_{END}^2} + \left(\frac{-Int_{END}}{Ctrl_{END}^2}\right)^2 \times \mathrm{var}(Ctrl_{END})$$

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Appendix VI

Articles included in meta-analyses

Chlorhexidine

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