From: Tomcik, Kamila
Sent: Tuesday, September 10, 2002 2:14 PM
To: 'Wolfe.Mary'
Subject: RESUBMISSION: Public Comments on Hexafluorosilicic acid (H2SiF6) and Sodium Flu orosilicate (Na2SiF6)

Fluoride background report Oct99anon.doc

Comments on Core Indicators 2002-Fluoride excerpt.doc

Please note: This submission, including the new attachments, is intended to replace the message I sent previously in its entirety.

Dear Dr. Wolfe,
I would like to submit the following documents in response to the request for public comments on the use of fluorosilicates for drinking water fluoridation. Although I am employed with a public health agency, I am making this submission as a private citizen. I hope that the committee will undertake a thorough review of the matter, and will also consider current ethical standards for medical treatment and medical research involving human subjects, in particular: issues of informed consent to medication, the primary responsibility to do no harm, and the obligation to disclose all known and potential risks of recommended/administered medication or treatment to affected individuals (including caregivers of minors and others who are unable to provide informed consent).

I am attaching two documents; one is an unpublished review of health effects and risks of fluoride exposures from an environmental health perspective, prepared between 1998-1999 for a public health agency; the other is an excerpt of my reviewer comments, as an invited subject matter expert, on the use of fluoride levels in water as a health indicator, submitted to a public health association earlier this year. I would again note that this is a personal submission, and does not claim or imply in any way to reflect the official position of my employer or any health organisation.

It is striking that the agencies and individuals responsible for adding HFS/FSA and attendant substances to drinking water seem to have so little knowledge of the composition and properties of the chemicals involved,

including the paucity of research on health and environmental effects, and that they generally assume - and tell the public - there can be no differences between these compounds and sodium fluoride, without any substantive evidence. Although there has been very little research on the mechanisms of action and the health effects of fluorosilicates, some published works have become available after I drafted my report. In particular, a Ph.D. thesis from a German university examined differences between NaF and fluorosilicates, particularly from a neuro-endocrine focus. You are also probably aware of the work published more recently by Masters & Coplan, which found a strong association between fluorosilicates in drinking water and elevated blood lead levels in US children. The studies are available via the internet, and will hopefully be submitted by other individuals.

Thank you for the opportunity to comment, and I look forward to receiving notification of the committee's findings. I would also be happy to provide further information or clarification.

Sincerely,

Kamila Tomcik
120 Fentiman Avenue
Ottawa, ON
Canada K1S 0T8

phone: (613) 730-4207 (res)/ (613) 724-4122, ext. 23492 (bus.)

September, 2002
NOTE; THE FOLLOWING IS AN EXCERPT OF MY INVITED COMMENTS ON THE USE
OF "FLUORIDE LEVELS IN DRINKING WATER" AS A PUBLIC HEALTH INDICATOR,
AS PROPOSED BY A HEALTH AGENCY. THE COMMENTS ARE BASED ON MY
PERSONAL FAMILIARITY WITH RESEARCH IN THIS AREA. THEY HAVE NOT BEEN
ENDORSED OR APROVED BY ANY AGENCY OR MEDICAL AUTHORITY.

Comments on "Environment and Health: Physical Environment" Section

1. Municipal Drinking Water Quality
b) Should add average fluoride concentrations, and whether natural or added (see detailed
comments below).

f) What is the purpose of the statement "fluoride is a *naturally occurring* mineral..."? Why
are there no similar statements for other naturally occurring contaminants such as ozone,
particulate matter, *E. coli*, arsenic, etc.? Even tobacco & nicotine are natural substances.
This is totally irrelevant to the circumstances and effects of this, or any, substance in drinking
water, and suggests bias. Moreover, this statement is misleading, in that (1) this chemical is
not naturally occurring at anything approaching 1 ppm in most (surface) water supplies, but
is deliberately added; and (ii) the naturally occurring mineral form of fluoride is *not* what is
used as the fluoridating agent in public water supplies, an entirely different species (see
below).

I would also revisit the current literature on the purported mechanism of action of fluoride
w.r.t.. caries reduction, and revise this section of the indicators accordingly. There is
currently no definitive understanding of the primary mechanism, and no scientific consensus
on the issue (summary in UNICEF report below; references in my attached literature review).
It seems likely that fluoride acts mainly as a bacterial inhibitor by disabling or disrupting
enzyme function (see USEPA statement referenced below).

h) Note that the recommended concentration range for adding F to drinking water was
revised by the Ontario Government in 2000, and is now 0.6-0.8 ppm.

i) With respect to the indicator for fluoride, what is it meant to "indicate"? To answer this
question, it is necessary to objectively examine current knowledge about this chemical
additive, and separate scientific fact from science fiction and wishful thinking. Several
"systematic reviews" have recently been completed, focusing on very narrowly defined
questions and studies. One was conducted by the Centre for Review and Dissemination in the
U.K, and another by Dr. David Locker (U of Toronto) for the Ontario Ministry of Health. A
third was by the CDC, and was so blatantly politically-driven (as publicly stated by a panel
member), inaccurate and slanted that it does not merit further consideration. The 2 former
efforts were genuine attempts to get at "the facts" from a very narrow "epidemiological"
angle (which did not consider the kind of information and data sources considered crucial for
examining other environmental exposures and toxicants). Links to the summarized findings,
and subsequent statements issued by the authors, are presented below. It is important to
realise what happened when these studies were publicly released: the findings were contrary
to the prevailing beliefs of public health officials, and were promptly "reinterpreted" by

vested interests, mainly dental and medical associations, which "spun" stories to the media and health practitioners. This in turn caused wide misrepresentation of the current "state of evidence" about both the "benefits" and the adverse effects of this long-standing practice – in the investigators' own publicly issued statements (see below).

What has become clear as a result of these reviews of human epidemiological studies only, is that:

1. there is little or no demonstrable benefit of drinking water fluoridation, especially in the Canadian context
2. whatever benefit there is, is very small (ca. 15%) and, due to the very low current background caries levels (w/o F), amounts to a fraction of one tooth – **from a statistical standpoint, most of the population gets no demonstrable benefit** (further supported by several recent studies where after cessation of fluoridation, caries levels declined to a greater extent than in fluoridated communities- see Locker article, among others) ;
3. after over 50 years of the practice, it has not been possible to demonstrate that fluoridation reduces dental inequalities – poor children do not benefit more
4. dental fluorosis has significantly increased, due to multiple sources, many of which stem from the addition of fluoride to drinking water also used in food processing, which concentrates fluorides in foods and beverages
5. water fluoridation is a major risk factor for dental fluorosis (this is consistently downplayed by dental professionals, who claim miraculous caries reduction benefits of water fluoridation on the one hand, while on the other blaming "other fluoride sources" for the undesired fluorosis effects)
6. dental <u>fluorosis is not a "cosmetic issue", but an adverse health effect,</u> often involving considerable distress and financial costs, and is likely a predisposing factor for tooth decay and tooth loss in later years (teeth are more brittle)
7. dental fluorosis results from the impairment (poisoning) of biological signalling systems (including G proteins) during tooth development, not from accumulation of F in tooth enamel as commonly believed - it is a symptom of systemic fluoride poisoning
8. any potential benefit of fluoride is topical (contact with teeth surfaces where bacteria live); <u>ingestion offers no known benefits,</u> but poses a number of actual (dental and skeletal fluorosis) and probable risks (some noted in the USEPA scientists' statement, Dr. Limeback's statement and the Boston Physicians' statement below)

However, there are a number of other important facts which must be considered:

9. fluoride is a bioaccumulative substance; according to Health Canada, between 50% to 90% of ingested fluoride is <u>retained in the body,</u> in bones as well as soft tissues
10. because fluoride is a general enzyme inhibitor, and also affects other bodily processes and systems including G proteins (which are key regulatory signaling mechanisms for a range of biological and developmental processes), it is completely unreasonable and illogical to assume that it acts only on the teeth when ingested
11. There has been virtually no testing of the pharmacological health effects of fluoride, as is required for any other pharmaceutical – see letter from USEPA below

