Submission
No 261

INQUIRY INTO DENTAL SERVICES IN NSW

Organisation: Professionals Against Water Fluoridation (PAWF)

Name: Dr John Ryan

Position: Chairman

Telephone:

Date Received: 24/01/2006

Theme:

Summary

PROFESSIONALS AGAINST WATER FLUORIDATION

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January 20, 2006

NSW Dental Inquiry
Standing Committee on Social Issues
The Hon Jan Burnswoods (Chair)
Legislative Council
Parliament House
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As Chairman of Professionals Against Water Fluoridation (PAWF), I wish to bring our concerns cocerning water fluoridation to your attention. PAWF represents over 3,000 doctors, dentists, scientists and other health professionals who view the fluoridation of our water supplies as a grave risk to health; we also challenge the efficacy of this practice.

There is no doubt that fluoride inhibits enzyme function and is toxic in small amounts. This enzyme toxicity is the principal cause for the very low margin of safety involved in fluoridating water. There are well documented effects on gene mutations, and water fluoridation is associated with cancer, cardiovascular dysfunction, neurotoxicity, bone pathology and dental fluorosis. Our opposition to fluoridation thus relates to whole body toxicity rather than teeth issues alone.

We view as irresponsible the commendation of the Dental and Medical Associations that fluoridation is safe even though studies recommended by the National Health and Medical Research Council have not been implemented. Compounding the risks in fluoridation are its toxicity, the high exposure from drinking water and inadequate testing; and yet it is proposed that it be added to drinking water for a lifetime.

Because of this well-known toxicity, the vast majority of developed nations with advanced standards of public health have rejected fluoridation and in most cases prohibit it. In the countries that have rejected fluoridation it is conspicuous that biochemists, pharmacologists, and enzymologists have been consulted and their concerns noted.

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PROFESSIONALS AGAINST WATER FLUORIDATION

In Sweden the government sought the opinion of the Nobel Medical Institute, one of the most prestigious in the world ; this Institute recommended against fluoridation, based largely on its toxicity, and Swedish water is not fluoridated. The French government consulted the Pasteur Institute which strongly recommended against fluoridation and France remains un-fluoridated. West Germany experimented with a few limited local fluoridation projects and then dropped the whole idea. Denmark adopted fluoridation a number of years ago, but then sharply reversed this and now strictly prohibits the addition of fluoride to public water supplies. In Belgium, fluoride tablets, fluoride drops and fluoride chewing gum are to be taken off the market because of the risks to physical and psychological wellbeing.

Queensland Child Dental Health Surveys of 2000 and 2001 (Figures Attached (1)) indicate some areas in non fluoridated Brisbane have slight better dental health than those of fluoridated Townsville and some areas slightly worse. Other studies including the Brunelle and Carlos study of 39,000 children in 1986-87 have shown very little difference (less than one surface of 128 total tooth surfaces) between fluoridated and non fluoridated subjects. This study was seen by the dental community as a justification for fluoridation even though the outcome showed comparatively little difference. In NSW, dental surveys have fared no better. In Western Australia (which is fluoridated), complaints against increasing dental decay has been in the news for some time. Topical fluoridation , rather than ingested fluoride , is the best avenue to use fluoride, according to the Center of Disease Control (1999, 2002).

The major causes of poor dental health are well established. Human tooth decay is linked to diet, sugar intake, tooth brushing technique, hours of sunlight, parental education, and family income, (See Attached (2) paper by Harris et al, 2004). Significantly, these have been neglected as confounding factors (eg Townsville Vs Brisbane study—where additional issues such as very large difference in populations , and movements of population ,were also ignored). These variables must be considered in the fight against tooth decay and in lowering of the financial burden associated with diminished oral and general health.

Arguments in Australia focus on the difference in fluoridated and non-fluoridated communities, but the results show negligible difference. Explicit in any research both in Australia and overseas are socioeconomic factors and it is well established that fluoride is ineffective at preventing the most common type of dental decay - pit & fissures, which accounts for upwards of 85% of all dental decay. Available public health funding would be better spent advising protocols for better dental health in classrooms.

Australian Dental and Medical Associations promote fluoridation as a harmless agent, putting their heads in the sand against a sea of knowledge on the adverse effects. I bring to you attention modern research indicating the harmful effects of fluoride when complexed with traces of aluminum (AlF) as in water fluoridation. The negative actions on G proteins affect numerous biological signalling systems that control our most important life functions. Struenecaka et al, 2002 (attached (3)) outline the effects to ecosystems as well as body systems and warn on the hidden dangers for health that has not been fully recognized. To ignore one or two negative studies may not be considered negligent, but to persistently ignore a plethora of studies from all over the world from scientists with no interest in politics or dentistry is dangerous science - and a dangerous precedent.

Of particular concern in all the evidence are young children. Dietary reference intakes for infants 0-6months recommend intakes not exceed 0.01ug/day, for babies 7-12 months the reference levels are 0.5g per day and for those one to three years the levels recommended are less than 0.7ug/day. (See attached (4), Dietary Reference Intakes: Elements, J Public Health, Fluorosis). These levels are unattainable in a fluoridated

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community and pose the risk of fluorosis and the risk to mental health. Also of concern are the risks of osteogenic sarcoma to young boys in fluoridated communities. The Northern Ireland community voted against fluoridation partly because of the evidence associated with higher levels of this type of cancer in the fluoridated Irish community as opposed to non fluoridated Ulster. Based on growing evidence the Environmental Working group in the USA have asked the National Toxicology Program (NTP) of the National Institutes of Health (NIH) to list fluoride in tap water in its authoritative Report on Carcinogens, based on its ability to cause this rare form of childhood bone cancer, osteosarcoma, in boys (see Attached (5)).

The evidence against fluoridation in recent years differs greatly from data proposed in previous years. Scientific analyses of water fluoridation studies and experiments fail to prove any caries prophylactic effects. Dentist and WHO experts predicted a very large increase ("a tide of caries") after termination of fluoridation in European countries; however, analysis of data reveal significant decrease in dental caries after suspension of water fluoridation in Japan, the Netherlands, Germany and the city of Prague.

There is no question that dental fluorosis is a clear risk for the children it is supposed to protect. There is a risk of bone cancer, negative thyroid effects and neurological effects. With exposure over a lifetime there is the risk bone fracture, arthritis and cardiovascular issues. Any risk is significant. That is why some of the world's oldest democracies and their top scientists refuse to use or promote water fluoridation.

Please feel free to contact me on any of the research mentioned above. I have purposely attached few papers in the hope that you read these with interest. Please call me at work 07 38628811 or mobile 0418 874 995; and e-mail: drjohnryan@hotmail.com should you require any further communication.

Yours Sincerely,

Dr John Ryan

Chairman, PAWF (Professionals Against Water Fluoridation)

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Risk factors for dental caries in young children: a systematic review of the literature

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Objective To conduct a systematic review of the literature on risk factors for dental caries in deciduous teeth of children aged six years and under, to give a scientific framework for the international collaborative studies on inequalities in childhood caries. ***Method*** Accepted guidelines were followed. Studies were identified by electronic searching and reviewed on the basis of key words, title and abstract by two reviewers to assess whether inclusion criteria were met. Copies of all articles were obtained and assessed for quality according to the study design. ***Results*** 1029 papers were identified from the electronic search, 260 met the *prima facie* inclusion criteria. 183 were excluded once full copies of these papers were obtained. Of the 77 studies included, 43 were cross sectional, 19 cohort studies, 8 case control studies and 7 interventional studies. Few obtained the highest quality scores. 106 risk factors were significantly related to the prevalence or incidence of caries. ***Conclusion*** There is a shortage of high quality studies using the optimum study design, i.e. a longitudinal study. The evidence suggests that children are most likely to develop caries if *Streptococcus Mutans* is acquired at an early age, although this may be partly compensated by other factors such as good oral hygiene and a non- cariogenic diet. Diet and oral hygiene may interact so that if there is a balance of 'good' habits by way of maintaining good plaque control and 'bad' habits by way of having a cariogenic diet, the development of caries may be controlled.

Key words: risk factors; dental caries; early childhood caries

Introduction

Dental caries is widely recognised as an infectious disease induced by diet. The main players in the aetiology of the disease are; a) cariogenic bacteria, b) fermentable carbohydrates, c) a susceptible tooth and host and d) time. However, in young children bacterial flora and host defence systems are in the process of being developed, tooth surfaces are newly erupted and may show hypoplastic defects, and their parents must negotiate the dietary transition through breast/bottle feeding, first solids and childhood tastes. Thus it is thought that there may be unique risk factors for caries in infants and young children (Seow, 1998).

It is disconcerting to see rampant caries in young children (Fass, 1962). The pattern of decay is typically that many teeth are affected, with caries developing rapidly, often soon after the teeth have erupted. Surfaces usually at low risk of developing caries are affected such as the buccal surfaces of maxillary incisors with the obvious consequence of affecting the child's facial appearance. It is this pattern of caries that has been labelled variously as 'baby bottle tooth decay', 'nursing caries' and 'night bottle mouth'. However, since these terms suggest that the prime cause of such caries is inappropriate bottle feeding and current evidence suggests that although use of a sugar-containing liquid in a bottle at night-time may be an important aetiological factor, it may not be the only or the most important factor, it is now recommended that the term 'early childhood caries' be used when describing any form of caries in infants and pre-school children (Reisine and Douglass, 1998).

The answer to the question 'What causes early childhood caries?' is an important, if a complex one. It concerns those in both developed (Holt *et al.*, 1996; Wendt *et al.*, 1996) and developing countries (Matee *et al.*, 1994; Ye *et al.*, 1999) where many children experience this pattern of disease. Understanding the aetiology of the disease has a direct influence on public policy. In the United Kingdom, the British Society of Paediatric Dentistry recommend a reduction in sugar intake by the whole child population in the country. The policy recommended by the equivalent professional society in America, on the other hand is that sugar restrictions can be relaxed in a society where fluoride is used frequently, particularly for children who have low or no caries (although this is not a universally held view amongst dentists in America), (British Society of Paediatric Dentistry, 1992; American Academy of Pediatric Dentistry, 1989). How aetiology is interpreted also influences the design of interventional programmes set up to prevent the disease.

Factors that may be implicated in giving rise to caries in young children have been described in a number of review papers (Federation Dentaire Internationale, 1988; Horowitz, 1998; Moss, 1996; Reisine and Douglass, 1998; Seow, 1998). However none of these have employed a systematic methodology. The body of evidence in this area is large, and without a systematic approach where strategies for identifying and selecting information are

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defined and data quality taken into account, reviews may be open to bias and be unreliable.

Therefore the primary objective of this systematic review is to identify risk factors for dental caries in the deciduous teeth of children aged six years and under, in order to help development of systematic approaches for preventive oral care programmes world-wide and in order to provide sound information for oral health promotion and public health care. A secondary objective is to describe the extent to which relationships between risk factors are explained by ethnic group and material deprivation.

Method

Context of the review

The review was undertaken as part of an international collaborative oral health research programme aiming to determine interventions which might reduce dental caries in children in disadvantaged communities and minimise the effects of exclusion from health care systems and disadvantage related to ethnic diversity. Whilst the primary objective of the review was to identify all risk factors for dental caries in the deciduous dentition, information on ethnicity and socio-economic descriptors was gathered for all studies included so that interactions between these and other risk factors could be taken into account. The review was conducted in general accordance with guidelines promulgated by The Cochrane Collaboration (1997).

Data sources

In view of the large body of literature the review is limited to studies identified by computer searching. Handsearching of journals and gathering of unpublished reports and conference proceedings was outside the scope of the review at this stage. The PubMed database was searched using the search strategy: dental caries AND child AND (risk OR disadvantage OR ethnic) AND (toothbrushing OR sugar {Text Word} OR diet, cariogenic OR dietary sucrose). The search included all literature published from 1966 onwards and was last updated in April 2002. The titles, authors and abstracts from all studies identified by the electronic search were printed and reviewed independently on the basis of keywords, title and abstract by two reviewers (CP, PA) to determine whether these met the inclusion criteria. In cases where there was uncertainty regarding the relevance of an article, a full copy of the article was obtained. A full copy of all relevant articles selected for the review was obtained prior to commencement of the analysis of the data.

Study selection

The outcome considered in the review is the presence and severity of caries in deciduous teeth. If the outcome was another measure, such as *Streptococcus Mutans* count, the study was not included. Where studies involved age ranges of samples extending beyond six years of age, data were extracted where caries in deciduous teeth was reported for children six years and under. If data could not be extracted the study was excluded. Papers in all languages were included.

Data extraction

Studies were divided into four types of study design: cross sectional, cohort, case-control and interventional studies. Table 1 describes the information extracted from each type of paper.

Data extraction was performed by the principal researcher (RH) using a previously prepared proforma.

Assessment of data quality

Since there were no generic quality checklists or scales relevant to this review, the following quality checklist was devised to delineate studies according to quality. Firstly a key question was used for each study: 'Was the sample size adequate and appropriate for the study design?' If the answer to this question was 'No' then no further quality score was applied. The decision not to include a study any further in the review on this basis was confirmed after discussion with a statistician. For the remaining studies, these were given a quality score of between 0 and 4 for cross sectional and case control studies and between 0 and 5 for cohort and interventional studies. The higher the score, the higher the quality of the study.

For cross sectional studies and case control studies the following four questions were asked:

1. Does any pre-selection of the study sample still allow the general applicability of the study findings i.e. is selection bias acceptable? Yes/No
2. Detection Bias, Caries: if more than one examiner was used, were examiners calibrated? Yes/No
3. Detection Bias, Psycho-social: for data gathered on dietary habits and other dental health related behaviour, were the measures validated? Yes/No
4. Were appropriate statistical methods used in the analysis? Yes/No

Studies were given a score of 1 for each of these quality ratings if the answer was 'Yes, giving a maximum quality score of 4. For cohort studies an additional quality dimension was included which was appropriate to longitudinal studies:

5. Was attrition bias acceptable? Yes/No

For cohort studies the maximum quality scoring was therefore 5.

For most interventional studies psycho-social measures were not used and selection bias was less relevant and therefore quality aspects scored of these types of studies were: detection bias (caries) (1), statistical analysis (2), and attrition bias (3), as outlined above, and in addition whether

4. Were subjects allocated to test and control groups randomly? Yes/No
5. Was blinding maximal depending on the intervention type? Yes/No

The maximum quality score for interventional studies was therefore 5.

The evaluation of quality items is affected by poor reporting of primary studies. If the authors did not report certain items, for example the number of examiners used, the study was rated as if that source of bias existed. To validate the scoring system a sample of 16 papers (four papers from each of the four study designs, covering a range of quality scores) were rated independently using

Table 1. Data extracted from each study by type of study design.

Cross sectional	*Cohort*	*Case-control*	*Interventional*
Ethnic group	Ethnic group	Ethnic group	Ethnic group
Social class	Social class	Social class	Social class
Age of subjects	Age at start	Age at start	Age at start
How sample was obtained	Age at follow up	Groups to be compared	Length of study
Sample size	How cases were obtained	Age at follow up	How cases were obtained
Selection bias	Sample size	How cases were obtained	Sample size
Detection bias (assessment of caries)	Selection bias	Sample size	Allocation to test and control groups
Detection bias (psychosocial measures)	Detection bias (assessment of caries)	Selection bias	Blinding
Statistical analysis	Detection bias (psychosocial measures)	Detection bias (assessment of caries)	Detection bias (assessment of caries)
Significant risk factors	Attrition bias(drop outs)	Detection bias (psychosocial measures)	Attrition bias
Risk factors tested but not found to be significant	Statistical analysis	Statistical analysis	Statistical analysis
	Significant risk factors	Significant risk factors	Outcome
	Risk factors tested but not found to be significant	Risk factors tested but not found to be significant	

this checklist by a second person. Differences in quality scoring between the two scorers were analysed using a weighted Kappa.