12. fluoride products have never received approval for use as pharmaceuticals by the usual regulatory agencies (US FDA, Health Canada)
13. the substance which is added to drinking water supplies is not "natural" fluoride (NaF or the relatively insoluble CaF_2 mineral), but a mixture of substances captured in waste streams during the processing of phosphates (usually fertilizers); this mixture, which contains hydrofluosilicic acid (HFS), the fluoridation agent, is chemically and compositionally very different from naturally-occurring fluorides
14. HFS (in either the "industrial grade" added to drinking water, or a purified or pharmaceutical grade) has not undergone testing by any governmental regulatory agencies; it is "approved" as a drinking water additive by the National Sanitation Foundation (NSF, a private NGO with industry & government agency members), which has been <u>unable to provide any evidence that it has followed its own requirements</u> (available on request<u>) for testing drinking water additives for this product</u> (see my letter, below, to the supplier of HSF requesting this information – I have so far received no reply)

15. HFS, being an industrial waste product captured by industrial scrubbers, contains other chemical contaminants, which usually include arsenic, but also radionuclides and a range of other industrial reagents and by-products
16. the epidemiological reviews noted above examined only human epidemiological studies of caries and dental fluorosis (the Locker report did a cursory review of a few additional studies such as hip fractures); they did not consider a broad range of important facts, including the toxicological and biological properties of fluorides: according to the US EPA, <u>fluoride is more toxic than lead, and only slightly less toxic than arsenic</u>; the maximum allowable concentrations in drinking water are: <u>arsenic</u>: Canada- 25 parts per *billion (IMAC);* USA-50 ppb- the US is likely to reduce this to 10 ppb in the near future; <u>lead</u>: 10 ppb; <u>fluoride</u>: 1.5 parts per *million* (Canada); 4 ppm (USA) – again, note that F is a bioaccumulator
17. current intakes of fluoride, as determined by agencies such as Health Canada and NIH/Institute of Medicine, are at levels which are known to pose risks of skeletal fluorosis, a serious, irreversible adverse effect, over the longer term (ingestion over 10 years or more; exposures now occur from before conception and people ingest these levels throughout a lifetime of over 70 years)

Summary of the findings of the NHS fluoridation review by the panel chair:

DEPARTMENT OF HEALTH STUDIES, Innovative Centre, York Science Park, University Road, YORK, YO10 5DG

10/12/2000

In my capacity of chair of the Advisory Group for the systematic review on the effects of water fluoridation recently conducted by the NHS Centre for Reviews and Dissemination the University of York and as it[s] founding director, I am concerned that the results of the review have been widely misrepresented. The review was exceptional in this field in that it was conducted by an independent group to the highest international scientific standards and a summary has been published in the British Medical Journal. It is particularly worrying then that statements which mislead the public about the review's findings have been made in press releases and briefings by the British Dental Association, the National Alliance for Equity in Dental Health and the British Fluoridation Society. I should like to correct some of these errors.

1 Whilst there is evidence that water fluoridation is effective at reducing caries, the quality of the studies was generally moderate and the size of the estimated benefit, only of the order of 15%, is far from "massive".

2 The review found water fluoridation to be significantly associated with high levels of dental fluorosis which was not characterised as "just a cosmetic issue".

3 The review did not show water fluoridation to be safe. The quality of the research was too poor to establish with confidence whether or not there are potentially important adverse effects in addition to the high levels of fluorosis. The report recommended that more research was needed.

4 There was little evidence to show that water fluoridation has reduced social inequalities in dental health.

5 The review could come to no conclusion as to the cost-effectiveness of water fluoridation or whether there are different effects between natural or artificial fluoridation.

6 Probably because of the rigour with which this review was conducted, these findings are more cautious and less conclusive than in most previous reviews.

7 The review team was surprised that in spite of the large number of studies carried out over several decades there is a dearth of reliable evidence with which to inform policy. Until high quality studies are undertaken providing more definite evidence, there will continue to be legitimate scientific controversy over the likely effects and costs of water fluoridation.

(Signed) T.A. Sheldon,
Professor Trevor Sheldon, MSc, MSc, DSc, FMedSci.

Excerpts from the British Fluoridation Society's summary of the York Review:

"The review was set up to establish whether fluoridation is still effective, and whether it is still safe, and the report is unequivocal: **water fluoridation is EFFECTIVE and SAFE.**" (emphasis in original).

"The review findings in relation to general health effects are unequivocal: there is no association between water fluoride and any adverse health effect."

"Importantly, the review also confirms that water fluoridation reduces inequalities in dental health. It narrows the dental health gap between young children living in poverty and their more affluent peers."

Recent article, with Canadian references, from David Locker (director of Community Dental Research at U of T):

<div align="center">

The Science and Ethics of Water Fluoridation
• **Howard Cohen**, BA, MA, PhD •
• **David Locker**, BDS, PhD •

</div>

http://www.cda-adc.ca/jcda/vol-67/issue-10/578.html

Irish Medical Journal editorial

Irish Medical Journal F editorial.url

Public statement from Dr. Hardy Limeback, former fluoride adviser to the Canadian Dental Association, and Head of Preventive Dentistry at U of T:
http://www.fluoridealert.org/limeback.htm

Statement of the US Environmental Protection Agency's Union of Scientists and Professionals:

http://www.fluoridation.com/epa2.htm

Publication by Dr. John Colquhoun former Principal Dental Officer, Auckland, NZ:
Perspectives in Biology & Medicine, reprinted in *Fluoride*
http://www.fluoride-journal.com/98-31-2/312103.htm

UNICEF report (includes current views on mechanism of action):
"It has long been known that excessive fluoride intake carries serious toxic effects. But scientists are now debating whether fluoride confers any benefit at all" - UNICEF
http://www.unicef.org/programme/wes/info/fluor.htm

Greater Boston Physicians for Social Responsibility report
appended after remaining comments

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
NATIONAL RISK MANAGEMENT RESEARCH LABORATORY
CINCINNATI, OH 45268

November 16, 2000

OFFICE OF
RESEARCH AND DEVELOPMENT

Roger D. Masters
Research Professor of Government
Dartmouth College
Department of Government
6108 Silsby Hall
Hanover, New Hampshire 03755-3547

Dear Professor Masters:

We have received your letter dated September 27, 2000, requesting empirical scientific data we may have on the health effects of fluosilicic acid or sodium silicofluoride and manganese neurotoxicity.

To answer your first question on whether we have in our possession empirical scientific data on the effects of fluosilicic acid or sodium silicofluoride on health and behavior, our answer is no. Health effects research is primarily conducted by our National Health and Environmental Effects Research Laboratory (NHEERL). We have contacted our colleagues at NHEERL and they report that with the exception of some acute toxicity data, they were unable to find any information on the effects of silicofluorides on health and behavior.

In answer to your question on empirical information we may have on manganese neurotoxicity, NHEERL scientists forwarded to us several manuscripts with reference sections that contain information on the neurotoxicity of manganese. These are enclosed for your information.

I apologize for the delay in responding to your request and hope you find the enclosed information useful.

Sincerely,

Robert C. Thurnau, Chief
Treatment Technology Evaluation Branch
Water Supply and Water Resources Division

Enclosures

My letter requesting info from supplier

3 October 2000

Mr. Mark E. Looney
Vice President - Inorganic Fluorides
Solvay Fluorides, Inc.
1630 Des Peres Rd., Suite 210
St. Louis, MO 63131

Dear Mr. Looney   **Re:**   **HFS Information - Follow-up**

Thank you for your response to my request for information (dated 19 April, 2000) about the hydrofluosilicic acid (HFS/FSA) product supplied by your company for use in the public drinking water supply in Ottawa-Carleton. I am writing in follow-up, as most of the information requested in my original letter was not made available. In previous correspondence, I had requested the following information:

(1) A certified analysis identifying all the "other materials" referred to in the product specification;

(2) Documentation (or published references) of toxicity testing for each substance identified in (1);

(3) A clarification of the statement that these "other materials" are "nontoxic", as the HFS/FSA supplied to our municipality contains arsenic, which is known to be a toxic substance;

(4) A clarification of the reason(s) for the following statement in Section V of the MSDS for the HFS/FSA supplied to our municipality: "Warning: This product contains detectable amounts of a chemical known to the State of California to cause cancer/birth defects or other reproductive harm";

(5) A description of the specific processes involved at each stage of the production of the HFS/FSA product supplied to our municipality;

(6) Clarification of whether it is possible that other substances (e.g., defoamers, reagents, etc.) may be introduced, directly or indirectly (e.g., via recycled water), into the commercial HFS/FSA product at various stages of the process; and

(7) Documentation (or published references) of toxicity testing for HFS/FSA.