Data synthesis

Heterogeneity among the studies, especially with respect to the varying quality and presentation of results, precluded use of statistical methods of pooling data such as meta-analysis.

Interpretation and discussion of results

A systematic review, with strict inclusion and quality assessment criteria as outlined above, aims to provide an unbiased overview of the literature, and therefore discussion related to individual studies is usually limited, because there may be bias from any interconnections made between various studies. Also, were further studies to be added to the body of literature over time, interpretation may change. However, since the main purpose of this review was to give a scientific framework for the international collaborative studies of childhood caries it was necessary to draw some conclusions from the review and therefore include some discussion of the results in the review. This paper includes a narrative review of the studies meeting the inclusion criteria, although it must be highlighted that interconnections made are subject to bias due to the authors' views. To clarify where these two approaches have been taken, the results section is divided into two parts: the systematic review and the narrative review. The narrative review contains references to all studies meeting the inclusion criteria regardless of the quality score given, and this must be taken into account when considering the weight of scientific evidence in this part of the review.

Results: systematic review

The electronic search identified 1029 papers. After review of the study title, keywords and abstracts, 260 papers were identified potentially meeting inclusion criteria. For the rest it was evident that they did not meet the inclusion criteria because they were reviews, subjects were too old or the outcome of the study was not caries. Once full copies of the papers were obtained, a further 71 studies were excluded because they were reviews, 58 were excluded because the outcome of the study was not caries, and 54 were excluded because they involved an older age range and the data could not be extracted for deciduous teeth. Of the 77 studies included in the review, 43 were cross sectional studies, 19 were cohort studies, 8 were case control studies and 7 were interventional studies. Two cross sectional and two case control studies were excluded from further consideration because the sample size was rated as insufficient.

Out of the 41 cross sectional studies included, 11 were based on low income groups, in 13 information in the paper indicated that the sample contained a range of socio-economic groups and in 17 there was no information on the socio-economic background of the subjects. In only seven out of the 19 reports of cohort was socio-economic background described. Three cohort studies were undertaken on low socio-economic groups and four on samples containing a range of socio-economic backgrounds. Only one case control study noted socio-economic background of the sample and none of the interventional studies. Thus of the 73 studies considered, in only 44 per cent (32) could socio-economic background be determined.

The ethnicity of the subjects could be determined in all 73 studies. Nineteen cross sectional and 15 cohort studies included children from ethnic groups differing from the majority ethnic group for the country in which the study was based. However, there were no children from ethnic minority groups in the respective countries studied in case control and interventional studies.

When comparing the quality scores for the sample of 16 papers scored to validate the scoring system, the agreement between the two independent scorers was

Table 2. Quality scores for papers meeting the inclusion criteria

Paper reference number, author, year of publication	*Selection bias*	*Detection bias (caries)*	*Detection bias (psycho-social)*	*Attrition bias*	*Statistical analysis*	*Blinding*	*Random allocation*	*Total quality score*
1. Aaltonen (1991)	√	X	X	X	X			1
2. Aaltonen & Tenovuo (1994)	√	X	√	√	X			3
3. al-Ghanim *et al.* (1998)	√	√	X		√			3
4. Angelillo *et al.* (1998)	√	√	X		X			2
5. Barnes *et al.* (1992)	√	√	√		X			3
6. Cleaton-Jones *et al.* (1984)	√	√	√		√			4
7. Creedon & O'Mullane (2001)	√	√	X		√			3
8. Dasanayake *et al.* (1995)	√	X	√		√			3
9. Douglass *et al.* (2001)	X	X	X		X			0
10. Ekman (1990)	X	X	X	X	√			1
11. Freeman *et al.* (1997)	√	√	X		√			3
12. Freeman *et al.* (1989)	√	X	X		X			1
13. Gibson & Williams (1999)	√	√	√		√			4
14. Grindefjord *et al.* (1995)	√	√	X	√	√			4
15. Grindefjord *et al.* (1996)	√	√	X	√	√			4
16. Grytten *et al.* (1988)	√		X	√	√			3
17. Hallonsten *et al.* (1995)	√	X	X		√			2
18. Harrison *et al.* (1997)	X	√	X		√			2
19. Holst *et al.* (1997)	X	√	X	√	√			3
20. Holt & Downer (1996)	√	√	X		√			3
21. Hu *et al.* (1998)		√		√	√	X	X	3
22. Isokangas *et al.* (2000)		X		√	√	X	√	3
23. Kalsbeek & Verrips (1994)	√	√	X		√			3
24. Karjalainen *et al.* (1997)		√		√	√	X	√	4
25. Karjalainen *et al.* (2001)	X	√	√	√	√			4
26. Kawabata *et al.* (1997)	√	X	√	X	√			3
27. Kendrick *et al.* (1998)	X	√	√		√			3
28. Kerusho *et al.* (1991)	√	√	X		√			3
29. Khan & Cleaton-Jones (1998)	X	√	X		√			2
30. Kowash *et al.* (2000)		√		√	√	X	√	4
31. Leverett *et al.* (1997)		X		√	√	√	√	4
32. Li *et al.* (1996)	√	√	X		√			3
33. Li *et al.* (2000)	√	X	√		√			3
34. Lin & Tsai (1999)	X	√	X		X			1
35. Lopez *et al.* (1998)	√	√	X		√			3
36. Lopez *et al.* (1999)		X		X	√	√	√	3
37. Maciel *et al.* (2001)	√	√	√		√			4
38. Al-Malik *et al.* (2001)	√	√	X		√			3
39. Marques & Messer (1992)	√	√	X		√			3
40. Matee *et al.* (1994)	X	√	√		√			3
41. Mattila *et al.* (1998)	√	√	X	X	√			3
42. Mattila *et al.* (2000)	√	√	X	X	√			3
43. Milen (1987)	√	X	X		√			2
44. Milgrom *et al.* (2000)	√	√	√		√			4
45. Moss (1999)	√	X	√		√			3
46. Muller (1996)	X	X	X		√			1
47. Ollila *et al.* (1998)	X	X	X	√	√			2
48. Paunio *et al.* (1993)	√	√	X	X	√			3
49. Petersen (1992)	√	X	X		√			2
50. Petersen & Esheng (1998)	√	X	X		√			2
51. Quinonez *et al.* (2001)	X	√	√		√			3
52. Ramos-Gomez *et al.* (1999)	X	√	X		√			2
53. Reisine *et al.* (1994)	X	√	X	X	√			2
54. Rodrigues & Sheiham (2000)	√	√	√	√	√			5
55. Rodriguez-Contreras *et al.* (1989)	√	X	X		√			2
56. Schroder *et al.* (1994)	√	X	X	√	√			3
57. Schwarz *et al.* (1998)		√		√	√	X	X	3
58. Seow *et al.* (1999)	√	√	X		X			2
59. Thibodeau & O'Sullivan (1999)	√	√	√	X	√			4
60. Toi *et al.* (1999)	X	√	√		√			3
61. Tsai *et al.* (2001)	X	√	X		√			2
62. Tsubouchi *et al.* (1994)	√	X	X		√			2
63. Venugopal *et al.* (1998)	√	√	X		X			2

cont'd

Paper reference number, author, year of publication	*Selection bias*	*Detection bias (caries)*	*Detection bias (psycho-social)*	*Attrition bias*	*Statistical analysis*	*Blinding*	*Random allocation*	*Total quality score*
64. Verrips *et al.* (1992)	√	√	X		√			3
65. Vidal & Schroder (1989)	X	X	X		√			1
66. Wendt *et al.* (1996)	√	√	X	√	√			4
67. Wendt *et al.* (1999)	√	√	√	√	X			4
68. Wetzel (1988)	√	X	X		X			1
69. Wetzel *et al.* (1989)	√	X	X		X			1
70. Williams & Hargreaves (1990)	√	√	X		√			3
71. Williams *et al.* (2000)	√	√	√		√			4
72. Ye *et al.* (1999)	√	X	X		√			2
73. Zoitopoulus *et al.* (1997)	√	√	√		√			4

Table 3. Quality scores by type of study.

Type of study	*Cross sectional*	*Cohort*	*Case control*	*Interventional*	*All studies*
No. papers with score 0	1	0	0	0	1
No. papers with score 1	6	2	0	0	8
No. papers with score 2	11	2	3	0	16
No. papers with score 3	17	8	3	4	32
No. papers with score 4	6	6	0	3	15
No. papers with score 5	–	1	–	0	1
Total no. papers	41	19	6	7	73

excellent, with a weighted Kappa of 0.84. Table 2 shows the quality scores for all 73 studies included in the review and Table 3 shows the quality scoring by study design. Only six cross sectional (Gibson and Williams, 1999; Maciel *et al.*, 2001; Milgrom *et al.*, 2000; Cleaton-Jones *et al.*, 1984; Zoitopoulos *et al.*, 1996; Williams *et al.*, 2000), and one cohort study (Rodrigues and Sheiham, 2000) reached a maximum quality score of 4 and 5 respectively. No case control or interventional studies reached the highest quality score of 4 and 5 respectively.

In order to weigh the evidence by the quality of the study from which the finding was generated, particularly taking into account study design, studies of particularly high quality and strong study design will be highlighted. Since cross sectional studies were considered the weakest study design in the hierarchy of scientific evidence only the six studies with the highest quality scores are discussed below. Cohort studies with quality scores of 4 and 5 are discussed and all the case control and interventional studies because of their stronger study design.

Cross sectional studies

The six cross sectional studies reaching the maximum quality score are described as follows. The study by Gibson and Williams (1999) used four-day weighed dietary records from a large sample of British pre-school-children to look for associations between caries and consumption of biscuits, cakes, sugar confectionery, chocolate confectionery, soft drinks and the percentage of energy from Non-Milk Extrinsic sugars. Sugar confectionery was the only variable found to be significant and even this was relatively less important than social class and toothbrushing. The strength of the association between social class and caries experience was twice that of the association between toothbrushing and caries and nearly three times that between sugar confectionery and caries.

Maciel *et al.* (2001) used a modified version of the Sweet Preference Inventory to test mother and child sweet preferences. This proved to be only very weakly associated with the child's caries experience. The authors suggested that since they had studied a low socio-economic group where sugar consumption was universally high, this consequently failed to explain the distribution of disease in the group. Gibson and Williams (1999) too suggest that the apparent weak relationship between sugar and caries in developed countries is because of the widespread use of sugar with other factors becoming more important discriminators of caries experience.

Although Milgrom *et al.* (2000) used a questionnaire to assess the child's dietary history, both reliability and validity of the questionnaire were tested. High Kappa statistics for specific food-related sections of the questionnaire were achieved. A high cariogenicity score (food cariogenicity combined with the frequency of consumption) was found to be a significant risk indicator. Cleaton-Jones *et al.* (1984) recorded sucrose intake by reference to food tables and achieved a high level of reproducibility in sucrose intake and frequency. They also collected data on dental plaque levels and found an interaction between plaque levels, sucrose consumption and ethnic group. This South African study found that in rural Black children where sucrose intake was low and the Debris Index high, there were relatively few caries free children compared to Urban White children who had a higher sucrose intake but lower Debris Index. One explanation might be that the amount of plaque present has a role to play in explaining why some children with apparently non-cariogenic diets develop dental caries whilst some children with cariogenic diets do not. On the other hand, high debris scores could also be evidence of a lower use of fluoride.

Zoitopoulos *et al.* (1996) in a cross sectional study

Table 4. Cohort studies with quality score 4 and 5

Reference	*Quality score*	*Age at start*	*Age at follow up*	*Setting*	*Significant risk factors*
Rodrigues & Sheiham (2000)	5	3 yrs old	4 yrs old	650 children in 29 nurseries in Brazil	Children living with >3 people Enamel hypoplasia Family income Use of fluoride gel Toothbrushing <1x/day Daily frequency and weight of sugar intake at nursery
Grindefjord *et al.* (1995) *significant variables in multivariate analysis	4	1 yr olds	3.5 yrs old	1,095 1 yr olds living in 8 suburbs of Stockholm	Immigrant mother's education Candy ≥ 1x/week Consumption sugary drinks ≥ 2x/day *Strep. Mutans* present at 1yrs of age Night time meals or drinks *Father unemployed *Toothbrushing <1x/day
Grindefjord *et al.* (1996)	4	1 yr olds	3.5 yr olds	1,095 1 yr olds living in 8 suburbs of Stockholm	For 1–2.5 yr olds: *Strep. Mutans* present Immigrant For 2.5–3.5 yr olds in addition: Mother's education Candy ≥ 1x/week Consumption sugary drinks ≥ 2x/day
Wendt *et al.* (1996)	4	1 yr olds	3 yrs old	632 1 yr olds living in area of 4 child welfare centres	Visible plaque Sugary liquid when thirsty at 1yr Sugary liquid during night at 2 yrs Soft drinks>2x/week at 2 yrs
Wendt *et al.* (1999)	4	3 yrs olds	6 yrs old	As Wendt *et al.* (1996), 575 children followed up for further 3 yrs	Immigrant status
Karjalainen *et al.* (2001)	4	3 yrs	6 yrs	Every 5th child in study of athero-sclerosis	Daily sucrose intake at 3 and 6 yrs Combination of visible plaque and sweet intake >1x/week
Thibodeau & O'Sullivan (1999)	4	3 yrs olds	9 yrs olds	240 children enrolled in Head Start Programme	High Strep. Mutans count at 3, 6, and 9 yrs of age

based in London also reported ethnic differences. Afro-Caribbean pre-schoolchildren were found to have less dental caries than their Caucasian counterparts, and *Mutans Streptococci* and *Lactobacilli* were also recovered less frequently from the Afro-Caribbean children than from the Caucasians. However, in both groups there were significant correlations between caries experience and *Mutans Streptococci* and ethnic differences were not seen in children where both *Mutans Streptococci* and *Lactobacilli* were isolated.

The work by Williams *et al.* (2000) was based on the same large database of British pre-schoolchildren as was used by Gibson and Williams (1999), except their study focussed on parental smoking as a possible risk factor for dental caries. The authors postulated that passive smoking might influence the child's growth and hence their nutritional status. Whilst maternal smoking was found to be significantly related to dental caries, even after controlling for social class, and there was found to be a relationship between maternal smoking and the child's nutritional status, no significant relationship was found when measurement of nutritional status was compared with maternal smoking directly.

Cohort studies

Table 4 describes the seven cohort studies with quality scores of 4 or 5. A combination of demographic, sugar consumption, oral hygiene factors and *Mutans Streptococci* count feature in each as significant risk factors for childhood caries. Five of these seven studies are Scandinavian studies and therefore based in a system supported by an effective universal dental access plan, which makes generalisation difficult. There are also relatively few conducted on subjects under three years of age. However, it is noticeable that factors relating to bottle and breast-feeding do not feature even though all three studies involving 1–3 year olds did use aspects of infant feeding as a variable in the analysis (Grindefjord *et al.*, 1995; Grindefjord *et al.*, 1996; Wendt *et al.*, 1996). In the study by Rodrigues and Sheiham (2000) 23% of the 3–4 year olds were still being bottle-fed during the study period. The authors suggest that the absence of an effect of current bottle-feeding on caries increment may be because by three years of age other dietary habits are more important in determining dental caries development than bottle-feeding.

Again the balance between sugar consumption and

Table 5. Case control studies

Reference	*Quality score*	*Age of subjects*	*Comparison of groups*	*Significant risk factors*
Hallonsten *et al.* (1995)	2	17–31 mths	49 with caries + not breast fed 11 with caries + breast fed 39 caries free + breast fed 101 caries free + not breast fed	Irrespective if breast fed: Higher number of cariogenic intakes per day % children with *Strep. Mutans* % children with *Lactobacilli*
al Ghanim *et al.* (1998)	3	3–5 yrs	231 children caries free 215 children with dmft ≥ 8	Debris Index Age at first dental visit Use of sweetened milk in a bottle Frequency of soft drinks Frequency of drinks taken
Ye *et al.* (1999)	2	2–5 yrs	400 children with ≥ 2 maxillary incisors with caries compared to 400 without rampant caries	Breast feeding at 6–12 mths Eating sweet food and candy Lack of a mother's care for a long period Drinking sweet liquids Bottle feeding with sweetened milk Eating sweet foods before sleeping High frequency of snacks in the day Duration of breast feeding
Matee *et al.* (1994)	3	1–4 yrs	116 children with ≥ 2 maxillary incisors with caries compared to 243 without rampant caries	Nocturnal breast feeding Linear hypoplasia
Tsai *et al.* (2001)	2	24–48 mths	41 children with ≥ 3 carious lesions compared to 49 caries free	Not having teeth cleaned at bed-time No regular paediatric check-ups Mothers with bad teeth Mother with non full-time jobs
Kendrick *et al.* (1998)	3	13–41 mths	67 children with cavitation on ≥ 2 incisors compared with 25 caries free	None (Toddler Temperament scale tested)

oral hygiene appears important. Wendt *et al.* (1996) found that if a risk behaviour such as giving a child a sugary liquid when thirsty was established at one year of age, the chance of his or her remaining caries free until three years of age is highest if good oral hygiene habits exist and no visible plaque is present at two years of age. Karjalainen *et al.* (2001) also found the combination of unsuitable dietary habits and poor oral hygiene to be important, for whilst sweet intake of more than once a week and the presence of visible plaque did not increase caries by themselves, the two combined gave a 1.7 fold caries risk as compared to children with neither habit.

Case control studies

Table 5 describes the six case control studies included in the review.

Breast and bottle feeding variables do feature in three of the studies (al Ghanim *et al.*, 1998; Ye *et al.*, 1999; Matee *et al.*, 1994), where children with a high level of caries (defined as caries affecting at least two maxillary incisors) are compared with caries free controls. However, other variables are also found to be significant, such as the Debris Index, linear hypoplasia and frequency of snacks and soft drinks taken. Two of the studies with the highest quality scores (al Ghanim *et al.*, 1998; Matee *et al.*, 1994) studied different groups of children. al Ghanim *et al.* (1998) studied Saudi Arabian children and produced a model with a sensitivity of 90% and specificity of 81% with the following risk factors: Debris Index, age of first dental visit, use of sweetened milk in a bottle, and frequency of soft drinks and drinks taken. The study of Tanzanian children by Matee *et al.* (1994) found that nocturnal breast-feeding and linear hypoplasia were significant factors for rampant caries. The differences between the findings in Tanzania and Saudi Arabia probably reflect the age of the subjects, background fluoride availability, cultural differences in infant feeding patterns, the prevalence of linear enamel hypoplasia in the population, and the fact that different variables were tested in the two studies (for example: Matee *et al.* (1994) did not collect data on the Debris Index).

Interventional studies

Table 6 shows the seven interventional studies included in the review. Five of the interventions resulted in the test group of children having less caries than the controls. In keeping with the fact that there are a number of different risk factors for early childhood dental caries, a number of different types of interventions were successful. The effect of using xylitol gum (Isokangas *et al.*, 2000) was attributed to the prevention of transmission of *Streptococcus Mutans* from mother to child. The study by Kowash *et al.* (2000) indicates that interventions involving individually tailored dental health education advice are successful irrespective of whether the emphasis in the advice session has been on reducing sugar or improving plaque control. Schwarz *et al.* (1998) showed an effect above the improvements that might be achieved through dental health education, by introducing daily toothbrushing in kindergartens. Some may suppose that the improvement related to toothbrushing is on account of the use of fluoride toothpaste rather than the removal of plaque, but the significant effect of visible plaque in the final regression model indicates that

Table 6. Interventional studies

Study	*Quality score*	*Age of subjects*	*Intervention*	*Outcome*
Hu *et al.* (1998)	3	2/3yrs at start, 6yrs at follow up	176 children in 2 test kindergartens given fluoride drops every morning in term time. 148 children in control kindergarten had none	Caries increment over 3 yrs was 1.8 mean dmft for test group and 3.9 for control
Isokangas *et al.* (2000)	3	3 mths at start to 5 yrs at follow up	120 mothers given xylitol gum from 3 mths after birth to 24 mths, 36 given fluoride varnish at 6, 12 and 18 mths after birth, and 32 given chlorhexidine varnish	Children with mothers in xylitol gum group had significantly less caries, than those in fluoride and chlorhexidine varnish groups
Leverett *et al.* (1997)	4	Second trimester of pregnancy to 3 yrs	Test group given 1 mg fluoride tablet daily from second trimester. Control group had placebo tablet	No significant difference in proportion caries free children between test and control group
Kowash *et al.* (2000)	4	8 mths at start to 3 yrs	Mother and child pairs assigned to 5 groups: Gp1: Dental health education (DHE) focussed on diet, Gp2. DHE focussed on oral hygiene, Gp3. DHE focused on diet + oral hygiene (Gps 1–3 had DHE every 3 mths for 2 yrs and twice in 3rd year), Gp4. DHE focussed on diet and oral hygiene once for each of 3 yrs, Gp5 had no DHE.	4% of children in groups 1–4 developed caries by 3 yrs whereas 33% of control children (Gp5) developed caries. No difference between emphasis of DHE or regularity of DHE sessions
Karjalainen *et al.* (1997)	4	7 mths to 3 yrs	Mothers of 78 babies had tailored instructions to reduce fat intake at 1.3, and 6 mth intervals, control gp given written information on a healthy diet	Test children had diets with more carbohydrate and less fat but there was no difference between the proportion caries free children
Lopez *et al.* (1999)	3	12–19 months	Randomised controlled trial of 31 babies attending a Welfare Clinic Puerto-Rico	Fewer from test group with topically applied povidine iodine developed caries
Schwarz *et al.* (1998)	3	3–4 yr olds	168 children in test kindergarten had DHE + daily toothbrushing with 1000ppm toothpaste after lunch whereas 121 children in control kindergartens had no toothbrushing, just DHE	At baseline 25% caries free in test and control. After 3yrs this reduced to 20% in test group and 12% in control

at least in a population with limited oral hygiene, the mechanical cleaning may add to the effect of fluoride.

Narrative review

A total of 106 factors were found to be significantly related to the prevalence or incidence of caries in the 73 studies included (Table 7). These could be grouped into 20 demographic factors, 29 dietary factors, 15 factors related to breast and/or bottle feeding, 9 factors related to oral hygiene habits, 4 related to oral bacteria flora and 29 related to other factors such as parental oral health and enamel hypoplasia. The reference number alongside the author and year of publication of the study is tabulated in Table 2.

Drawing from all 73 of these studies, there are some important issues that emerge across the different types of studies and these are addressed in the following sections.

The importance of oral hygiene

Studies have collected information on oral hygiene habits either by means of reported behaviour or more directly by using a plaque or debris index. Table 7 shows the range of variables that have been found to be a significant risk indicator or risk factor for dental caries in this respect. There is evidence in more studies that toothbrushing once a day or more as opposed to less than once daily and the presence of visible plaque is important, than for other factors such as the frequency of toothbrushing comparing brushing three, twice and once daily, age at which toothbrushing was started, parental supervision of toothbrushing , not having teeth brushed at bed-time and the use of a fluoride as opposed to a non-fluoridated toothpaste.

Gibson and Williams (1999), comparing children in Britain from families where the head of the household is employed in manual (Manual) as opposed to non-manual

Table 7. Factors found to be significantly related to the prevalence and/or incidence of deciduous caries in children age 6 years and under.

Socio-demographic factors	*Dietary factors*	*Oral hygiene*	*Factors related to breast/bottle feeding*	*Oral bacterial flora*	*Other factors*
Gender of child (male)[64,37]	High frequency, high sugar foods/ day[72,16,1,6,12,41,7]	Daily tooth-brushing[13,43,49,64,19, 14,1,48]	Bottle as opposed to breast fed[63]	Presence of *Strep. Mutans*[14,15, 17,33,2,73,8,65]	Few hours child sleeps[35]
Public rather than private school[38]	High number of between meals sugary food/drink[28, 55,65]	Frequency tooth-brushing: more often[53,9] &less often[54]	Duration of breast feeding[32,33,3,72,40,26,18, 68]	Presence of *Lactobacilli*[65,17,2,73]	Mother irregular toothbrusher[42]
Family income[43,54, 32,49]	No set time for snacks[62]	Age brushing started[3,72,7,38,64]	Nocturnal breast feeding[40]	*Strep. Mutans* count[53,58,44,59,60,73]	Mother does not floss teeth[42]
Asian/Non-Asian[20,	Cariostat score[62]	Visible Plaque[48,6,58, 3,19,66,41]	Night-time bottle use[9,12,7,34]	Rare transfer of maternal saliva to baby[1,2]	Mother missing teeth[16]
Father unemployed[14]	High pocket money for sweets[49]	Combined frequency brushing and parental supervision[23]	Use of sugar/cereal in the bottle[3,72,66,1,70]		Mean DMFS mother[37]
Low parental education[64,49,29]	High age of weaning[12]	Adults involved in brushing[12,9]	Frequency of breast feeding[72]		Father seldom uses toothpaste[42]
Low maternal education[41,12,72,14,15, 16,23,38]	Not eating fruit as a snack[12]	Lack of use of fluoride toothpaste [1,35]	Bottle with sugary drink at bed-time[65]		High mean DT mother[2]
Single mother[37]	High sugar/fat snacks[12]	Not having teeth cleaned at bed-time[61]	Bottle/breast fed to stop baby crying at night[35]		Level of water fluoride at home[7,55]
Occupation of head of household[13,28,46]	5x daily sweet snacks[23]	High gingival score[65,56]	Duration bottle feeding with fruit juice[2]		Father's high caries increment[42]
High number children per family[46]	Candy ≥1x/week[14]		Breast fed or plain milk in bottle at night[3,58]		Lack of care by mother for long period[72]
3+ adults in household[35]			Still bottle/breast fed at 18mths[26,62]		Age at first dental check[3]
Rural[42,5] or Urban[50] domicile	Low Magnesium Intake[39]		Bottle at night ≥24mths[47]		Parents wear full dentures[10]
Mother with non full-time jobs[61]	High iron intake[39]		Duration breast or bottle feeding[51]		Mother's dental attendance[12,16]
Birth order[12]	High cariogenicity score[56,44,17,52]		If child slept with bottle or breast at 12 months[52]		Occurrence of headache in the child[42]
Immigrant background[14,15,67,23]	High daily frequency of sugar intake at nursery[54]		Bottle carried around during the day[18]		Medication with saliva inhibiting drug[19]
Mother's young age[42]	High daily weight of sugar intake at nursery[54]				Illness for 1 week >4x/year[19]
2+ children living in household[35,64]	>6 eatings/ drinkings per day[19]				Lack of paediatric check ups[61]
Cohabitation of parents[42]	Food before sleeping[72]				Enamel hypoplasia[40, 54,32,44,58]
Ethnicity[58,5,73,51]	Fruit juice at bed-time[48,38]				Previous experience of dentistry[39]
Parental occupation[4]	Sugary bed-time drink[12] Drinks carbonated, drinks at bed-time[38]				Blood lead[45] Irregularity of dental attendance[20]

cont'd

Socio-demographic factors	*Dietary factors*	*Oral hygiene*	*Factors related to breast/bottle feeding*	*Oral bacterial flora*	*Other factors*
	Daily sucrose intake[25]				Sub-optimal use of fluoride[43,39,19,2,54]
	Night-time meals/drinks[14]				Duration child watches TV[42,26]
	Night-time juice[48]				Use of sweetened comforter[20,38]
	Frequency of consumption of diluted syrup[38]				Sweetened medicine at bedtime[46]
	Milk intake score[12]				Maternal smoking[71]
	Dates eaten daily[38]				Child's shyness[51]
	Frequency consumption of sugary drinks[3,72,14,26,15,66]				Low height for age[32]
	Frequent consumption of carbonated drinks[12,38]				Pacifier sucking ≥24 months[47]
	Amount and frequency of sweet consumption[3,2,41,13,49]				

work (Non-Manual), found that the impact of toothbrushing frequency did not reach significance among children from the Manual group. A relationship was however evident among children from Non-Manual groups. Children from families with a Non-Manual head of household were more likely to have parental help in toothbrushing and the authors suggest that toothbrushing undertaken in these families was a more effective means of plaque removal and this was more important a factor than how many times a day toothbrushing was carried out. Other explanations are possible, such that for Manual groups deleterious dietary patterns are such that oral hygiene becomes secondary. It is interesting to note that 96% of children in the study used a fluoride toothpaste, and manual children were less likely to use a low fluoride variety – which lends support to the apparent importance of plaque removal in toothbrushing in addition to the fluoride effect of the toothpaste.

The importance of dietary factors

The importance of dietary factors is evident from the long list of this type of risk factors that have been found to be significantly related to childhood caries (Table 7). Some factors such as a low Magnesium intake, high iron intake (Marques and Messer, 1992) and low milk intake (Freeman *et al.*, 1989) have been considered and found to be significant in only a few studies, but in the main, most dietary factors found to be significant are related to the consumption of sugar – either its amount, frequency or timing of consumption.

There is a problem in comparing studies to reach a consensus view on which of these indicators is the most reliable predictor of childhood caries since most studies rely on parental recall of dietary habits in either questionnaires or interviews, and very few studies have used standardised or validated questions. The study by Ekman (1990) shows how unreliable some of this reported data may be. In this longitudinal study of Finnish children in Sweden the frequency of consumption of sugar-containing products was one of the variables tested but found not to be significantly correlated with caries. However whilst 44% of children stated that they had three or more snacks between meals per day, only 16% of parents gave this answer.

The study by Gibson and Williams (1999) is a particularly good example of the effect of assessing sugar consumption using four day weighed dietary records as opposed to reliance on interview data about the frequency of consuming various foods. The authors used the same data from the British National Diet and Nutrition Survey as had been reported by Hinds and Gregory (1995), but used weighed dietary record data instead of the questionnaire data on sugar consumption which is prone to recall bias, and came to a different conclusion. Hinds and Gregory (1995) had reported that 'overall, the benefits of frequent brushing of teeth did not outweigh the damaging effect of frequent sugar consumption', whereas Gibson and Williams (1999) concluded 'for children who brushed their teeth twice a day or more, consumption of sugars and sugary foods did not appear to be associated with caries'.