Your company has previously indicated that the HFS product it supplies to our municipality is National Sanitation Foundation (NSF) certified. Because such certification (NSF Certification Standard 60) requires the submission of documentation addressing most of the above issues, it is unclear why there appears to be some difficulty with providing the requested information. I would very much appreciate it if you would supply whatever information is readily available, as well as some specific direction (including contact names and phone numbers) on how to obtain other pertinent documents or some explanation of why this is not possible. It does not seem unreasonable to request copies of documents which have already been submitted to the certifying agency and which were considered satisfactory to obtain certification.

As regards the steps in the production process generating the HFS product, my previous inquiry was also referred to the U.S. EPA and to NSF. However, these agencies have been unable to provide assistance, as they have no involvement in the manufacturing process. It appears that a full and accurate description of the production process will need to be obtained from the producer (in this instance, Solvay Fluorides Inc.).

I hope that you will be able to provide the requested information and/or documentation concerning the points outlined above, at your earliest convenience. The information would be most helpful in enabling the delivery of mandated services. Thank you kindly for your co-operation and assistance.
Yours truly,
Kamila Tomcik   cc: (*deleted*)

To return to the original question: **what is the indicator for fluoride in drinking water meant to indicate?** It does not appear to be a useful or relevant measure of dental health or caries risk. It is a measure of exposure to an environmental toxicant, and can be used to estimate F intake levels (Health Canada). It is also a **direct measure of risk for one adverse health effect, dental fluorosis, as fluoridated drinking water is typically the major F source, contributing 35%-65% of children's daily fluoride intake (Health Canada, 1996).** Dental fluorosis rates are estimated at 15%-45% in unfluoridated communities, and 35%-60% (even higher rates have been reported) in fluoridated communities. Therefore, at least one **new indicator** should be added to this section: the prevalence and severity of dental fluorosis. This is certainly a more appropriate and relevant cross-reference than, say, asthma prevalence and outdoor air pollutant levels. In fact, of all the indicators in this document, the health effect fluorosis is the only one with a direct, unique and causal relation to a specific environmental constituent. **There is no scientific justification for excluding dental fluorosis as an indicator in this section,** particularly when one-third to two-thirds of children in fluoridated areas have demonstrably been permanently affected by systemic toxicity, attributable to one specific contaminant.

However, given the number of other health effects which can reasonably be causally linked with fluoride exposures (check Medline, if documentation provided thus far is not convincing enough), priority consideration should be given to including other health indicators such as osteosarcoma incidence- and possibly other cancers, neurobehavioural diagnoses and related medication prescription in children (e.g., AHDD, autism; related drug prescriptions); incidence/prevalence and age of diagnosis of dementia/alzheimer's disease and arthritis; age of onset of puberty; tooth eruption data, and children's blood lead levels. Further review and analysis of scientific studies, with references, can be found in my unpublished background report (attached separately), which reviewed the health effects and risks of fluoride for the preparation of a State of Environment Report on drinking water quality.

From: CHAPTER 6: Known and Suspected Developmental Neurotoxicants

Pages 90 to 92

Fluoride

Since the 1950's, in many communities throughout the US and other areas of the world, fluoride has been added to community drinking water supplies with the intention of reducing tooth decay. Controversy about the safety of that practice centers around concerns about increased risks of tooth staining and brittleness (dental fluorosis), bone brittleness (skeletal fluorosis), bone cancer, hormone disruption (melatonin), premature puberty, and altered neurological developmental. In addition, some critics argue that fluoridating the water supply has a minimal impact on tooth decay. The practice has been staunchly defended by the American Dental Association and heralded by the Centers for Disease Control and Prevention as one of the major public health success stories of the 20 th century. We do not intend to review the entire controversy here. Recent reviews are found elsewhere (149 150 151). Rather, here we comment briefly on concerns about neurodevelopmental impacts of prenatal exposure to fluoride.

The US EPA sets a Recommended Maximum Contaminant Level of 4.0 ppm fluoride in drinking water. The National Institute for Dental Research considers fluoride at 1 ppm optimal for preventing dental caries. This level may be exceeded in some communities. Additional sources of fluoride, including topical fluoride treatments, fluoride tablets, and fluoride toothpaste, add to the total fluoride burden.

In an animal study, pregnant rats were given 0.13 mg sodium fluoride/kg by injection on 9 separate occasions from days 14-18 or 17-19 during pregnancy (152). Offspring of treated animals and controls were monitored by videotape that was then computer-analyzed in order to quantify various behavioral characteristics. Offspring exposed to fluoride on days 17-19 of pregnancy showed significant hyperactivity. They tended to move from one activity to another more frequently than unexposed animals. This study has been criticized for using excessive fluoride exposures. The authors respond by noting that the blood levels of fluoride in the treated animals were similar to the levels measured in people who are exposed through fluoridated water. Another criticism centered on the lack of biological plausibility that the results would differ in the two groups exposed at similar times during pregnancy (153). The authors, however,

point out that vulnerable developmental stages change rapidly during this time window and argue that the findings are entirely plausible (154).

Another study found that the offspring of rats given 5, 15, 50 ppm fluoride in drinking water during pregnancy and lactation had significantly elevated acetylcholinesterase levels when tested at 80 days of age (155). Maternal acetylcholinesterase levels were also increased. Though not measured in this study, a likely result of elevated acetylcholinesterase activity is decreased acetylcholine levels. As we have noted, the enzyme, acetylcholinesterase, and the neurotransmitter, acetylcholine, play important roles in brain development. Changes in the concentrations of any neurotransmitter during development may have permanent neurological consequences. The largest effect was seen at 5 ppm, decreasing at the higher levels.

Two reports from China identify significantly lower childhood IQs in communities where fluoride exposure is elevated. In one community, where drinking water naturally contains 4.12 ppm fluoride, IQs were significantly lower than in a nearby community with fluoride levels at 0.91 ppm (average IQ 98 vs. 105)(156). This difference persisted when the study population was controlled for parental educational level. The authors describe similar occupations, living standards, and social customs in the two communities. The ecologic design of this study imposes some limits on the conclusions that may be drawn since the exposure (fluoride) and outcome (IQ) were compared on a population-wide basis without any attempt to associate individual fluoride exposure levels with individual IQs. Nonetheless, an IQ shift of 7 points in an entire population has large population-wide implications, as well as impacting individual members, and these results deserve close attention.

In the other study, investigators used dental fluorosis and urinary fluoride levels to stratify children into four quartiles(157). Elevated fluoride exposures were associated with decreased IQs in this population. That is, the distribution of IQ scores in children in each quartile of fluoride exposure shifted progressively down-ward as the fluoride exposures increased.

Conclusion
Studies in animals and human populations suggest that fluoride exposure, at levels that are experienced by a significant proportion of the population whose drinking water is fluoridated, may have adverse impacts on the developing brain. Though no final conclusions may be reached from available data, the findings are provocative and of significant public health concern. Perhaps most surprising is the relative sparseness of data addressing the central question of whether or not this chemical, which is intentionally added to drinking water, may interfere with normal brain development and function. Focused research should address this important matter urgently.

In the sidebar:
Studies in animals and human populations suggest that fluoride exposure, at levels that are experienced by a significant proportion of the population whose drinking water is fluoridated, may have adverse impacts on the developing brain.

References:

149 Hileman B. Fluoridation of water. Chem Eng News 66:26-42, 1988.

150 Connett P. Fluoride: a statement of concern. Waste Not #459. Canton NY.

151 CDC. Fluoridation of drinking water to prevent dental caries. MMWR 48:986-993, 1999.

152 Mullenix PJ, Denbesten PK, Schunior A, Kernan W. Neurotoxicity of sodium fluoride in rats. Neurotoxicol Teratol17(2):169-177, 1995.

153 Ross J, Daston G. Letter to the editor. Neurotoxicol Teratol 17(6):685-686, 1995.

154 Mullenix P, Denbesten P, Schunior A, Kernan W. Reply. Neurotoxicol Teratol 17(6):687-688, 1995.

155 Zhao XL, Wu JH. Actions of sodium fluoride on acetylcholinesterase activities in rats. Biomed Environ Sci 11(1):1-6, 1998.

156 Zhao LB Liang GH, Zhang DN, et al. Effect of a high fluoride water supply on children's intelligence. Fluoride 29(4):190-192, 1996.

157 Li XS, Zhi JL, Gao RO, Effect of fluoride exposure on intelligence in children. Fluoride 28(4):189-192, 1995.

NOTE (appended September 2002): THE FOLLOWING REPORT PRESENTS AN UNPUBLISHED REVIEW OF FLUORIDE RESEARCH AS IT RELATES TO HUMAN HEALTH RISKS. IT CONTAINS PUBLICLY-AVAILABLE INFORMATION (REFERENCES ATTACHED) FROM VARIOUS SOURCES. THE REPORT HAS BEEN PEER-REVIEWED, BUT <u>HAS NOT BEEN APPROVED OR ENDORSED BY ANY AGENCY OR MEDICAL AUTHORITY</u>.

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Executive Summary

The following report was prepared as background material for the (*deleted text*) State of Environment (SOE) Report on Drinking Water Quality, one of a series of reports examining health risks from environmental exposures. The background report is not intended as a comprehensive review of all relevant literature on fluoride, as that would be beyond the scope of the report series. Rather, it attempts to summarise recent scientific information on the health effects of fluoride in drinking water, with particular emphasis on health risks. The Health Department's SOE reports are directed to examine existing and potential environmental risk areas and populations, pursuant to the Mandatory Programs and Services of the Ontario Ministry of Health.

Fluoride is a unique constituent of the Regional drinking water supply, as it is the only substance which is added as a medication at the end of the treatment process. Fluoride is an element found naturally in rocks and soils, and used widely in industry. People ingest fluoride from a variety of sources, including foods, dental products and (naturally or artificially) fluoridated drinking water. Although fluoride has been classified as a beneficial element in the past, Health Canada and the U.S. Public Health Service now state that there is no evidence that it is required for growth or reproduction (Health Canada, 1996; Department of National Health and Welfare, 1990; U.S. PHS, 1991). The concentration of fluoride in the Ottawa River, the source of the Regional piped supply, is low (0.03 mg/L in 1997). Fluoride has been added to the Regional drinking water supply since the mid-1960s in the belief that it is a preventive agent against tooth decay. Fluoride is a very reactive chemical, which can interact with a wide range of bodily tissues and their biochemical processes[1], discussed briefly below.

Fluoride intakes and sources

In Canada, average total fluoride intake is approximately 4.4 mg/day, depending on age (Health Canada, 1996; reviewed by Nosal, 1998). Typical intakes in areas with 1 ppm fluoride in the drinking water range from 3 to 9 mg/day (U.S Department of Health and Human Services, 1991[2]). This represents an increase of an order of magnitude (10-fold)

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[1] If fluoride did not have biological effects, it could not be expected to affect caries rates.

[2] The U.S. Public Health Service estimates F intakes as 2.1-9.1. mg/day in fluoridated areas (at 0.7-1.2 mg/L) and 1.1-2.8 mg/day in non-fluoridated (<0.3 mg/L) areas. (Foulkes, RG. Review of "Dietary

compared with average fluoride intakes in the 1940s, when fluoridation of drinking water was first introduced (ibid.). For children, the two major sources of ingested fluoride are drinking water, which contributes 35% to 65% of fluoride intake, and dental products such as toothpaste (Minister of Supply and Services Canada, 1993; Clark, 1993). For adults in fluoridated communities, drinking water is the single main source of fluoride, contributing 30-45% of total daily intake (Minister of Supply and Services Canada, 1993; reviewed by Nosal, 1998). Health Canada estimates of daily fluoride intakes in fluoridated and unfluoridated communities for various age groups are shown in Table 2. Fluoridated water contributes fluoride to foods cooked in it and to processed foods such as juices, soups and other beverages which are reconstituted with fluoridated water (ibid.; Minister of Supply and Services Canada, 1993).

Fluoride is eliminated from the body mainly by the kidneys, at rate dependent on age. Over 90% of ingested fluoride is absorbed (Health Canada, 1996). Up to 75% of absorbed fluoride is deposited in calcified tissues (bones and teeth), which account for 99% of total body fluoride (ibid.). Although fluoride accumulates in calcified tissues, it is also found in soft tissues and organs (Waldbott, 1976). Fluoride readily passes from mother to foetus across the placenta (Health Canada, 1996). In people with reduced kidney function (including diabetics), the ability to excrete fluoride decreases significantly (Minister of Supply and Services Canada, 1993; Kono, 1994). Wide variations in excretion rates have also been documented in "normal" individuals (Waldbott, 1962).

Health effects

The health benefits and risks of fluoride exposure have been and continue to be under scientific debate. Studies cited by agencies such as the World Health Organization and dental and medical associations in Canada, the U.S., and elsewhere claim that fluoride in water can help prevent tooth decay, especially in children (Foulkes, 1997; Health Canada, 1996; Department of National Health and Welfare, 1990; Hileman, 1988). The suggested "optimal daily requirement" of fluoride ingestion for these benefits is 1 mg/day (e.g., Clark, 1993). It is now generally recognised that any beneficial effect of fluoride is due to the topical contact of fluoride with the teeth, not to ingestion and incorporation into tooth enamel (Limeback, 1999; Colquhoun, 1997; Gray, 1987; A. Burry, Ottawa-Carleton Health Dept., personal communication). Topical fluoride is thought to affect the rate of enamel demineralization and remineralization, thereby possibly strengthening tooth surfaces (Gray, 1987). Fluoride likely also inhibits bacterial growth in the mouth by disrupting enzymes or other physiological functions (NTEU, 1999). The benefit which is ascribed to fluoridated water currently amounts to less than one decayed tooth per person, on average (Diesendorf et al., 1997; Gray, 1987).

Reference Intakes, calcium, phosphorus, magnesium, vitamin D and fluoride" by the Institute of Medicine, National Academy of Science, Washington, DC. In: Fluoride 30:4, 1997).

On the other hand, many scientific studies in various countries have been unable to show that drinking water fluoridation reduces caries rates. For instance, in British Columbia, school districts with the highest caries-free rates were totally unfluoridated (Gray, 1987). Similar findings were reported in Alberta, where there was no statistically significant difference in caries rates between unfluoridated Calgary and fluoridated Edmonton (City of Calgary, 1998, p. 27) and in Nova Scotia (Ismail, 1993). Examinations of over 39,000 U.S. schoolchildren (aged 5-17) by dentists trained by the National Institute of Dental Research found no statistically significant differences between decay rates of permanent teeth or the percentages of decay-free children in fluoridated and unfluoridated areas[3] (Yiamouyiannis, 1990). Examples in other countries include New Zealand (Colquhoun, 1997), India (Teotia and Teotia, 1994), several European countries and Japan (Ziegelbecker, 1998; Diesendorf, 1986), the U.S. (New York State Dept. of Health. 1999; Glass, 1981; DePaola, 1982; Zacherl & Long, 1979), South Africa (Hartshorne et al., 1994), England and Sri Lanka (Nunn et al., 1994) and Australia (Diesendorf, 1986). Caries rates have been declining over the past 40-50 years in many parts of the world, prior to and independently of fluoridation (Diesendorf, 1986; Hileman, 1988; Gray, 1987; Colquhoun, 1997; Ziegelbecker, 1998; Angelillo et al., 1999) Locations where fluoridation of drinking water has been stopped[4] and dental caries rates have continued to decline include Germany (Ziegelbecker, 1998, Kunzel, 1997), Japan (Takahashi, 1998, Ziegelbecker, 1998), Finland (Seppa et al., 1998), and the Netherlands (Kalsbeek et al., 1993, Ziegelbecker, 1998).