The importance of oral bacterial flora

Streptococcus Mutans is viewed as the principal bacterial species initiating dental caries. Although *Streptococcus Mutans* is not usually detectable in infants' mouths before tooth eruption, several studies utilising a range of study designs (cross sectional, cohort and case control studies) have shown that the age at which these bacteria are acquired by the child is a significant indicator of caries risk (Table 7). The presence of *Lactobacillus* at a young age has also been found to be significant,

although in all these studies *Streptococcus Mutans* was also a significant risk factor. High levels of *Streptococcus Mutans* in plaque and saliva have also been associated with an increased caries experience (Table 7). Failure to correlate *Lactobacilli* count with caries experience may indicate that *Lactobacilli* acting alone may not initiate caries, but that it may initiate caries, together with *Streptococcus Mutans*, possibly by increasing the acid production in plaque (Toi *et al.*, 1999). The success of a randomised controlled trial using topical application of an iodine agent to dental surfaces of children at risk for early childhood caries also points to the importance of *Mutans Streptococci*, for by suppressing dental levels of *Streptococci Mutans* by using an antimicrobial, caries was reduced (Lopez *et al.*, 1999).

Since the earlier the infection of mouth with *Streptococcus Mutans*, the greater is the caries risk of the deciduous dentition, and that since salivary transfer is required to spread the infection, nurturing habits such as cleaning a pacifier by putting in the mother's mouth before it is given to the child, kissing the child directly on the mouth, and pre-tasting food before it is given to the child have been studied (Aaltonen, 1991; Aaltonen and Tenovuo, 1994). However these studies have shown that a frequent transfer of saliva to the mouth of the baby from the mother is actually protective. Children with a high frequency of maternal salivary contact before tooth eruption had lower numbers of *Streptococcus Mutans* and less dental caries than those with rare contact, possibly because the infant's exposure to cariogenic bacteria prior to tooth eruption might have increased the child's immunological resistance to the infection.

A link between *Streptococcus Mutans* and dental caries has been found in studies involving a wide range of ethnic groups: Finnish (Aaltonen, 1991; Aaltonen and Tenovuo, 1994), Australian aborigines (Seow *et al.*, 1999), African-American and Hispanics (Reisine *et al.*, 1994; Thibodeau and O'Sullivan, 1999; Dasanayake *et al.*, 1995), Latin Americans in Sweden (Vidal and Schroder, 1989), Swedish (Grindefjord *et al.*, 1995; Grindefjord *et al.*, 1996), Black and Coloureds in South Africa (Toi *et al.*, 1999), Afro-Caribbean and White Caucasians in Britain (Zoitopoulos *et al.*, 1996), and Chinese (Li *et al.*, 2000). Some studies have shown that ethnic differences exist in the proportion of children from whom *Mutans Streptococci* can be isolated (Toi *et al.*, 1999; Zoitopoulos *et al.*, 1996) even after controlling for age and caries experience (Zoitopoulos *et al.*, 1996). However, if *Mutans Streptococci* are present, whatever the ethnic group, this does seem to be a strong indicator of caries risk. Ethnic differences though in the acquisition of cariogenic bacteria may explain some ethnic differences in the prevalence of dental caries.

Although *Mutans Streptococci* are considered the aetiological agent of dental caries in children, bacterial infection is a *necessary* but not *sufficient* factor for developing clinical disease. *Mutans Streptococci* and *Lactobacilli* are also found to be present in children who are caries free (Toi *et al.*, 1999).

Ethnicity

Despite the comprehensive reporting of ethnicity of subjects, relatively few studies (Holt *et al.*, 1996; Milgrom *et al.*, 2000; Verrips *et al.*, 1992; Grindefjord *et al.*, 1995; Grindefjord *et al.*, 1996; Quinonez *et al.*, 2001; Zoitopoulos *et al.*, 1996) used ethnic background as a variable in their analyses. Verrips *et al.* (1992) studying different ethnic groups in Amsterdam found that whilst the level of education of the parents, level of fluency in Dutch, gender of the child and ethnicity were all significant risk indicators for childhood caries, the level of education was the most important.

Enamel Hypoplasia

Relatively few studies included enamel hypoplasia as a potential risk factor in the study (Milgrom *et al.*, 2000; Seow *et al.*, 1999; Rodrigues and Sheiham, 2000; Matee *et al.*, 1994; Li *et al.*, 1996). Milgrom *et al.* (2000), Seow *et al.* (1999), Matee *et al.* (1994) and Li *et al.* (1996) all found that if enamel hypoplasia was present the odds of having dental caries was greatly increased. Rodrigues and Sheiham (2000) on the other hand found a weaker association between enamel hypoplasia and caries. They found that whilst enamel hypoplasia was significantly related to caries increment, the effect disappeared after controlling for socio-demographic and socio-economic variables, although this weaker association between enamel hypoplasia and caries could be due to the fact that relatively few children in the study had hypoplasia. Conversely, the study by Li *et al.* (1996) showed that enamel hypoplasia was a stronger predictor of caries than parental income or the country of residence (the study was undertaken in two rural counties of China, one with a relatively high economic development and one with a low level of economic development). The authors also found that the higher the dental defect score, the more caries was experienced by the children. The studies including the presence of enamel hypoplasia appear to have been based on samples of children from developing countries and so the generalisability of the findings concerning the importance of enamel hypoplasia as a predisposing factor for dental caries, to groups of children from other situations may be limited.

Discussion

The purpose of a systematic review is to locate, appraise and synthesise evidence from scientific studies in order to provide informative empirical answers to scientific research questions. The central question of this review is 'what are the risk factors for dental caries in the deciduous teeth of a child aged six years and under?', and a structured approach has been taken to identify relevant literature in order to minimise any bias in the selection of studies included. Many systematic reviews include a variety of methods for identifying relevant studies such as hand-searching journals as well as computerised literature searches. However, the body of literature is significant in this area and handsearching was beyond the scope of this review. The review was therefore limited to computerised searching. However, this may mean that some relevant studies may not have been encompassed by the search terms used and therefore may have been omitted on this basis. The comprehensiveness of the review could be improved by the addition of papers identified by handsearching.

However, in order to maintain the unbiased nature of the review, handsearching would need to be undertaken in a systematic way, and is a significant task in itself.

Since by definition a risk factor must clearly establish that the exposure has occurred before the outcome, or before the conditions are established that make the outcome likely, longitudinal studies are necessary to demonstrate risk factors. An exposure associated with an outcome in a cross sectional study can only be viewed as a risk indicator. A risk indicator may be a probable, or putative risk factor, but the cross sectional data upon which it is based must be viewed as weaker than the longitudinal data provided by cohort, case control and interventional studies. Thus whilst data from cross sectional studies were considered in the review, more weight must be given to the longitudinal studies.

The review shows that whilst many studies have looked at predictors of dental caries in young children, about half of these are cross sectional studies, which is not the ideal study design. Although several cohort and case control studies have been undertaken, very few have high quality scores. There is a large reliance on recalled health behaviour, particularly in the area of dietary habits. Many of the studies would have been improved by using validated measures to collect psychosocial information on dietary and oral hygiene habits.

The balance between the deleterious effects of sucrose consumption and the benefit of toothbrushing is an important theme, with the interaction between these two factors explaining some apparent inconsistencies in study findings. A balance between 'good' habits by way of maintaining good plaque control and 'bad' habits by way of having a highly cariogenic diet, appear to be important with regard to caries (Wendt *et al.*,1996). Any interpretation of study findings is hampered by the large number of different measures used to assess very similar factors (for example toothbrushing frequency with various cut off points, supervision of toothbrushing, age toothbrushing started). Use of fewer, validated measures, such as visible plaque present would help improve comparability.

The review identified a large range of significant risk factors, many of which may act as confounding variables, the use of multivariate statistical analysis is therefore appropriate. Many studies in the review used stepwise logistic regression analysis, although this relies on the use of dichotomised data and therefore means that the categorisations used may be as important as the number of the variables tested. For example one study might analyse toothbrushing frequency comparing 'less than once daily as opposed to once or more times daily', whereas another might compare 'less than twice daily and twice and three times daily', and come to different conclusions.

The more widespread a causative factor is, the less it explains the distribution of a disease. Thus if a feature such as sugar consumption is homogenous within the population it will fail to discriminate between those with or without caries in the population. It is therefore useful to conduct similar studies in a number of different situations and countries. The study by Kerosuo and Honkala (1991) is a good example of this where data on the caries experience of Tanzanian and Finnish children are compared. Whereas the consumption of sweet snacks was a significant risk indicator in the Tanzanian group, it was not for the Finnish group, where the overall level of consumption of sweet snacks was higher, and fluoride toothpaste and other products are available and regularly used.

Conclusion

Whilst many studies have looked for predictors of caries in young children, many have not used the optimum study design, which is a longitudinal study. There is also a shortage of high quality studies, particularly those using validated measures for dietary and oral hygiene habits. A wide range of risk factors have been found to be significantly related to early childhood caries, and whilst factors relating to breast and bottle-feeding do feature, they are by no means the only factors. The evidence points most consistently to a young child being most likely to develop caries if they acquire *Streptococcus Mutans* at a young age. It appears that a high level of *Streptococcus Mutans* may be partly compensated by other parameters such as good oral hygiene and a non-cariogenic diet. Less than daily toothbrushing (or visible plaque) and a highly cariogenic diet are thus important risk factors, but they may interact so that if there is a balance of good and bad habits the development of caries may be controlled. Enamel hypoplasia is also a predisposing factor.

It is noteworthy that no studies were found that evaluated the impact of parental beliefs and attitudes about toothbrushing and sugar snacking on the presence of childhood caries. Further studies, conducted in different countries, on different social and ethnic groups, but using standardised data collection will help in understanding how socio-economic background and ethnicity help determine which young children develop dental caries.

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TOOTH DECAY – TOWNSVILLE & BRISBANE 2000 & 2001

The Child Dental Health Survey – Queensland 2000 & 2001, report the following:

2000 – Table 12: Five-six-year old deciduous caries experience by Health Zone by Health Service District (page 19).

DISTRICT	NUMBERS	MEAN dmft
Townsville	199	1.95
Brisbane North	254	1.64
Bayside	104	1.97
QE11	280	2.51
West Morton	43	2.98
Logan/Beaudesert	118	1.92
Sunshine Coast	129	1.91
Gold Coast	1832	1.94

2000 – Table 13, Twelve-year old permanent caries experience by Health Zone by Health Service District (page 20).

DISTRICT	NUMBER	MEAN DMFT
Townsville	73	1.01
Brisbane North	114	0.78
Bayside	40	0.65
QE11	104	1.13
West Morton	25	1.08
Logan/Beaudesert	34	1.15
Sunshine Coast	52	1.52
Gold Coast	1334	0.86

2001 – Table 12: Five-six-year-old deciduous caries experience by Health Zone by Health Service District (page 17).

DISTRICT	NUMBER	MEAN dmft
Townsville	161	1.90
Brisbane North	264	1.88
Bayside	52	1.96
QE11	282	2.38
West Moreton	94	2.18
Logan/Beaudesert	135	2.61
Sunshine Coast	147	2.56
Gold Coast	1695	2.21

2001. Table 13: Twelve-year-old permanent caries experience by Health Zone by Health Service District. (page 18).

DISTRICT	NUMBER	MEAN DMFT
Townsville	59	1.15
Brisbane North	97	1.30
Bayside	22	1.05
QE11	83	0.82
West Morton	16	0.31
Logan/Beaudesert	51	1.59
Sunshine Coast	49	1.37
Gold Coast	1363	1.07

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MINIREVIEW

Fluoride Plus Aluminum: Useful Tools in Laboratory Investigations, but Messengers of False Information

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Summary

Aluminofluoride complexes (AlF_x) form spontaneously in aqueous solutions containing fluoride and traces of aluminum ions and appear to act as phosphate analogs. These complexes have become widely utilized in laboratory investigations of various guanine nucleotide-binding proteins. Reflecting on many laboratory studies, a new mechanism of fluoride and aluminum action on the cellular level is being suggested. The long-term synergistic effects of these ions in living environment and their hidden danger for human health are not yet fully recognized.

Key words

Aluminum • Fluoride • Aluminofluoride complexes • G-protein • Second messenger

Introduction

The transfer of phosphate groups is the basic mechanism in the regulation of the activity of numerous enzymes, energy metabolism, cell signaling, and cell growth. Phosphate is an important component of phospholipids in cell membranes. In view of the ubiquity of phosphate in cell metabolism, a phosphoryl analog might represent a useful tool for laboratory investigations, but also a strong potential danger for living organisms including humans. Such a compound has been already found, described and opted for molecule of the month in March 1997 (Chabre 1990, Wittinghofer 1997). Moreover, the new phosphate analog has been used in experimental work in numerous laboratories (Strunecká and Patočka 1999ab).

Scientists need not buy this powerful compound through any catalog or drug store. Fluoride anions, generally introduced as NaF solutions, have long been known to influence the activity of various enzymes and guanine nucleotide-binding proteins (G-proteins). Sternweis and Gilman (1982) reported that fluoride activation of the purified guanine nucleotide-binding regulatory component of adenylate cyclase depends on the presence of aluminum traces. This fact had at first been ignored because aluminum is a normal component of glass, from which it is etched by a solution with fluoride. Aluminofluoride complexes (AlF_x) form spontaneously in aqueous solutions containing fluoride and traces of aluminum ions. However, the exact structure and the proportions of species such as AlF_3 and AlF_4^{1-} able to simulate PO_4^{3-} group in many biochemical reactions are still disputed.

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AlF_x – analog of a phosphate group

Analogies between the phosphate group and the aluminofluoride complex consist in their atomic and molecular similarities. The fluorine atom has the same size and the same valence orbital as oxygen. Aluminum is close to phosphorus; their valence electrons are in the same shell. An Al-F bond is the same length as the P-O bond in phosphate, i.e. 1.5 to 1.6 Å. Like phosphorus, aluminum has possible coordination numbers of 1 - 6, due to the possible hybridization of its outer shell 3p electrons with the 3d orbital. The complexation state depends on the pH of the solution (Chabre 1990, Schlichting and Reinstein 1999). In aqueous solutions with a pH of less than 5.5, aluminum exists as the octahedral hexahydrate $Al(H_2O)_6^{3+}$, usually abbreviated to Al^{3+}. At pH values above 6.2, $Al(H_2O)_6^{3+}$ undergoes successive deprotonation, becoming tetrahedral aluminate $Al(OH)_4^{1-}$. If fluoride is added, the four equatorial water molecules of the hexahydrate are replaced by fluoride to give $AlF_4(H_2O)_2^{1-}$. At pH values above 6.2, the tetrahedral aluminate species predominates with a varying composition of hydroxyls and fluoride. The theoretical calculation of the aluminum (Al^{3+})-fluoride predominance is demonstrated in Figure 1. We calculated the dependency of the complexation state on pH and fluoride concentration for Al^{3+} 10 μmol.l^{-1}.

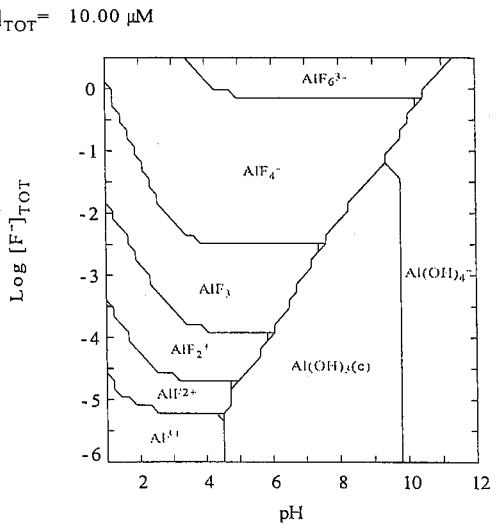

Fig. 1. *Aluminum (Al^{3+})-fluoride predominance diagram. The dependency on pH and fluoride concentration has been calculated for Al^{3+} 10 μM.*

Chabre and co-workers (Bigay *et al.* 1987, Chabre 1990, Antonny and Chabre 1992) suggested that AlF_4^{1-} is probably the active species, which mimics the role of the γ-phosphate. They suggested that the high concentration of fluoride in the solution induces the formation of a soluble tetracoordinated state of aluminum, which has the same geometry, size and coordinance as a phosphate. Their assumption was reinforced by the earlier observation of Sternweis and Gilman (1982). These authors reported that the requirement of fluoride activation of adenylate cyclase for aluminum is highly specific. Of 28 other metals tested, only beryllium could substitute for aluminum. This fact supported the assumption that aluminum acts through its tetrahedral phosphate-like complex AlF_4^{1-}, because all beryllium complexes are tetracoordinated.