There are several possible explanations for the different conclusions about whether or not fluoride protects teeth from decay, ranging from poor study design and analysis errors in most early studies, to confounding factors including environmental and social changes such as eating habits and nutrition, personal hygiene and health care (Diesendorf, 1986; Ismail, 1998; Colquhoun, 1987; Ziegelbecker, 1998; Hileman, 1988; Moss et al., 1999). Some suggest that caries has declined in areas which do not fluoridate drinking water due to a "halo effect", meaning the introduction of fluoride into beverages and foods processed with fluoridated water (Limeback, 1993; Gray, 1987) and the use of fluoridated dental products and salt (Gray, 1987; Nosal, 1998). However, this cannot be the only factor responsible, as caries reductions have also been observed in areas without such products (Diesendorf, 1986; Colquhoun, 1997; Nosal, 1998). Moreover, caries has continued to decline in areas where all children had been exposed to fluoride for all their lives, which means that fluoride cannot be the chief factor responsible (Diesendorf, 1986; Colquhoun, 1997). Another consideration is the finding that the most prevalent type of caries in

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[3] Fluoridation status information was based on reports published by the U.S. Public Health Service, with some local agency verification.

[4] These have included: the Federal Republic of Germany (fluoridation introduced in 1952, stopped 1971), Sweden (introduced 1952, stopped 1971); the Netherlands (introduced 1953, stopped 1976); Czechoslovakia (introduced 1958, stopped 1988/90); the German Democratic Republic ()introduced 1959, stopped 1990 (1993 in Spremberg)); the Union of Soviet Socialist Republics (introduced 1960, stopped 1990); Finland (introduced 1959, stopped 1993); Japan (introduced 1952, stopped 1972). In most cases, fluoridation was stopped for ethical, legal and health reasons (Hileman, 1988; Ziegelbecker, 1998).

North America (pit and fissure) are not considered treatable by fluorides (Gray, 1987, cites Becker, 1967). Yet another possible factor is that fluoride merely delays tooth decay, instead of preventing it (Sutton, 1980 - cites Weaver, 1944, 1948; Pauley, 1957; Carlsson, 1978; Royal College of Physicians, 1976). There is some evidence that this delay might be due, at least in part, to delayed tooth eruption (Szelag, 1990; Yiamouyiannis, 1990; Sutton, 1980 - cites Feltman & Kosel, 1961, Dr. JW Benfield's clinical observations, New York; Krook and Maylin, 1979). Lastly, any initial caries protective effect of fluoride may be offset by the development of fluorosis, which can result in tooth damage and decay (Kim, 1984; Waldbott, 1978, ch. 12).

Although the ingestion of fluoride confers no known benefits (Health Canada, 1996; U.S. Public Health Service, 1991), it can pose acute and chronic health risks. Risks from short-term exposure to concentrations below 2 ppm in water can include gastrointestinal, dermal and neurological symptoms, at levels as low as 0.25 mg F⁻ ion (Canadian Compendium of Pharmaceuticals and Specialties, 1989) or lower (Grimbergen, 1974; Petraborg, 1974; Waldbott, 1998; Susheela et al., 1992, 1993; Shea et al. 1967; Burgstahler et al., 1998; Desarathy et al., 1996; Waldbott, 1956, 1998; Hileman, 1988). The proportion of the population which is hypersensitive to fluoride has not been determined.

The ability of fluoride to accumulate in the body can pose health risks from chronic exposure to low levels. The most widely recognised risks involve toxicity to teeth, bones and connective tissues, termed fluorosis (e.g., ATSDR, 1999; Health Canada,1996). Dental fluorosis, the first visible sign of systemic fluoride toxicity, is a disturbance in the formation of tooth enamel by ameloblasts, the tooth-forming cells in the jaw during tooth development (i.e., in children). It is a progressive effect: the mildest forms are a barely noticeable mottling of tooth surfaces, while more severe cases result in staining and pitting of the teeth. Dental fluorosis can also cause increased brittleness and caries, resulting in a higher rate of tooth loss due to fluorosis in adulthood in comparison with normal teeth[5] (New York State Dept. of Health, 1999; Kim, 1984; Waldbott, 1978, ch. 12, cites Smith and Smith, 1940). Several fluoride-induced effects may be involved in the etiology of fluorosis, but current evidence suggests that inhibition of enzymatic degradation of amelogenins, causing delay their removal from the developing enamel and impaired crystal growth, may be the primary mechanism (Whitford, 1997).

Dental fluorosis rates have been increasing (Gray, 1987; Limeback, 1993; Clark, 1993; Colquhoun, 1997); fluoride intakes are currently much higher (10 times or more) than they were when fluoridation was first introduced in the 1940s and 1950s (Burgstahler et al., 1998; Nosal, 1998, Gray, 1987). The majority of dental fluorosis cases in Canada are

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[5] The former Director of the Office of Drinking Water, U.S. EPA, wrote: "It is difficult to conclude *a priori* that teeth which spontaneously pit are stronger teeth. Further, data suggest that the effects of fluorosis are not merely discoloration and pitting, but fracturing, caries and tooth loss as well...it is difficult to conclude... that such effects are not adverse" (Kim, 1984).

mild, but the prevalence of moderate to severe fluorosis also appears to be rising (Clark, 1993). In Canada, dental fluorosis typically affects between 35% - 60% of children in communities with fluoridated water, and 15% - 45% in communities with nonfluoridated water, depending on the extent of water fluoridation, proximity to water fluoridation and consistency of the recommended concentration of fluoride in water systems (Clark, 1993). Similar ranges have been reported in other countries (Angelillo et al., 1999). A 1998 survey of Ontario children reported up to 30% incidence of moderate fluorosis, and up to 10% of severe fluorosis (Cutter, 1998). The extent, severity and rate of increase of dental fluorosis in Ottawa-Carleton children have not been assessed (Cutter, 1998), but are consistent with national trends (A. Burry, Ottawa-Carleton Health Dept, 1999, pers. comm.). It has been found that dental fluorosis rates are typically about two times higher in fluoridated areas than in areas where water is not fluoridated (Clark, 1993; Weeks et al., 1993). Because fluoride is also present in various foods and beverages, many children and nearly all adults are consuming fluoride at levels far in excess of the "optimum daily requirement" of 1 mg/day (Health Canada, 1996; Lewis and Limeback, 1996). Fluoride in drinking water contributes significantly (35%-65%) to children's daily intakes (Health Canada, 1996), and thus to associated fluorosis risks.[6]

Fluoride build-up in bones can lead to gradual calcification of bones, joints and ligaments, with symptoms such as arthritis and bone brittleness (Roholm, 1937; Hileman, 1988; Whitford, 1996). The early stages of skeletal fluorosis can occur at intake levels of 2-5 mg/day (Whitford, 1996; Hileman, 1988). Several well-designed epidemiological studies have found a strong association between fluoridation and bone fractures in the elderly, although not all studies have reported such associations (major publications reviewed by Nosal, 1998 - cites Danielson, 1992; Jacobsen 1990/92; Jacqmin-Gadda, 1995; Sowers, 1986, 1991; Suarez-Almazor, 1993). Health and Environment Canada state that the weight of evidence in ecological studies indicates that there may be an association between the consumption of fluoridated drinking water and an increased incidence of hip fracture, particularly among the elderly (Minister of Supply and Services Canada, 1993 - additional studies cited include Cooper et al, 1991, Keller, 1991, and May and Wilson, 1991, both cited in Gordon and Corbin, 1992). The effects of fluoride on childhood bone development are also largely unknown, but there is evidence of an association between bone and collagen abnormalities and dental fluorosis in children (Chlebna-Sokol & Czerwinski, 1993; Yiamouyiannis, 1993; Shen et al., 1992). Fluoride compounds have been found to cause changes in collagen (Pawlowska et al., 1998, cite: Ammintzbool et al., 1988; Grucka-Mamczar et al., 1992; Veron and Couble, 1992), and glycosaminoglycan metabolism (Susheela and Kharb, 1990; Pawlowska et al., 1998, cite: Ammintzbool et al., 1988; Grucka-Mamczar et al., 1992) in animal and in tissue culture experiments. Collagen is a major structural component of skin, bones, teeth, ligaments, tendons, muscles and cartilage. Accumulation of fluoride in heart tissues may be implicated in cardiovascular

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[6] For children, swallowing toothpaste and other dental products such as rinses is a significant additional risk factor for excessive fluoride intake.

disease, although very little research has been done in this area (Waldbott, 1962, 1978; Burgstahler, 1974).