In earlier reviews, Martin (1988) had also admitted that the active species might be simply the aluminum ion Al^{3+} and that fluoride complexation would only be needed to allow the penetration of aluminum across the plasma membrane. Once corrected for these effects, Antonny and Chabre (1992) proposed that $AlF_3(OH)^{1-}$ is the main activating species and that the bound form of the complex is tetracoordinated GDP-AlF_3. The studies of the crystal structures of nucleotide binding-proteins complexed with AlF_x indicate that factors other than pH, such as the location of positively charged amino acid of the active site of the phosphoryl-transferring enzyme, may cause deviation from the strict pH dependence of AlF_3 versus AlF_4^{1-} in biological systems. AlF_4^{1-} was determined as the active site species in the case of G-proteins, myosin-S1 and nitrogenase, whereas AlF_3 was bound e.g. in nucleoside diphosphate kinase and uridylate monophosphate kinase (Wittinghofer 1997). It is not clear whether these differences reflect some differences between proteins or are due to technicalities. Schlichting and Reinstein (1999) compared the coordination numbers at different crystallization conditions and suggested that the different coordination numbers originate mainly from the difference in pH at which the enzymes were crystallized. According to these authors AlF_x occurs, as AlF_3 at pH 7.5-8.5 but as AlF_4^{1-} at pH below 7.

Aluminofluoride complexes can bind to proteins by hydrogen bonds to the fluorine atom just as to oxygen atoms of a phosphate ion. AlF_x was found as a good analog of a γ-phosphate for a number of ATP- and GTP-converting enzymes. However, an important functional difference between a phosphate group and the structurally analogous AlF_x exists (Chabre 1990). In the phosphate,

oxygen is covalently bound to the phosphorus and does not exchange with oxygen from the solvent. In AlF_x, ionic bonds are formed between the electropositive aluminum and the highly electronegative fluorine. While the reaction of a bound phosphate compound with orthophosphate is endergonic and slow, the corresponding reaction with AlF_x is rapid and spontaneous. AlF_x bind ionically to the terminal oxygen of GDP β-phosphate. Enzyme-bound GDP or ADP could therefore form a complex with AlF_x that imitates ATP or GTP in its effect on protein conformation. The analogy with beryllium bonding that is strictly tetrahedral led Chabre (1990) to suggest that AlF_x cannot follow this route, since AlF_x causes a structural change that locks the site and prevents the dissociation of the trisphosphate.

The ATPase or GTPase pathway must go through a pentacoordinated transition state for the γ-phosphate. Wittinghofer (1997) described in his article an admirable methodological and intellectual approach, which led to further understanding of the mechanism of phosphoryl transfer reactions using AlF_x and proved that AlF_x acts as the pentacoordinated phosphoryl transfer transition state analog.

Table 1. The effects of fluoride (used as NaF in millimolar concentrations) and aluminum (used as $AlCl_3$ in micromolar concentrations) on levels of the second messenger molecules.

Cell or tissue	Free cytosolic Ca^{2+}	Inositol 1,4,5,P_3	cAMP	References
Hepatocytes	increased	increased		Blackmore *et al.* 1988
Kidney	increased		increased	Zhou *et al.* 1990
Thrombocytes	increased	increased		Deckmyn 1991
Red blood cells		increased		Strunecká *et al.* 1991
Fibroblasts	increased	increased	decreased	Magnaldo *et al.* 1988, Harootunian *et al.* 1991
Osteoclasts	increased	increased	decreased	Moonga *et al.* 1993
Neurons	increased	increased		Nadakavukaren *et al.* 1990,
or brain				Candura *et al.* 1991
		decreased[a]		[a]Sarri and Claro 1999

AlF_x in laboratory studies

The availability of fluoride and aluminum soluble salts probably contributed to the fact that their use became widely spread in laboratory studies of G-proteins (Wittinghofer 1997, Strunecká and Patočka 1999ab). These studies provided a great deal of knowledge about the involvement of G-proteins in cell signaling. Numerous papers presented evidence that AlF_x influence various functions and biochemical reactions of many cells and tissues of the animal or human organisms. Fluoride in the presence of trace amounts of aluminum affects blood elements, endothelial cells and blood circulation, the function of lymphocytes and cells of the immune system, bone cells, fibroblasts and keratinocytes, ion transport, calcium influx and mobilization, processes of neurotransmission, metabolism of the liver, cell growth and differentiation, protein phosphorylation and cytoskeletal proteins (Strunecká and Patočka 2002). This is not surprising if we consider the role of G-proteins in the cell. Physiological agonists of G-protein-coupled receptors include neurotransmitters and hormones, such as dopamine, epinephrine, norepinephrine, serotonin, acetylcholine, glucagon, vasopressin, melatonin, TSH, neuropeptides, opioids, excitatory amino acids, prostanoids, purines, photons and odorants.

Numerous laboratory studies demonstrated that AlF_x interacts with all known G-protein-activated effector enzymes. Fluorides in the presence of aluminum ions affect the levels of second messenger molecules, including cAMP, inositol phosphates and cytosolic calcium level (Table 1). In the liver, for example, it was concluded that AlF_x mimics the effects of Ca^{2+} mobilizing hormones by activating the G-protein, which couples the hormone receptor to phospholipase C. AlF_x potentiated the effects of submaximal doses of glucagon, vasopressin, angiotensin II and α_1-adrenergic agonists (Blackmore and Exton 1986). Fluoride anions in the presence of aluminum turn the liver metabolism to catabolic

processes such as glycogenolysis, fatty acid oxidation and lipolysis.

The observation that AlF_x complexes activate G-proteins has been useful for studying the mechanism of G-protein activation, for understanding the biochemical mechanism of GTP hydrolysis, and for the elucidation of three-dimensional structures of several GTPases, including the discovery of the GTPase-activating proteins (GAPs). Biochemical evidence showed that GAPs bind with a higher affinity to G·GDP·AlF_x complex than to the triphosphate state of G-protein. GAPs also stabilize the GTPase active conformation of G-proteins. These observations further support the view that AlF_x stabilize the transition state (Wittinghofer 1997).

Moreover, the phosphoryl-transfer analog model of AlF_x may be extended to small G-proteins (for review see Wittinghofer 1997, Strunecká and Patočka 2002). For example, the proto-oncogene product Ras is a component of intracellular signaling pathways involved in cell growth and division. It has a very low intrinsic GTPase reaction rate that is stimulated 105-fold by RasGAPs that downregulate the accumulation of Ras·GTP. The determination of the structure of a complex between RasGAP and Ras·GDP in the presence of aluminum ions and fluoride shows that AlF_3 forms a pentagonal bipyramid, with the fluorides forming the trigonal base with two apical oxygen ligands. Similar studies demonstrated that several classes of small GTPases can stably interact with their respective GAPs in the presence of AlF_3, suggesting that the aluminofluoride complex could bind to a wide variety of GTPases. These observations demonstrate that the GTP hydrolysis mechanism is similar for both small GTPases and Gα- subunits.

The phosphate-analog models of AlF_x action have been accepted for G-proteins but may be extended to all enzymes that bind the phosphate or nucleoside-polyphosphate (Wittinghofer 1997, Schlichting and Reinstein 1999). Regarding the role of phosphoryl transfer reactions in cell metabolism, we can predict hundreds of reactions, which might be influenced. It has been reported, for example, that aluminofluoride complexes impair the polymerization-depolymerization cycle of tubulin (Bigay *et al.* 1987). Changes in shape and disorganization of the spectrin network were observed after addition of 1 mM NaF and 10 μM $AlCl_3$ in human red blood cells (Strunecká *et al.* 1991, 2000). Rapid and dynamic changes of the cytoskeletal network are of vital importance for many cells. AlF_x also binds at phosphate sites of various ATPases and phosphatases. These actions may potentially complicate the interpretation of results regarding modulation of signaling systems by aluminofluoride complexes, particularly when dealing with intact tissue or cell preparations.

The interpretation of laboratory investigations using isolated animal and human cells or tissues in an intact multicellular organism could be discussed. Nevertheless, many ecological and clinical studies brought forth evidence about the detrimental effects of synergistic action of fluoride and aluminum ions in animals and humans.

Fluoride and aluminum in ecosystems

Aluminum, a metal of the earth's lithosphere, is everywhere: in water sources, in nourishment, in different food additives and also in air in the form of dust particles. It has, until relatively recently, existed in forms not generally available to living organisms, and was therefore regarded as non-toxic. Aluminum concentration in fresh waters with the neutral pH is negligible and mostly in the form of insoluble $Al(OH)_3$. With the appearance of acid rains and the use of aluminum in industry, the increase in the amount of uncomplexed aluminum in ecosystems has been observed.

Evidence about the detrimental effects of aluminum on several aquatic species has accumulated. The concentrations of Al^{3+} in the range of 100-800 $\mu g.l^{-1}$ in fresh-water lakes were reported (Jones and Benett, 1985). The reproduction toxicity test using *Daphnia magna* was elaborated. The aluminum concentration of 5 $\mu g.l^{-1}$ caused 50 % mortality of daphnias in the course of 10 h, the concentration of 20 $\mu g.l^{-1}$ caused 100 % mortality in the course of 20 h (Muller 1982). Aluminum ions have been toxic after short-term exposure of juvenile trouts to 75 $\mu g.l^{-1}$ in fresh water. Exley *et al.* (1996) designed a laboratory bioassay to expose fish to kinetically determined differences in aluminum hydroxide solution chemistry. They investigated the hitherto unexpected observations of the acute aluminum toxicity in the fish body at pH 6.5. Supporting experiments have demonstrated that the mechanism of toxicity at this pH was probably asphyxiation brought about by aluminum-induced changes in the rheological and diffusional properties of the mucus lining of the gill epithelium.

Fluoride comes from fluoridated water, from medicines, dental products, pesticides, fertilizers and fuels. About 143 000 tons are pumped yearly into drinking water supplies in the U.S.A. 500 000 tons a year

go into fresh waters and the sea, 155 000 tons of fluoride are released annually into the atmosphere (Hatterslay 1999). Industrial fertilizers and pesticides increase the amount of this element in agricultural products and food sources.

Various physiological ligands, such as citrate, phosphate, and silicic acid, or the low absorption in the gastrointestinal tract, are effective natural barrier systems for aluminum preventing the increased accumulation of this metal under natural conditions. Mullenix *et al.* (1995) demonstrated that the presence of fluoride caused more aluminum to cross the blood-brain barrier and be deposited in the brain of rats. Fluoridation of public water treated with aluminum salts together with the wide use of fluoride and aluminum in medicine, industry and agriculture, increase the loading of living organisms with these ions as never before.

Evidence about the action of AlF_x in humans

Most of the ill effects caused by fluoride were first recognized among workers in aluminum factories, where fluoride and aluminum are present in high concentrations. The levels of fluoride in the serum, urine and hair of these workers are higher than in control subjects. Osteoarthritis and related disorders in such workers have been reported since the 1930's (McClure 1933). Observation of industrial fluorosis (osteosclerosis) led to the use of fluoride as a treatment to increase bone mass in osteoporosis patients. Psychiatric disturbances were also reported in aluminum smelter workers. The study of persons living near an enamel factory reports a distinct decline in mental activity, poorer memory, and inability to coordinate thoughts and reduced ability to write. Those living further away from the factory were less affected and had a lower urinary fluoride content (Spittle 1994). Fluoride intoxication with multiple nonspecific symptoms has been observed in chronic hemodialysis patients (Arnow *et al.* 1994). In some regions, the water used for the dialysis also contained a high content of aluminum. Some patients used aluminum-containing medications. Moreover, patients with renal failure cannot remove aluminum from the blood. Elevated aluminum levels have also been implicated as the cause of dialysis encephalopathy or dementia (Altman *et al.* 1999). Speech disorders precede dementia and convulsions.

Fluoride has been used in the prevention of tooth decay for over 50 years. Many studies reporting and evaluating the risks and adverse effects of fluoride on the human organism were published during the same period (Waldbott *et al.* 1978, Hatterslay 1999). Some of them demonstrated a positive correlation between the higher intake of fluoride and osteoarthritis, changes in bone structure, and various non-specific symptoms. Lower intelligence of children, various psychiatric symptoms in adults, such as memory impairment, and difficulties with concentration and thinking were reported (Hatterslay 1999, Spittle 2000, Lu *et al.* 2000). Elevated fluoride content was found in embryonic brain tissues obtained from required abortions in areas where fluorosis was prevalent. These studies showed poor differentiation of brain nerve cells and delayed brain development (Hatterslay 1999).

Endocrine glands such as the parathyroid gland, the thyroid, the pituitary gland and the pineal gland are extremely sensitive to fluoride. Of particular importance relating to G-protein activation is the ability of fluorides to clone the role of the thyroid stimulating hormone (TSH). Fluoride is used in laboratory animals specifically to substitute for TSH. The synergistic action of the thyroid on fluoride toxicity has been reported since 1940. Fluoride effects on thyroid hormone synthesis can be observed on many different levels. There is a direct dose-response relationship with iodine: the higher the fluoride intake – the lower the iodine in the system. The major areas of iodine deficiency are identical to endemic fluorosis areas. The functional changes of the hypophysis-thyroid gland system caused by disorders of the regulatory chain and fluorine impact on thyroid hormones metabolism at the level of target cells were reported and the comparison of fluoride toxicity symptoms and symptoms of thyroid disorders has been reviewed (Schuld 2000). Regarding the crucial role of the thyroid in the regulation of growth, development and metabolism of many tissues, AlF_x might influence the proper function of the entire human body.

Chronic exposure of humans to AlF_x begins in the fetus. High fluoride exposure appears to weaken mental functions among children, as well as adults. In respect to the etiology of Alzheimer's disease, the long-term action of AlF_x also represents a serious and potent risk factor for the development of this new epidemic threat to human civilization (Strunecká 1999, Strunecká and Patočka 1999b). AlF_x may affect all pathological hallmarks of this disease: processes of neuro-transmission, β amyloid generation, plaque formation, metabolism of apolipoprotein E, protein tau phosphorylation, cytoskeletal protein organization,

transport of ions, energy metabolism, and calcium homeostasis Řípová and Strunecká 2001).