Other studies suggest that fluoride can have much broader effects, including disruption of homeostatic and signalling mechanisms, and hormones, particularly in males (Luke, 1994; Boeckhabisch et al., 1997; Susheela and Jethanandani, 1996; Kranwar et al., 1983). Fluoride is a powerful enzyme inhibitor, and is able to form complex ions with nearly all metal ions other than group I metals (the sodium family) (ATSDR, 1993; Connett, 1998; Baykov et al., 1992). It can also form very strong hydrogen bonds with the amide function in proteins and nucleic acids (ibid.), and can replace hydroxyl (OH$^-$) groups in molecules, including hydroxylapatite in teeth and bones (Health Canada, 1996).

Of particular note is the ability of fluoride-aluminum complexes to interact with G proteins[7], which transduce signals for over 1,000 proteins, hormones, neurotransmitters, chemokines, local mediators and sensory stimuli (Hamm, 1998; Farfel et al., 1999). G proteins help to regulate ion channels, metabolism, gene expression, and cytoskeletal structures (ibid.). Fluoroaluminate (AlF$_4^-$ or AlF$_3$(OH)$^-$) can activate certain G proteins[8] causing osteoblast proliferation and differentiation, and modulation of the adhesion properties of osteoblasts which in turn affects cellular differentiation, migration and apoptosis (programmed cellular death) (Susa, 1999).[9] Several studies have found fluoride to be carcinogenic, but the issue has not been resolved (Toft, 1960; Hileman, 1988; NTP. 1990; Yiamouyiannis, 1993; Marcus, 1990; New Jersey Dept. of Health, 1992; Health Canada, 1996). Fluoride can damage chromosomes at a concentration of 10 parts per billion and is a recognised mutagen; mutagens are in general considered potential carcinogens (Health Canada, 1996). Of particular interest is that Ras oncogenes, implicated in 25% of human cancers, are G proteins and regulate many vital cellular processes (Sprang, 1997; Ferrante et al., 1999; Fahraeus et al., 1999; Agapova et al., 1999; Davidson et al., 1999; Beaupre and Kurzrock, 1999; Bourne, 1997). Because G proteins are involved in the regulation of so many physiological processes, there is a strong possibility that their interaction with fluoroaluminate can play a role in many health conditions and symptoms. As well, AlF$_4^-$ binding can either enhance or reduce normal G protein signal transmission (Bourne, 1997). Both increased and decreased G protein signal transmission has been linked to human diseases (Farfel et al., 1999).

Recent convergent animal and human studies strongly suggest that fluoride may be neurotoxic, both during foetal and early childhood development and in the aging process (dementia) (Grimbergen 1974; Hileman, 1988; Waldbott, 1956, 1962, 1976, 1998; Liu,

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[7] Guanine nucleotide binding proteins.
[8] Pertussis toxin-insensitive proteins, probably from Ga 12 class (Susa, 1999).
[9] It is noteworthy that activation of kinases and protein phosphorylation are key steps in the activation of some oncogenes. Increased cellular proliferation, altered cell differentiation, and changes in cell adhesion and migration properties are important features of carcinogenic processes.

1989; Li et al., 1994, 1995; Yang et al., 1994; Zhao et al., 1994; Mullenix et al., 1995; Varner et al., 1993, 1995; 1998; Guan et al., 1998; Zhao and Wu, 1998; Spittle, 1994). This effect may arise as a result of fluoride's complexation with aluminum, at levels as low as 1 ppm in water (Varner et al., 1995, 1998; Guan et al, 1998; Isaacson, 1997; Ahn et al., 1995), which may affect the permeability of membranes including the blood-brain barrier (Machoy-Mokrzynska and Machoy, 1992; Guan et al., 1998; Kumari & Rao, 1991; Susheela & Kumar, 1991; Varner et al., 1998).

Guideline

The Maximum Acceptable Concentration of fluoride in drinking water was recently reviewed and maintained at 1.5 mg/L. The "optimum range" was lowered from 1.0-1.2 to 0.8-1.0 mg/L (Health Canada, 1996). The fluoride concentration in the Regional drinking water supply was dropped from 1.0 to 0.8 ppm in June, 1999. This makes fluoride one of the few drinking water contaminants for which the Maximum Acceptable Concentration (MAC, 1.5 ppm) does not include a margin of safety of one or more orders of magnitude.[10] For most toxic substances[11], safety factors (the difference between exposure level and expected toxic level) of at least two orders of magnitude are considered necessary, especially for chronic exposures (Hodge, 1963; examples in ATSDR, 1997).

In the table below, the Health Canada Tolerable Daily Intake, the ATSDR Minimal Risk Level for chronic ingestion, and the U.S. Public Health Service Minimal Toxic Dose (acute ingestion) (Calabrese et al., 1999 (1997)), are compared with fluoride intakes (Health Canada, 1996) in fluoridated and unfluoridated Canadian communities.

Fluoride Intakes, Guidelines and Minimal Toxic Doses - in μg/kg bw/day

Tolerable Daily Intake*	Minimal Risk Level** Chronic	Minimal Toxic Dose Acute	Average total daily intake - children: 7 mo-4 yr.	Average total daily intake - adults
122	50	40	**87-160** (fluoridated) 45-96 (unfluoridated)	**47-58** (fluoridated) **32-36** (unfluoridated)

* based on risk of moderate to severe dental fluorosis; ** based on musculoskeletal toxicity

The maximum recommended guideline levels for fluoride intakes set by government agencies and documented toxicity thresholds are being consistently and substantially exceeded in fluoridated areas, especially by children. At current fluoride intake levels in fluoridated communities, at least a portion of the adult population is likely to be at risk

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[10] As elaborated in section 4.7 of the Health Department's SOE Report on Drinking Water Quality, 1999.

[11] Fluoride is designated as a toxic substance under the Canadian Environmental Protection Act.

skeletal fluorosis over the longer term. Fluoride intakes by young children exceed the MRL and MTD levels by 200% to 400%. Average intakes by many young children from drinking water alone[12] exceed the MTD (40 µg/kg bw/day) by more than two-fold. Intakes by many adults also exceed both the MTD and the MRL. Health Canada lists the Tolerable Daily Intake (TDI) for fluoride as 122 µg/kg bw/day, on the basis that it is unlikely to produce moderate to severe dental fluorosis in children 22-26 months old (Health Canada, 1996). Mild dental fluorosis is the result of fluoride toxicity to tooth-forming cells; thus the TDI level is set at a toxic endpoint. Health Canada acknowledges that some children in fluoridated communities exceed the TDI for fluoride (Health Canada, 1996). Health and Environment Canada state that "average daily intakes [of fluoride] are at least 20% less than the level at which adverse effects upon the skeleton are anticipated" (Health Canada, 1996, p. 13; Minister of Supply and Services Canada, 1993). The anticipated effects for people with above-average intakes, factors such as variations in fluoride metabolism and hypersensitivity (which are dependent on such factors as age, nutritional and health status), and recent publications linking fluoride ingestion to neurotoxicity were not explicitly considered in this estimation.

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[12] Fluoridated water contributes 35% to 65% of children's total fluoride intake (Health Canada, 1996d).

NOTE (appended September 2002): THE FOLLOWING REPORT
PRESENTS AN UNPUBLISHED REVIEW OF FLUORIDE
RESEARCH AS IT RELATES TO HUMAN HEALTH RISKS. IT
CONTAINS PUBLICLY-AVAILABLE INFORMATION (REFERENCES
ATTACHED) FROM VARIOUS SOURCES. THE REPORT HAS BEEN
PEER-REVIEWED, BUT <u>HAS NOT BEEN APPROVED OR
ENDORSED BY ANY AGENCY OR MEDICAL AUTHORITY</u>.

Fluoride in Drinking Water: A Focus on Health Risks

Introduction

The following report was prepared as background material for the (*deleted*) State of
Environment (SOE) Report on Drinking Water Quality, one of a series of reports
examining health risks from environmental exposures. The background report is not
intended as a comprehensive review of all relevant literature on fluoride, as that would be
beyond the scope of the report series. Rather, it attempts to summarise recent scientific
information on the health effects of fluoride in drinking water, with <u>particular emphasis
on health risks</u>. The Health Department's SOE reports are directed to examine existing
and potential environmental risk areas and populations, pursuant to the Mandatory
Programs and Services of the Ontario Ministry of Health.

Fluoride is a unique constituent of the Regional drinking water supply as it is the only
substance which is added as a medication at the end of the treatment process, without the
intent of improving the safety of the finished drinking water.

Fluoride is an element found naturally in rocks and soils, and used widely in industry.
People ingest fluoride from a variety of sources, including foods, dental products and
(naturally or artificially) fluoridated drinking water. Fluoride levels in water are highly
variable. The concentration of fluoride in the Ottawa River, the source of the Regional
piped supply, is low (0.03 mg/L in 1997). Fluoride has been added to the Regional
drinking water supply (and to various dental products) since the 1960s in the belief that it
is a preventive agent against tooth decay. Wells in rural areas contain variable
concentrations of fluoride, as discussed in section 4.9.5.1 of the SOE Report on Drinking
Water Quality.

Although Health Canada had classified fluoride as a beneficial element in the past, it now
states that "attempts to demonstrate its essentiality for growth and reproduction in
experimental animals have not been successful" (Health Canada, 1996; Department of
National Health and Welfare, 1990). The U.S. Public Health Service states that "...there

is no conclusive evidence that fluorine or any of the fluoride compounds are essential for human homeostasis or growth" (U.S. PHS, 1991).

Overview of fluoride metabolism

Fluoride is an extremely reactive chemical, which can interact with a wide range of bodily tissues and their biochemical processes[13]. The fluoride ion is very small and has a high charge density (very electronegative), enabling penetration into virtually all cells and chemical reactions with other ions (Waldbott, 1976). Fluoride is a powerful enzyme inhibitor, and is able to form complex ions with nearly all metal ions other than group I metals (the sodium family) (Connett, 1998). It can also form very strong hydrogen bonds with the amide function in proteins and nucleic acids (ibid.), and can replace hydroxyl (OH⁻) groups in molecules, including hydroxylapatite in teeth and bones (Health Canada, 1996). Although fluoride accumulates in calcified tissues, it is also found in soft tissues and organs (Waldbott, 1976).

Fluoride is eliminated from the body mainly by the kidneys, at rate dependent on age. Over 90% of ingested fluoride is absorbed (Health Canada, 1996). Up to 75% of absorbed fluoride is deposited in calcified tissues (bones and teeth), which account for 99% of total body fluoride (ibid.). As much as 75% of daily fluoride intake may be incorporated into bones, especially in children with active bone growth and tooth development and in people consuming unfluoridated drinking water (Minister of Supply and Services Canada, 1993). It is incorporated into the developing bone lattice by replacing hydroxyl (OH⁻) groups in hydroxylapatite, forming fluorapatite (ibid.). Fluoride can be mobilized from bone through ion-exchange or bone remodelling, for instance, during menopause (ibid.). Soft tissues and organs can also accumulate significant amounts of fluoride, with wide individual variations in concentration levels (Waldbott, 1962). Fluoride readily passes from mother to foetus across the placenta (ibid.). In people with reduced kidney function (including diabetics), the ability to excrete fluoride decreases significantly (Minister of Supply and Services Canada, 1993; Kono, 1994). Wide variations in excretion rates have also been documented in apparently "normal" individuals (Waldbott, 1962).

Fluoride intakes and sources

In Canada, average total fluoride intake is approximately 4.4 mg/day, depending on age (Health Canada, 1996; reviewed by Nosal, 1998). Typical intakes in areas with 1 ppm fluoride in the drinking water range from 3 to 9 mg/day (Nosal, 1998 - cites U.S Department of Health and Human Services, 1991[14]). This represents an increase of an

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[13] If fluoride did not have biological effects, it could not be expected to affect caries rates.

[14] The U.S. PHS estimates F intakes as 2.1-9.1. mg/day in fluoridated areas (at 0.7-1.2 mg/L) and 1.1-2.8 mg/day in non-fluoridated (<0.3 mg/L) areas.(Foulkes, RG. Review of "Dietary Reference Intakes,

order of magnitude (10-fold) compared with average fluoride intakes in the 1940s, when fluoridation of drinking water was first introduced (ibid.).

Uptake and absorption of inorganic fluorides by aquatic and terrestrial animals and in humans tends to be higher from water than from food (Waldbott, 1962; Minister of Supply and Services Canada, 1993). It has been reported that some ions, in particular calcium, may be strong antagonists of fluoride and may inhibit its intestinal absorption (Waldbott, 1962; Teotia and Teotia, 1994). Foods contain varying amounts of inorganic fluoride; it has been reported that between 34% and 79% of the total fluoride content of food is inorganic (ibid., cites Singer and Ophaug, 1983). Certain foods, particularly some seafood, certain meats and eggs, and tea, can contain over 4 ppm fluoride, although concentrations can be more than 10 times below this level (ibid.; Waldbott, 1962). Human breast milk is naturally low in fluoride, even when the mother ingests high levels of fluoride (Minister of Supply and Services Canada, 1993; Health Canada,1996d). Fluoride concentrations in toothpastes range from 1000 to 1500 ppm (Whitford, 1987, cited in Minister of Supply and Services Canada, 1993). One gram of toothpaste, the approximate amount used for brushing, therefore contains at least 1 mg fluoride (Gray, 1987). Fluoride excretion is highly variable among individuals, and even for a given individual over time (Waldbott, 1962).

For children, the two major sources of ingested fluoride are drinking water and dental products such as toothpaste (Clark, 1993). For adults in fluoridated communities, drinking water is the single main source of fluoride, contributing 30-45% of total daily intake by adults (Minister of Supply and Services Canada, 1993; reviewed by Nosal, 1998). Various foods together contribute the major portion of total fluoride, as seen in Table II below (Health Canada, 1996). Fluoridated water contributes fluoride to foods cooked in it and to processed foods such as juices, soups and other beverages which are reconstituted with fluoridated water (ibid.; Minister of Supply and Services Canada, 1993).

Children's water consumption patterns, and corresponding fluoride intakes are summarised in Table I below. It should be noted that the body weights listed are calculated averages, so that intakes in terms of body weight will actually be significantly higher in a portion of the younger group (especially young infants who are not exclusively breast-fed), and lower in a portion of the older group.

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calcium, phosphorus, magnesium, vitamin D and fluoride" by the Institute of Medicine, National Academy of Science, Washington, DC. In: Fluoride 30:4, 1997).

Table I   **Water consumption and average fluoride intakes in children**

Age range	Average body weight* (kg)	Daily water consumption (90% of population) (L)**	Daily fluoride intake from drinking water at 1 ppm fluoride (90% of population) (mg)	Typical daily fluoride intake from water, in µg/ kg of body weight
0-<6 months (not breast fed)***	7	0.75	0.75	107
6 months-3 years	13	0.87	0.87	67
3-5 years*	13*	1.5	1.5	115

- *13 kg based on average for children 7 months to 4 years old (Canadian Council of Ministers of the Environment (CCME), 1996). This number is also used in the Health Canada estimates listed in II. The value of 7 kg for 0-6 month old infants is from Health Canada (1996d).

- **As reported by Health Canada (1996d) for 0-6 month-olds and Clark (1993), for the other 2 groups.

- *** Estimated daily intake from breast milk is 0.47 to 1.05 µg/kg bw (Health Canada, 1996).