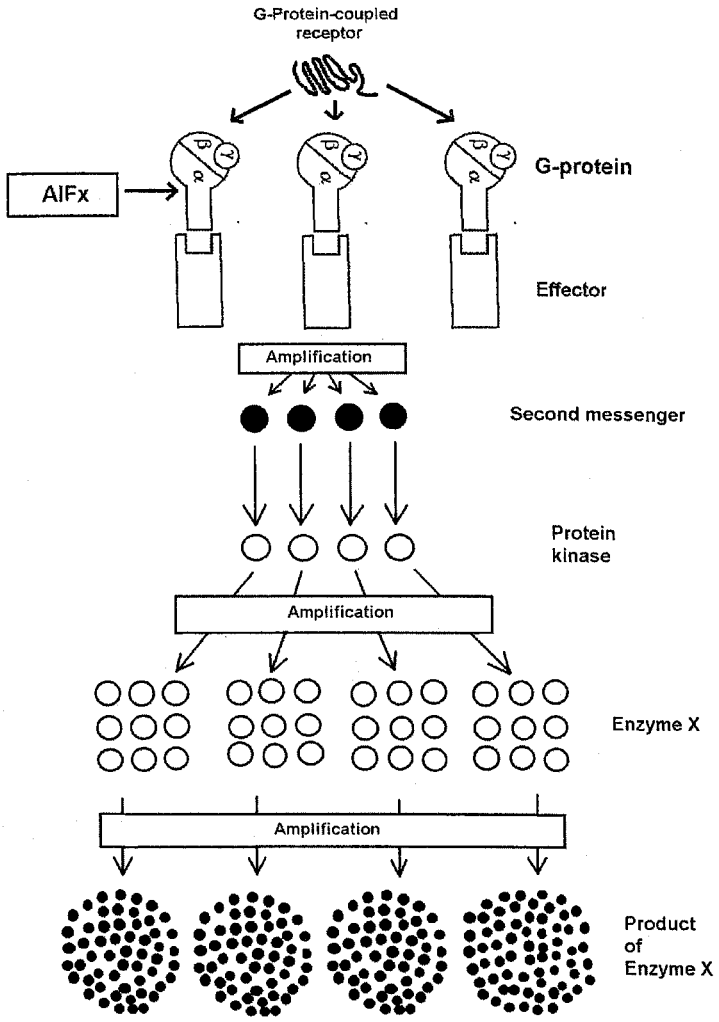

Fig. 2. *AlF_x acts as a messenger of false information. Its message is greatly amplified during the conversion into the functional response of a cell. Effectors are molecules such as cyclases phospholipases C and ion channels. The second messenger molecule could be cAMP, 1,4,5-IP_3, and DAG. Moreover, AlF_x can participate as the analog in the phosphoryl-transfer reactions involved in the signaling cascade.*

Conclusion

The discovery of AlF_x as a new class of phosphate analog has brought numerous demonstrations of their use as the ubiquitous tool in laboratory investigations but also the demonstration of their pharmacological efficacy. It is not surprising with respect to the role of G-proteins in signal transduction. G-proteins take part in numerous biological signaling systems, helping to control almost all important life processes. It has been demonstrated that AlF_x may clone or potentiate the action of numerous extracellular signals. The principle of amplification of the initial signal during its conversion into a functional response has been a widely accepted tenet in cell physiology (Fig. 2). It is evident that AlF_x is a molecule providing false information, which is amplified by processes of signal transmission. Biological signaling pathways interact with one another to form complex networks. Yet, it seems that we shall probably not find any physiological process which is not potentially influenced by AlF_x. These interactions may potentially complicate interpretation of the results.

Understanding the role of phosphate and G-proteins in cell signaling makes it possible to suggest a hypothesis that the synergistic action of fluoride and aluminum in the environment, water and food chains can evoke various and multiple pathological symptoms. AlF_x might induce the alterations of homeostasis, metabolism, growth and differentiation of the living organism. The hidden danger of their long-term action for human health is not yet fully recognized at present.

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Dietary Reference Intakes: Elements

Nutrient	Function	Life Stage Group	RDA/AI*	UL[a]	Selected Food Sources	Adverse effects of excessive consumption	Special Considerations
Arsenic	No biological function in humans although animal data indicate a requirement	Infants 0–6 mo 7–12 mo Children 1–3 y 4–8 y Males 9–13 y 14–18 y 19–30 y 31-50 y 50-70 y > 70 y Females 9–13 y 14–18 y 19–30 y 31-50 y 50-70 y > 70 y Pregnancy ≤ 18 y 19-30y 31-50 y Lactation ≤ 18 y 19-30y 31–50 y	 ND[b] ND ND ND ND ND ND ND ND ND ND ND ND ND ND ND ND ND ND ND ND ND	 ND ND ND ND ND ND ND ND ND ND ND ND ND ND ND ND ND ND ND ND ND ND	Dairy products, meat, poultry, fish, grains and cereal	No data on the possible adverse effects of organic arsenic compounds in food were found. Inorganic arsenic is a known toxic substance. Although the UL was not determined for arsenic, there is no justification for adding arsenic to food or supplements.	None
Boron	No clear biological function in humans although animal data indicate a functional role	Infants 0–6 mo 7–12 mo Children 1–3 y 4–8 y Males 9–13 y 14–18 y 19–30 y 31-50 y 50-70 y > 70 y Females 9–13 y 14–18 y 19–30 y 31-50 y 50-70 y > 70 y Pregnancy ≤ 18 y 19-30y 31-50 y Lactation ≤ 18 y 19-30y 31–50 y	 ND ND ND ND ND ND ND ND ND ND ND ND ND ND ND ND ND ND ND ND ND ND	(mg/d) ND ND 3 6 11 17 20 20 20 20 11 17 20 20 20 20 17 20 20 17 20 20	Fruit-based beverages and products, potatoes, legumes, milk, avocado, peanut butter, peanuts	Reproductive and developmental effects as observed in animal studies.	None

NOTE: The table is adapted from the DRI reports, see www.nap.edu. It represents Recommended Dietary Allowances (RDAs) in **bold type**, Adequate Intakes (AIs) in ordinary type followed by an asterisk (*), and Tolerable Upper Intake Levels (ULs)[a]. RDAs and AIs may both be used as goals for individual intake. RDAs are set to meet the needs of almost all (97 to 98 percent) individuals in a group. For healthy breastfed infants, the AI is the mean intake. The AI for other life stage and gender groups is believed to cover the needs of all individuals in the group, but lack of data prevent being able to specify with confidence the percentage of individuals covered by this intake.

[a]UL = The maximum level of daily nutrient intake that is likely to pose no risk of adverse effects. Unless otherwise specified, the UL represents total intake from food, water, and supplements. Due to lack of suitable data, ULs could not be established for vitamin K, thiamin, riboflavin, vitamin B_{12}, pantothenic acid, biotin, or carotenoids. In the absence of ULs, extra caution may be warranted in consuming levels above recommended intakes.

[b]ND = Not determinable due to lack of data of adverse effects in this age group and concern with regard to lack of ability to handle excess amounts. Source of intake should be from food only to prevent high levels of intake.

SOURCES: *Dietary Reference Intakes for Calcium, Phosphorous, Magnesium, Vitamin D, and Fluoride* (1997); *Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B_6, Folate, Vitamin B_{12}, Pantothenic Acid, Biotin, and Choline* (1998); *Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids* (2000); and *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc* (2001). These reports may be accessed via www.nap.edu.

Dietary Reference Intakes: Elements

Nutrient	Function	Life Stage Group	RDA/AI*	UL[a]	Selected Food Sources	Adverse effects of excessive consumption	Special Considerations
Calcium	Essential role in blood clotting, muscle contraction, nerve transmission, and bone and tooth formation	Infants	(mg/d)	(mg/d)	Milk, cheese, yogurt, corn tortillas, calcium-set tofu, Chinese cabbage, kale, broccoli	Kidney stones, hypercalcemia, milk alkali syndrome, and renal insufficiency	Amenorrheic women (exercise- or anorexia nervosa-induced) have reduced net calcium absorption. There is no consistent data to support that a high protein intake increases calcium requirement.
		0–6 mo	210*	ND[b]			
		7–12 mo	270*	ND			
		Children					
		1–3 y	500*	2,500			
		4–8 y	800*	2,500			
		Males					
		9–13 y	1,300*	2,500			
		14–18 y	1,300*	2,500			
		19–30 y	1,000*	2,500			
		31-50 y	1,000*	2,500			
		50-70 y	1,200*	2,500			
		> 70 y	1,200*	2,500			
		Females					
		9–13 y	1,300*	2,500			
		14–18 y	1,300*	2,500			
		19–30 y	1,000*	2,500			
		31-50 y	1,000*	2,500			
		50-70 y	1,200*	2,500			
		> 70 y	1,200*	2,500			
		Pregnancy					
		≤ 18 y	1,300*	2,500			
		19-30y	1,000*	2,500			
		31-50 y	1,000*	2,500			
		Lactation					
		≤ 18 y	1,300*	2,500			
		19-30y	1,000*	2,500			
		31–50 y	1,000*	2,500			
Chromium	Helps to maintain normal blood glucose levels	Infants	(µg/d)		Some cereals, meats, poultry, fish, beer	Chronic renal failure	None
		0–6 mo	0.2*	ND			
		7–12 mo	5.5*	ND			
		Children					
		1–3 y	11*	ND			
		4–8 y	15*	ND			
		Males					
		9–13 y	25*	ND			
		14–18 y	35*	ND			
		19–30 y	35*	ND			
		31-50 y	35*	ND			
		50-70 y	30*	ND			
		> 70 y	30*	ND			
		Females					
		9–13 y	21*	ND			
		14–18 y	24*	ND			
		19–30 y	25*	ND			
		31-50 y	25*	ND			
		50-70 y	20*	ND			
		> 70 y	20*	ND			
		Pregnancy					
		≤ 18 y	29*	ND			
		19-30y	30*	ND			
		31-50 y	30*	ND			
		Lactation					
		≤ 18 y	44*	ND			
		19-30y	45*	ND			
		31–50 y	45*	ND			

NOTE: The table is adapted from the DRI reports, see www.nap.edu. It represents Recommended Dietary Allowances (RDAs) in **bold type**, Adequate Intakes (AIs) in ordinary type followed by an asterisk (*), and Tolerable Upper Intake Levels (ULs)[a]. RDAs and AIs may both be used as goals for individual intake. RDAs are set to meet the needs of almost all (97 to 98 percent) individuals in a group. For healthy breastfed infants, the AI is the mean intake. The AI for other life stage and gender groups is believed to cover the needs of all individuals in the group, but lack of data prevent being able to specify with confidence the percentage of individuals covered by this intake.

[a]UL = The maximum level of daily nutrient intake that is likely to pose no risk of adverse effects. Unless otherwise specified, the UL represents total intake from food, water, and supplements. Due to lack of suitable data, ULs could not be established for vitamin K, thiamin, riboflavin, vitamin B_{12}, pantothenic acid, biotin, or carotenoids. In the absence of ULs, extra caution may be warranted in consuming levels above recommended intakes.

[b]ND = Not determinable due to lack of data of adverse effects in this age group and concern with regard to lack of ability to handle excess amounts. Source of intake should be from food only to prevent high levels of intake.

SOURCES: *Dietary Reference Intakes for Calcium, Phosphorous, Magnesium, Vitamin D, and Fluoride* (1997); *Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B_6, Folate, Vitamin B_{12}, Pantothenic Acid, Biotin, and Choline* (1998); *Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids* (2000); and *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc* (2001). These reports may be accessed via www.nap.edu.

Dietary Reference Intakes: Elements

Nutrient	Function	Life Stage Group	RDA/AI*	UL[a]	Selected Food Sources	Adverse effects of excessive consumption	Special Considerations
Copper	Component of enzymes in iron metabolism	Infants	(µg/d)	(µg/d)	Organ meats, seafood, nuts, seeds, wheat bran cereals, whole grain products, cocoa products	Gastrointestinal distress, liver damage	Individuals with Wilson's disease, Indian childhood cirrhosis and idiopathic copper toxicosis may be at increased risk of adverse effects from excess copper intake.
		0–6 mo	200*	ND[b]			
		7–12 mo	220*	ND			
		Children					
		1–3 y	**340**	1,000			
		4–8 y	**440**	3,000			
		Males					
		9–13 y	**700**	5,000			
		14–18 y	**890**	8,000			
		19–30 y	**900**	10,000			
		31-50 y	**900**	10,000			
		50-70 y	**900**	10,000			
		> 70 y	**900**	10,000			
		Females					
		9–13 y	**700**	5,000			
		14–18 y	**890**	8,000			
		19–30 y	**900**	10,000			
		31-50 y	**900**	10,000			
		50-70 y	**900**	10,000			
		> 70 y	**900**	10,000			
		Pregnancy					
		≤ 18 y	**1000**	8,000			
		19-30y	**1000**	10,000			
		31-50 y	**1000**	10,000			
		Lactation					
		≤ 18 y	**1300**	8,000			
		19-30y	**1300**	10,000			
		31–50 y	**1300**	10,000			
Fluoride	Inhibits the initiation and progression of dental caries and stimulates new bone formation	Infants	(mg/d)	(mg/d)	Fluoridated water, teas, marine fish, fluoridated dental products	Enamel and skeletal fluorosis	None
		0–6 mo	0.01*	0.7			
		7–12 mo	0.5*	0.9			
		Children					
		1–3 y	0.7*	1.3			
		4–8 y	1*	2.2			
		Males					
		9–13 y	2*	10			
		14–18 y	3*	10			
		19–30 y	4*	10			
		31-50 y	4*	10			
		50-70 y	4*	10			
		> 70 y	4*	10			
		Females					
		9–13 y	2*	10			
		14–18 y	3*	10			
		19–30 y	3*	10			
		31-50 y	3*	10			
		50-70 y	3*	10			
		> 70 y	3*	10			
		Pregnancy					
		≤ 18 y	3*	10			
		19-30y	3*	10			
		31-50 y	3*	10			
		Lactation					
		≤ 18 y	3*	10			
		19-30y	3*	10			
		31–50 y	3*	10			

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[b]ND = Not determinable due to lack of data of adverse effects in this age group and concern with regard to lack of ability to handle excess amounts. Source of intake should be from food only to prevent high levels of intake.

SOURCES: *Dietary Reference Intakes for Calcium, Phosphorous, Magnesium, Vitamin D, and Fluoride* (1997); *Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B_6, Folate, Vitamin B_{12}, Pantothenic Acid, Biotin, and Choline* (1998); *Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids* (2000); and *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc* (2001). These reports may be accessed via www.nap.edu.

Dietary Reference Intakes: Elements

Nutrient	Function	Life Stage Group	RDA/AI*	UL[a]	Selected Food Sources	Adverse effects of excessive consumption	Special Considerations
Iodine	Component of the thyroid hormones; and prevents goiter and cretinism	Infants	(µg/d)	(µg/d)	Marine origin, processed foods, iodized salt	Elevated thyroid stimulating hormone (TSH) concentration	Individuals with autoimmune thyroid disease, previous iodine deficiency, or nodular goiter are distinctly susceptible to the adverse effect of excess iodine intake. Therefore, individuals with these conditions may not be protected by the UL for iodine intake for the general population.
		0–6 mo	110*	ND[b]			
		7–12 mo	130*	ND			
		Children					
		1–3 y	**90**	200			
		4–8 y	**90**	300			
		Males					
		9–13 y	**120**	600			
		14–18 y	**150**	900			
		19–30 y	**150**	1,100			
		31-50 y	**150**	1,100			
		50-70 y	**150**	1,100			
		> 70 y	**150**	1,100			
		Females					
		9–13 y	**120**	600			
		14–18 y	**150**	900			
		19–30 y	**150**	1,100			
		31-50 y	**150**	1,100			
		50-70 y	**150**	1,100			
		> 70 y	**150**	1,100			
		Pregnancy					
		≤ 18 y	**220**	900			
		19-30y	**220**	1,100			
		31-50 y	**220**	1,100			
		Lactation					
		≤ 18 y	**290**	900			
		19-30y	**290**	1,100			
		31–50 y	**290**	1,100			
Iron (mg/d)	Component of hemoglobin and numerous enzymes; prevents microcytic hypochromic anemia	Infants	(mg/d)	(mg/d)	Fruits, vegetables and fortified bread and grain products such as cereal (non-heme iron sources), meat and poultry (heme iron sources)	Gastrointestinal distress	Non-heme iron absorption is lower for those consuming vegetarian diets than for those eating nonvegetarian diets. Therefore, it has been suggested that the iron requirement for those consuming a vegetarian diet is approximately 2-fold greater than for those consuming a nonvegetarian diet. Recommended intake assumes 75% of iron is from heme iron sources.
		0–6 mo	0.27*	40			
		7–12 mo	**11**	40			
		Children					
		1–3 y	**7**	40			
		4–8 y	**10**	40			
		Males					
		9–13 y	**8**	40			
		14–18 y	**11**	45			
		19–30 y	**8**	45			
		31-50 y	**8**	45			
		50-70 y	**8**	45			
		> 70 y	**8**	45			
		Females					
		9–13 y	**8**	40			
		14–18 y	**15**	45			
		19–30 y	**18**	45			
		31-50 y	**18**	45			
		50-70 y	**8**	45			
		> 70 y	**8**	45			
		Pregnancy					
		≤ 18 y	**27**	45			
		19-30y	**27**	45			
		31-50 y	**27**	45			
		Lactation					
		≤ 18 y	**10**	45			
		19-30y	**9**	45			
		31–50 y	**9**	45			

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[b]ND = Not determinable due to lack of data of adverse effects in this age group and concern with regard to lack of ability to handle excess amounts. Source of intake should be from food only to prevent high levels of intake.

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Dietary Reference Intakes: Elements

Nutrient	Function	Life Stage Group	RDA/AI*	UL[a]	Selected Food Sources	Adverse effects of excessive consumption	Special Considerations
Magnesium	Cofactor for enzyme systems	Infants 0–6 mo 7–12 mo Children 1–3 y 4–8 y Males 9–13 y 14–18 y 19–30 y 31-50 y 50-70 y > 70 y Females 9–13 y 14–18 y 19–30 y 31-50 y 50-70 y > 70 y Pregnancy ≤ 18 y 19-30y 31-50 y Lactation ≤ 18 y 19-30y 31–50 y	(mg/d) 30* 75* **80** **130** **240** **410** **400** **420** **420** **420** **240** **360** **310** **320** **320** **320** **400** **350** **360** **360** **310** **320**	(mg/d) ND[b] ND 65 110 350 350 350 350 350 350 350 350 350 350 350 350 350 350 350 350 350 350	Green leafy vegetables, unpolished grains, nuts, meat, starches, milk	There is no evidence of adverse effects from the consumption of naturally occurring magnesium in foods. Adverse effects from magnesium containing supplements may include osmotic diarrhea. The UL for magnesium represents intake from a pharmacological agent only and does not include intake from food and water.	None
Manganese	Involved in the formation of bone, as well as in enzymes involved in amino acid, cholesterol, and carbohydrate metabolism	Infants 0–6 mo 7–12 mo Children 1–3 y 4–8 y Males 9–13 y 14–18 y 19–30 y 31-50 y 50-70 y > 70 y Females 9–13 y 14–18 y 19–30 y 31-50 y 50-70 y > 70 y Pregnancy ≤ 18 y 19-30y 31-50 y Lactation ≤ 18 y 19-30y 31–50 y	(mg/d) 0.003* 0.6* 1.2* 1.5* 1.9* 2.2* 2.3* 2.3* 2.3* 2.3* 1.6* 1.6* 1.8* 1.8* 1.8* 1.8* 2.0* 2.0* 2.0* 2.6* 2.6* 2.6*	(mg/d) ND ND 2 3 6 9 11 11 11 11 6 9 11 11 11 11 9 11 11 9 11 11	Nuts, legumes, tea, and whole grains	Elevated blood concentration and neurotoxicity	Because manganese in drinking water and supplements may be more bioavailable than manganese from food, caution should be taken when using manganese supplements especially among those persons already consuming large amounts of manganese from diets high in plant products. In addition, individuals with liver disease may be distinctly susceptible to the adverse effects of excess manganese intake.

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[b]ND = Not determinable due to lack of data of adverse effects in this age group and concern with regard to lack of ability to handle excess amounts. Source of intake should be from food only to prevent high levels of intake.

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Dietary Reference Intakes: Elements

Nutrient	Function	Life Stage Group	RDA/AI*	UL[a]	Selected Food Sources	Adverse effects of excessive consumption	Special Considerations
Molybdenum	Cofactor for enzymes involved in catabolism of sulfur amino acids, purines and pyridines.	Infants	(µg/d)	(µg/d)	Legumes, grain products and nuts	Reproductive effects as observed in animal studies.	Individuals who are deficient in dietary copper intake or have some dysfunction in copper metabolism that makes them copper-deficient could be at increased risk of molybdenum toxicity.
		0–6 mo	2*	ND[b]			
		7–12 mo	3*	ND			
		Children					
		1–3 y	**17**	300			
		4–8 y	**22**	600			
		Males					
		9–13 y	**34**	1,100			
		14–18 y	**43**	1,700			
		19–30 y	**45**	2,000			
		31-50 y	**45**	2,000			
		50-70 y	**45**	2,000			
		> 70 y	**45**	2,000			
		Females					
		9–13 y	**34**	1,100			
		14–18 y	**43**	1,700			
		19–30 y	**45**	2,000			
		31-50 y	**45**	2,000			
		50-70 y	**45**	2,000			
		> 70 y	**45**	2,000			
		Pregnancy					
		≤ 18 y	**50**	1,700			
		19-30y	**50**	2,000			
		31-50 y	**50**	2,000			
		Lactation					
		≤ 18 y	**50**	1,700			
		19-30y	**50**	2,000			
		31–50 y	**50**	2,000			
Nickel	No clear biological function in humans has been identified. May serve as a cofactor of metalloenzymes and facilitate iron absorption or metabolism in microorganisms.	Infants		(mg/d)	Nuts, legumes, cereals, sweeteners, chocolate milk powder, chocolate candy	Decreased body weight gain Note: As observed in animal studies	Individuals with preexisting nickel hypersensitivity (from previous dermal exposure) and kidney dysfunction are distinctly susceptible to the adverse effects of excess nickel intake
		0–6 mo	ND	ND			
		7–12 mo	ND	ND			
		Children					
		1–3 y	ND	0.2			
		4–8 y	ND	0.3			
		Males					
		9–13 y	ND	0.6			
		14–18 y	ND	1.0			
		19–30 y	ND	1.0			
		31-50 y	ND	1.0			
		50-70 y	ND	1.0			
		> 70 y	ND	1.0			
		Females					
		9–13 y	ND	0.6			
		14–18 y	ND	1.0			
		19–30 y	ND	1.0			
		31-50 y	ND	1.0			
		50-70 y	ND	1.0			
		> 70 y	ND	1.0			
		Pregnancy					
		≤ 18 y	ND	1.0			
		19-30y	ND	1.0			
		31-50 y	ND	1.0			
		Lactation					
		≤ 18 y	ND	1.0			
		19-30y	ND	1.0			
		31–50 y	ND	1.0			

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Dietary Reference Intakes: Elements

Nutrient	Function	Life Stage Group	RDA/AI*	UL[a]	Selected Food Sources	Adverse effects of excessive consumption	Special Considerations
Phosphorus	Maintenance of pH, storage and transfer of energy and nucleotide synthesis	Infants	(mg/d)	(mg/d)	Milk, yogurt, ice cream, cheese, peas, meat, eggs, some cereals and breads	Metastatic calcification, skeletal porosity, interference with calcium absorption	Athletes and others with high energy expenditure frequently consume amounts from food greater than the UL without apparent effect.
		0–6 mo	100*	ND[b]			
		7–12 mo	275*	ND			
		Children					
		1–3 y	**460**	3,000			
		4–8 y	**500**	3,000			
		Males					
		9–13 y	**1,250**	4,000			
		14–18 y	**1,250**	4,000			
		19–30 y	**700**	4,000			
		31-50 y	**700**	4,000			
		50-70 y	**700**	4,000			
		> 70 y	**700**	3,000			
		Females					
		9–13 y	**1,250**	4,000			
		14–18 y	**1,250**	4,000			
		19–30 y	**700**	4,000			
		31-50 y	**700**	4,000			
		50-70 y	**700**	4,000			
		> 70 y	**700**	3,000			
		Pregnancy					
		≤ 18 y	**1,250**	3,500			
		19-30y	**700**	3,500			
		31-50 y	**700**	3,500			
		Lactation					
		≤ 18 y	**1,250**	4,000			
		19-30y	**700**	4,000			
		31–50 y	**700**	4,000			
Selenium	Defense against oxidative stress and regulation of thyroid hormone action, and the reduction and oxidation status of vitamin C and other molecules	Infants	(µg/d)	(µg/d)	Organ meats, seafood, plants (depending on soil selenium content)	Hair and nail brittleness and loss	None
		0–6 mo	15*	45			
		7–12 mo	20*	60			
		Children					
		1–3 y	**20**	90			
		4–8 y	**30**	150			
		Males					
		9–13 y	**40**	280			
		14–18 y	**55**	400			
		19–30 y	**55**	400			
		31-50 y	**55**	400			
		50-70 y	**55**	400			
		> 70 y	**55**	400			
		Females					
		9–13 y	**40**	280			
		14–18 y	**55**	400			
		19–30 y	**55**	400			
		31-50 y	**55**	400			
		50-70 y	**55**	400			
		> 70 y	**55**	400			
		Pregnancy					
		≤ 18 y	**60**	400			
		19-30y	**60**	400			
		31-50 y	**60**	400			
		Lactation					
		≤ 18 y	**70**	400			
		19-30y	**70**	400			
		31–50 y	**70**	400			

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Dietary Reference Intakes: Elements

Nutrient	Function	Life Stage Group	RDA/AI*	UL[a]	Selected Food Sources	Adverse effects of excessive consumption	Special Considerations
Silicon	No biological function in humans has been identified. Involved in bone function in animal studies.	Infants 0–6 mo 7–12 mo Children 1–3 y 4–8 y Males 9–13 y 14–18 y 19–30 y 31-50 y 50-70 y > 70 y Females 9–13 y 14–18 y 19–30 y 31-50 y 50-70 y > 70 y Pregnancy ≤ 18 y 19-30y 31-50 y Lactation ≤ 18 y 19-30y 31–50 y	 ND[b] ND ND ND ND ND ND ND ND ND ND ND ND ND ND ND ND ND ND ND ND ND	 ND ND ND ND ND ND ND ND ND ND ND ND ND ND ND ND ND ND ND ND ND ND	Plant-based foods	There is no evidence that silicon that occurs naturally in food and water produces adverse health effects.	None
Vanadium	No biological function in humans has been identified.	Infants 0–6 mo 7–12 mo Children 1–3 y 4–8 y Males 9–13 y 14–18 y 19–30 y 31-50 y 50-70 y > 70 y Females 9–13 y 14–18 y 19–30 y 31-50 y 50-70 y > 70 y Pregnancy ≤ 18 y 19-30y 31-50 y Lactation ≤ 18 y 19-30y 31–50 y	 ND ND ND ND ND ND ND ND ND ND ND ND ND ND ND ND ND ND ND ND ND ND	(mg/d) ND ND ND ND ND ND 1.8 1.8 1.8 1.8 ND ND 1.8 1.8 1.8 1.8 ND ND ND ND ND ND	Mushrooms, shellfish, black pepper, parsley, and dill seed.	Renal lesions as observed in animal studies.	None

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Dietary Reference Intakes: Elements

Nutrient	Function	Life Stage Group	RDA/AI*	UL[a]	Selected Food Sources	Adverse effects of excessive consumption	Special Considerations
Zinc	Component of multiple enzymes and proteins; involved in the regulation of gene expression.	Infants	(mg/d)	(mg/d)	Fortified cereals, red meats, certain seafood	Reduced copper status	Zinc absorption is lower for those consuming vegetarian diets than for those eating nonvegetarian diets. Therefore, it has been suggested that the zinc requirement for those consuming a vegetarian diet is approximately 2-fold greater than for those consuming a nonvegetarian diet.
		0–6 mo	2*	4			
		7–12 mo	**3**	5			
		Children					
		1–3 y	**3**	7			
		4–8 y	**5**	12			
		Males					
		9–13 y	**8**	23			
		14–18 y	**11**	34			
		19–30 y	**11**	40			
		31-50 y	**11**	40			
		50-70 y	**11**	40			
		> 70 y	**11**	40			
		Females					
		9–13 y	**8**	23			
		14–18 y	**9**	34			
		19–30 y	**8**	40			
		31-50 y	**8**	40			
		50-70 y	**8**	40			
		> 70 y	**8**	40			
		Pregnancy					
		≤ 18 y	**12**	34			
		19-30y	**11**	40			
		31-50 y	**11**	40			
		Lactation					
		≤ 18 y	**13**	34			
		19-30y	**12**	40			
		31–50 y	**12**	40			

NOTE: The table is adapted from the DRI reports, see www.nap.edu. It represents Recommended Dietary Allowances (RDAs) in **bold type**, Adequate Intakes (AIs) in ordinary type followed by an asterisk (*), and Tolerable Upper Intake Levels (ULs)[a]. RDAs and AIs may both be used as goals for individual intake. RDAs are set to meet the needs of almost all (97 to 98 percent) individuals in a group. For healthy breastfed infants, the AI is the mean intake. The AI for other life stage and gender groups is believed to cover the needs of all individuals in the group, but lack of data prevent being able to specify with confidence the percentage of individuals covered by this intake.

[a]UL = The maximum level of daily nutrient intake that is likely to pose no risk of adverse effects. Unless otherwise specified, the UL represents total intake from food, water, and supplements. Due to lack of suitable data, ULs could not be established for vitamin K, thiamin, riboflavin, vitamin B_{12}, pantothenic acid, biotin, or carotenoids. In the absence of ULs, extra caution may be warranted in consuming levels above recommended intakes.

[b]ND = Not determinable due to lack of data of adverse effects in this age group and concern with regard to lack of ability to handle excess amounts. Source of intake should be from food only to prevent high levels of intake.

SOURCES: *Dietary Reference Intakes for Calcium, Phosphorous, Magnesium, Vitamin D, and Fluoride* (1997); *Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B_6, Folate, Vitamin B_{12}, Pantothenic Acid, Biotin, and Choline* (1998); *Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids* (2000); and *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc* (2001). These reports may be accessed via www.nap.edu.

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June 6, 2005

Dr. C. W. Jameson
National Toxicology Program, Report on Carcinogens
P.O. Box 12233
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MD-EC-14
Research Triangle Park, NC 27709

Dear Dr. Jameson:

We are writing to nominate fluoride in tap water for inclusion in the Report on Carcinogens based on its ability to cause osteosarcoma in males less than 20 years of age.

The science supporting the link between fluoride and bone cancer in boys is compelling, and much of this science is recent and not reflected in current drinking water contaminant limits or the overall risk-benefit equation underlying the decision to add fluoride to the tap water of 170 million people. This widespread exposure to fluoride in tap water ensures that millions of boys are exposed during critical periods of development and growth that are relevant to the cancer in question.

EWG is aware of the value of fluoride to dentistry, yet a substantial and growing body of peer-reviewed science strongly suggests that adding fluoride to tap water may not be the safest way to achieve the dental health benefits of fluoridation. Based on a number of serious health concerns with fluoride, in 2002, the EPA commissioned a general review of the toxicity of fluoride by the National Research Council (NRC) of the National Academy of Sciences (NAS). Although the NRC panel will consider cancer effects in its comprehensive review, the committee is not charged with making a basic determination of fluoride's ability to cause bone cancer in boys. The NRC panel is comprised of individuals from a wide range of disciplines including dentistry, reproductive toxicology, neuroscience, biophysics, and epidemiology. Consequently it does not have the depth of expertise in carcinogenicity, the resources, or the mandate that the National Toxicology Program can bring to bear on this specific question. Only the NTP is in a position to undertake a thorough review of the total weight of the evidence supporting fluoride carcinogenicity – from the mechanistic data, through genotoxicity, animal cancer bioassays, and human epidemiologic studies.

Summary of the science

The overall weight of the evidence strongly supports the conclusion that exposure to fluoride in tap water during the mid-childhood growth spurt between ages 5 and 10 increases the incidence of osteosarcoma in boys ages 10 through 19.

Biologically, the link between fluoride in tap water and bone cancer in boys is highly plausible. Fifty percent of ingested fluoride is deposited in bones, and fluoride is a mitogen that stimulates bone growth in the growing ends of the bones where the osteosarcoma occurs. Fluoride is also a confirmed mutagenic agent in humans, which suggests that fluoride can cause genetic damage in bone cells where it is actively deposited, in this case precisely where the osteosarcoma arises. Animal studies add further credence to the potential link between fluoride and bone cancer in males. The only two animal cancer bioassays conducted with fluoride both show rare bone tumors, many of which were malignant, in male as opposed to female test animals. And finally, three high quality epidemiology studies each show a strong association between fluoride in tap water and osteosarcoma in boys. While several epidemiology studies have failed to find an association between fluoride and osteosarcoma in boys, these studies typically did not look for a relationship between age of exposure to fluoride and the incidence of bone cancer in young males.

Osteosarcoma accounts for about 3 percent of all childhood cancers. The five-year mortality rate is around 50 percent, and nearly all survivors have limbs amputated, usually legs.

Early concerns about cancer

Concern about the ability of fluoride to cause bone cancer arose first in a 1977 NAS review of fluoride safety, where the academy committee expressed concerns about a high (13.5 percent) incidence in bone structure defects in the population of one of the nation's first fluoridated communities, Newburgh, New York compared to a 7 percent rate in the non-fluoridated Kingston community. At that time, the NAS recommended a full study of fluoride's potential to cause osteosarcoma in young boys. The resulting U.S. Public Health Service (USPHS) study was completed in 1991 and found a significant association between fluoride exposure and bone cancer in boys.

The 1991 USPHS study was based on data collected by the National Cancer Institute from 1973 through 1987. The first phase compared osteosarcoma rates in males under 20 years of age in fluoridated communities, with non-fluoridated communities in Iowa and around Seattle. The researchers found a 79 percent increase in osteosarcoma from 1973 through 1987 in fluoridated communities, compared to a 4 percent decrease over the same time period in non-fluoridated communities. A second phase of the study expanded the analysis nationwide, and found that the rates of osteosarcoma were 57 percent higher in the fluoridated communities than in communities with non-fluoridated water supplies (Hoover 1991).

As a follow-up to the USPHS study, the New Jersey Department of Health (NJDH) commissioned a similar study at the municipal level based on an individual's residence at the time of osteosarcoma diagnosis. The NJDH found that young males living in fluoridated communities had significantly higher rates of osteosarcoma than young males living in non-fluoridated areas; males 10-19 years old in fluoridated areas were 6.9 times more likely to develop osteosarcoma than those in non-fluoridated areas. According to the study authors, the findings "support the importance of investigating the possible link between osteosarcoma and overall ingestion of fluoride" (Cohn 1992).

Some experts questioned the significance of the NTP study findings when it was published citing the lack of an association between osteosarcoma and the length of time that individuals were exposed to fluoride in tap water. The overall weight of the scientific evidence, however, including a doctoral thesis from Harvard discussed below that closely examined timing of exposure in relationship to osteosarcoma incidence, provides compelling evidence that fluoride exposure during distinct mid-childhood periods of rapid bone growth is a much better indicator of osteosarcoma risk, than total duration, or average lifetime exposure.

Of the studies that have failed to find an association between fluoride in tap water and bone cancer (Operskalski 1987; McGuire 1991; Freni and Gaylor 1992; Moss 1995; Gelberg 1995), most have basic methodological issues that readily explain the negative findings. For instance, four of the five studies referenced above failed to analyze for age-specific effects, making it impossible for them to find such an association. The other (Operskalski) used friends and neighbors as controls, which according to one expert, Dr. Elise Bassin, produced a phenomenon called overmatching, where "detecting a benefit or risk for fluoride would be unlikely" (Bassin 2001, pg 78). Overall, as summarized by Bassin, "Prior studies have primarily evaluated fluoride exposure at the time of diagnosis or as an average lifetime exposure, and have not evaluated exposures at specific ages during growth and development when cell division is occurring rapidly" (Bassin 2001, pg 69).

New Harvard doctoral thesis supports fluoride-bone cancer link

Environmental Working Group (EWG) has attached to this petition, key portions of a doctoral dissertation from the Harvard School of Dental Medicine that found a strong, statistically significant relationship between fluoride in tap water at levels commonly found in American water supplies, and the rare but often fatal form of bone cancer, osteosarcoma, in boys. The association is particularly strong when exposure occurs during periods of rapid bone growth that take place between ages five and ten. The findings confirm the results of earlier studies by the U.S. Public Health Service and the New Jersey Department of Health that found an association between fluoride in tap water and bone cancer in males under age 20.

The dissertation by Elise Bassin is titled "Association between fluoride in drinking water during growth and development and the incidence of osteosarcoma for children and adolescents". Bassin was awarded a doctorate by the Harvard School of Dental Medicine in 2001. The research findings from her doctoral dissertation, however, have not yet been published.

The study came to the attention of EWG as a result of a failed attempt to obtain the full doctoral thesis by the staff of the National Research Council committee on fluoride safety. After being repeatedly denied a copy of the thesis, the NRC committee instead sent a committee member to the Harvard Countway Library of Medicine to read the entire document and report back to the committee. Environmental Working Group obtained a copy of the results section of the document from the Fluoride Action Network, who sent two researchers to the library, each of whom were allowed to copy 10 percent of the document.

Dr. Bassin's study measured the risk of osteosarcoma before age 20 based on exposures to fluoride in drinking water during each year of age in childhood. The methodology employed is rigorous and fluoride levels in tap water for each study participant were confirmed for each year of exposure during childhood. The analysis shows significantly elevated risks of bone cancer in boys exposed to fluoridated water during a window of vulnerability, from ages five through ten, with a peak risk associated with exposures at seven years of age.

Elevated bone cancer risks were identified by Bassin at fluoride levels that are commonly found in American water supplies. For drinking water systems with fluoride levels from 30 to 99 percent of the amount recommended by the Centers for Disease Control and Prevention (CDC), Bassin reports elevated risks for exposure from ages five through ten, with a five-fold risk of osteosarcoma for those exposed at age seven (4.94 (1.23-19.8) at 95% CI)). At 100 percent or more of the recommended level (and still far below legal maximum levels), the risk for exposure at seven years old rises to 7.2-fold (1.73-30.0) at the 95% CI (Bassin 2001, pg 95 – see results section attached).

The CDC's recommended fluoride levels are well below what is legally allowed in tap water. The EPA's maximum contaminant limit, or MCL, for fluoride in tap water is 4 parts per million. The CDC recommends optimal fluoride levels ranging from 0.7-1.2 parts per million based on average annual air temperatures and corresponding water consumption rates.

Notably, Bassin's doctoral dissertation was based on a reanalysis of data from another study that found no association between drinking water fluoride levels and bone cancer, co-authored by Harvard Department Chair Dr. Chester Douglass (McGuire 1995). In her reanalysis, Bassin examined the same cases and controls used by Douglass in 1995. Dr. Bassin, however, refined the analysis by limiting cases to individuals exposed at less than 20 years old and conducted a more detailed analysis of fluoride exposure and age-specific effects. The result was a very strong correlation between fluoride exposure and bone cancer, particularly for boys exposed at ages 6 through 8.

Fluoride/cancer link in epidemiology studies is strongly supported by additional data

When the results of USPHS, New Jersey, and Harvard (Bassin) studies are combined with the results of animal tests, human genotoxicity studies, and the known biochemistry and metabolism of fluoride, the overall weight of the evidence strongly supports a conclusion that fluoride causes the rare and often fatal bone cancer osteosarcoma in boys. Beyond human epidemiologic studies, the core supporting evidence includes the following:

- The two animal cancer bioassays conducted to date each found an increase in extremely rare bone tumors among male test animals in two species, rats and mice, exposed to fluoride (Maurer 1990; Maurer et al 1993; NTP 1990).

- Six separate studies have found that fluoride causes genetic mutations in humans (Meng 1995, 1997; Lazutka 1999; Sheth 1994; Wu 1995; Joseph 2000);

additional studies show that humans appear to be more sensitive to the genotoxicity of fluoride than rodents (Kishi 1993).

- The link between fluoride and osteosarcoma during periods of rapid growth is biologically highly plausible. Fluoride is a proven mitogen, meaning that it increases the proliferation of osteoblasts (bone formation) during periods of rapid skeletal growth (Gruber 1991; Kleerekoper 1996; Whitford 1996). As put by Dr. Bassin in her doctoral thesis: "It is biologically plausible that fluoride increases the rate of osteosarcoma, and that this effect would be strongest during periods of rapid growth, particularly in males" (Bassin 2001, pg 79).

- Over ninety percent of fluoride in the human body is stored in the bones; 50 percent of fluoride ingested is deposited directly into bones or teeth.

Animal studies found bone cancer in male test animals

Only two long-term animal cancer bioassays with fluoride have ever been conducted; one by the National Toxicology Program (NTP), and another by Procter and Gamble, which involved both rats and mice. Both found an increase in rare bone tumors among male animals exposed to fluoride.

In the NTP study, a dose-dependent increase of osteosarcoma was seen in the bones of fluoride-treated male rats (NTP 1990). These findings are highly significant for a number of reasons:

- Osteosarcoma is extremely difficult to produce in rats; the only other environmental agent known to induce osteosarcoma in rats is high doses of radiation;

- The levels of fluoride in the treated rats' bones were in the same range as fluoride found in human bones;

- Bones are the site of fluoride accumulation, and;

- The osteosarcomas were evident before the end of the study, indicating an age dependent vulnerability similar to that seen in human males.

The study authors were unequivocal about their findings: "The neoplasms were clearly malignant (one metastasized to the lung) and there was complete agreement concerning the diagnoses at both the quality assessment and Pathology Working Group stages of the histopathology review."

Curiously, a 1993 National Research Council (NRC) review appeared to miss the importance of the findings. In characterizing the significance of the findings the NRC stated simply: "The equivocal result of osteosarcoma in male rats was not supported by results in females in the same study" (NRC 1993). This is an extraordinary statement given the prescient concerns for young males raised 16 years earlier by the NAS (in 1977), and the available epidemiologic data available at that time (Hoover 1991; Cohn

1992). Increased osteosarcoma in males, as identified in the Hoover and Cohn studies, is precisely the result that the 1977 NAS panel was concerned about.

In a 2002 review of fluoride toxicity the World Health Organization offered a more reasoned assessment of the results of the NTP rat study: "Such a (dose-dependent) trend associated with the occurrence of a rare tumour in the tissue in which fluoride is known to accumulate cannot be casually dismissed" (WHO 2002).

An additional animal study was conducted by Procter & Gamble, using both mice and rats. The study found a large, dose-dependent increase in rare bone tumors (osteomas) in fluoride-treated mice (Maurer 1993). The second part of the study, in rats, again found bone tumors and a rare tooth tumor in the treated rats but not the controls (Maurer et al. 1990). Apparently this study was discounted because most of the tumors, although rare, were not yet malignant.

Fluoride causes genetic damage in humans

A compound's ability to cause genetic damage is considered an important indicator of cancer-causing potential. Many studies have investigated and found positive evidence of fluoride's genotoxicity. Notable among these is a 1996 study that reported that sodium fluoride was mutagenic to rat cortical bone, the same tissue in which osteosarcoma forms (Mihashi and Tsutsui 1996).

Since 1994, six of eight published genotoxicity studies have found an increased incidence of genetic damage in humans exposed to fluorides. Three were from exposure to airborne fluorides (Meng 1995, 1997; Lazutka 1999), and three others from exposure to fluoride in drinking water (Sheth 1994; Wu 1995; Joseph 2000). In two of the three drinking water studies (Sheth 1994 and Joseph 2000) exposure levels were well within legal limits for fluoride in tap water in the United States (1.9 - 2.2 parts per million (ppm) and 1.6 - 3.5 ppm respectively). The third was at 4 to 15 ppm. Two?additional studies reported no increase in mutagenic damage or decrease in damage among humans drinking excess fluoride in water (Li 1995; Jackson 1997).

The most commonly observed genetic effect has been increased sister-chromatid exchange (SCE), a measure of how often the ends of DNA strands break off and the pieces switch positions when they reattach themselves (see: Sheth 1994; Meng 1995, Wu 1995; Lazutka 1999; Joseph 2000). Wu, who found an increase of SCE among humans drinking water with 4 - 15 ppm fluoride, described the significance of SCE as follows:

> "In recent years, SCE analysis has been considered to be a sensitive method for detecting DNA damage. There is a clear relationship between a substance's ability to induce DNA damage, mutate chromosomes, and cause cancers. The SCE frequency in the human body in peripheral blood lymphocytes is very steady, and does not vary with age or sex. Any increase of the SCE frequency is primarily due to chromosome damage. Thus using a method to detect SCE for exploring the toxicity and harm caused by fluoride is of great importance" (Wu 1995).

The finding of increased SCE in fluoride-exposed humans has reinforced the possibility — as suggested by numerous in vitro studies — that fluoride is a mutagenic agent.

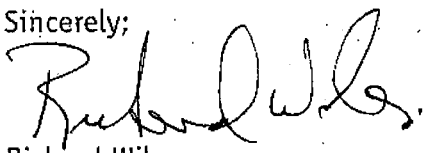
Human sensitivity

The mutagenicity of fluoride was compared in cells taken from rodents with the mutagenicity of fluoride in cells taken from great apes and humans (Kishi 1993). The conclusion of the study was that the ape and human cells showed greater susceptibility to fluoride's mutagenic effects than the rodent cells. These findings suggest that humans may be more susceptible to fluoride's mutagenic properties, and consequently, more susceptible to a potential carcinogenic effect. They may also explain the findings of mutagenic damage in humans' drinking water with relatively low fluoride concentrations: 1.9 - 2.2 ppm and 1.6 - 3.5 ppm (Sheth 1994; Joseph 2000).

Recommendations

The safety of fluoride in America's tap water is a pressing health concern. More than 170 million people live in cities and towns with fluoridated water, and the weight of the evidence strongly supports the conclusion that millions of boys in these communities are at significantly increased risk of developing bone cancer as a result. EWG urges the National Toxicology Program to put fluoride into an expedited review for inclusion in its Report on Carcinogens.

Sincerely;



Richard Wiles
Sr. Vice President
Environmental Working Group

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