Health Canada estimates daily fluoride intakes from various sources as follows (Health Canada, 1996):

Table II   **Estimated daily intake of fluoride for children and adults in Canada (µg/kg bw/day)**

Age group	Type of community	Air	Soil	Food	Tooth-paste	Drinking water	Total	Percent of daily intake from drinking water
7 months-4 years	Non-fluoridated	0.01	0.02-1.19	22.3	20-60	3.08-12.92	**45.41-96.42**	4%-23% avg. 15%
7 months-4 years	Fluoridated	0.01	0.02-1.19	22.3	20-60	44.92-76.92	**87.25-160.42**	35%-65% avg. 50%
20+ years	Non-fluoridated	0.01	0.002-0.09	30.08	1.14	1.07-4.50	**32.30-35.82**	3%-13% avg. 8%
20+ years	Fluoridated	0.01	0.002-0.09	30.08	1.14	15.64-26.79	**46.87-58.11**	33%-46% avg. 40%

Health effects

Beneficial effects

The health benefits and risks of fluoride exposure have been and continue to be under scientific debate. Many studies cited by agencies such as the World Health Organization and dental and medical associations in Canada, the U.S., and elsewhere claim that fluoride in water can help prevent tooth decay, especially in children (Foulkes, 1997; Health Canada, 1996; Department of National Health and Welfare, 1990; Hileman, 1988). The suggested "optimal daily requirement" of fluoride ingestion for these benefits is 1 mg/day (e.g., Clark, 1993). It is now generally recognised that any beneficial effect of fluoride is due to the topical contact of fluoride with the teeth, not to systemic effects due to incorporation of ingested fluoride into tooth enamel (Limeback, 1999; Colquhoun, 1997; Gray, 1987; A. Burry, Ottawa-Carleton Health Dept., personal communication). Hence, fluoride ingestion offers no known benefits. Topical fluoride is thought to affect the rate of enamel demineralization and remineralization, thereby possibly strengthening tooth surfaces (Gray, 1987). There is also some indication that fluoride may inhibit bacterial growth in the mouth by disrupting enzymes or other physiological functions (NTEU, 1999).

Nonetheless, many other studies have not found fluoride in drinking water to be beneficial in preventing tooth decay. This large body of published scientific research is usually omitted from the numerous publications promoting fluoridation. For example, the position statements on fluoridation listed on the websites of the Canadian Dental Association and the American Dental Association, the Canadian Medical Association and the Canadian Pediatric Society (attached in Appendix 1) make no mention of the scientific evidence casting doubt on fluoridation benefits. Neither was this evidence mentioned or reviewed in the Supporting Documentation for Health Canada's Guideline for Canadian Drinking Water Quality for Fluoride (Health Canada, 1996). From a health standpoint, this is an important issue, as fluoride is added as a prophylactic medication, not to improve the safety of drinking water. Therefore, more references are listed in the following section than for the previous section on fluoride benefits.

Many scientific studies and large-scale whole-population studies in various countries have been unable to demonstrate that drinking water fluoridation, or the use of fluoridated dental products, reduces caries rates. For instance, in British Columbia, school districts with the highest caries-free rates were totally unfluoridated (Gray, 1987). In Alberta, there was no statistically significant difference in caries rates between unfluoridated Calgary and fluoridated Edmonton (City of Calgary, 1998, p. 27). In Nova Scotia, the percentage of caries-free children was slightly higher in unfluoridated Truro than in fluoridated Kentville, and the mean number of caries did not differ significantly between the two areas (Ismail, 1993). Examinations of over 39,000 U.S. schoolchildren (aged 5-17) by dentists trained by the National Institute of Dental Research, conducted in 1986-7, found no statistically significant differences between decay rates of permanent teeth or the percentages of decay-free children in fluoridated and unfluoridated areas[15]. In addition, children who were exposed to fluoridated water for a portion of their childhood had slightly higher DMFT rates than either totally fluoridated or totally unfluoridated children, which is inconsistent with the hypothesis that water fluoridation reduces tooth decay (as these "partially fluoridated" children would be expected to have lower decay rates than unfluoridated children) (ibid.) (Yiamouyiannis, 1990).

Examples in other countries include New Zealand (Colquhoun, 1997), India (a 30-year study of over 400,000 children by Teotia and Teotia, 1994), several European countries and Japan (Ziegelbecker, 1998 - additional 42 references cited, listed in Appendix 2; Diesendorf, 1986), the U.S. (Glass, 1981; DePaola, 1982; Zacherl & Long, 1979), South Africa (Hartshorne et al., 1994), England and Sri Lanka (Nunn et al., 1994) and Australia (Diesendorf, 1986). Caries rates have been declining over the past 40-50 years in many parts of the world, prior to and independently of fluoridation (Diesendorf, 1986; Hileman, 1988; Gray, 1987; Colquhoun, 1997; Ziegelbecker, 1998; Angelillo et al., 1999)

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[15] Fluoridation status information was based on reports published by the U.S. Public Health Service, wth some local agency verification.

A large World Health Organization study of naturally fluoridated water in several countries found no significant differences in caries prevalence with water fluoride content ranging from 0-1 ppm (Ziegelbecker and Ziegelbecker, 1993), as seen below in Figure 1.

Furthermore, in most, if not all, cases where fluoridation of drinking water has been stopped[16], dental caries rates have continued to decline (Germany - Ziegelbecker, 1998, Kunzel, 1997; Japan -Takahashi, 1998, Ziegelbecker, 1998; Finland - Seppa et al., 1998; the Netherlands - Kalsbeek et al., 1993, Ziegelbecker, 1998 (cited references attached in Appendix 2). Many scientists, particularly overseas, contend that the temporal association between caries reduction and fluoridation is not causal (Diesendorf, 1986; Colquhoun, 1997; Ziegelbecker, 1998).

Figure 1   **WHO data on Correlation Between Water Fluoride Levels and Tooth Decay in 5 Countries**

Source: Ziegelbecker & Ziegelbecker, 1993.

* DMFT = decayed, missing, filled permanent teeth

There are several possible explanations for the different conclusions about whether or not fluoride protects teeth from decay (Colquhoun, 1987; Diesendorf, 1986; Ziegelbecker, 1998; Hileman, 1988). Analysis of many of the earlier studies which reported beneficial effects has revealed methodological and statistical problems   - for example, study

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[16] These have included: the Federal Republic of Germany (fluoridation introduced in 1952, stopped 1971), Sweden (introduced 1952, stopped 1971); the Netherlands (introduced 1953, stopped 1976); Czechoslovakia (introduced 1958, stopped 1988/90); the German Democratic Republic ()introduced 1959, stopped 1990 (1993 in Spremberg)); the Union of Soviet Socialist Republics (introduced 1960, stopped 1990); Finland (introduced 1959, stopped 1993); Japan (introduced 1952, stopped 1972). In most cases, fluoridation was stopped for ethical, legal and health reasons (Hileman, 1988; Ziegelbecker, 1998).

populations were not always randomly selected; none of the early epidemiological studies adequately controlled for most non-fluoride variables; and most trials were not "blind" (ibid.). There have been concomitant environmental and social changes in populations - factors such as eating habits and nutrition, personal hygiene and health care. For instance, a recent study has reported that exposure to lead may significantly contribute to the development of tooth decay (Moss et al, 1999). There is considerable evidence that malnutrition and inadequate vitamin intake, as well as sugar consumption, particularly during early childhood, are important factors influencing caries risk (Ismail, 1998).

Some suggest that caries has declined in areas which do not fluoridate drinking water due to a "halo effect", meaning the introduction of fluoride into beverages and foods processed with fluoridated water (Limeback, 1993; Gray, 1987) and the use of fluoridated dental products and salt (Gray, 1987; Nosal, 1998). However, this cannot be the only factor responsible, as caries reductions have also been observed in areas without such products and even without fluoridated toothpaste (Diesendorf, 1987; Colquhoun, 1997; Nosal, 1998). This is illustrated in Figure 2, showing caries trends in 5-year-old New Zealand children from 1930-1990, before and after the introduction of water fluoridation and fluoridated toothpaste (Colquhoun, 1997). Moreover, caries has continued to decline in areas where all children had been exposed to fluoride for all their lives, which means that fluoride cannot be the chief factor responsible (Diesendorf, 1986; Colquhoun, 1997). Another consideration is the finding that the most prevalent type of caries in North America (pit and fissure) are not considered treatable by fluorides (Gray, 1987, cites Becker, 1967).

Yet another possible factor is that fluoride merely delays tooth decay, instead of preventing it (Sutton, 1980 - cites Weaver, 1944, 1948; Pauley, 1957; Carlsson, 1978; Royal College of Physicians, 1976). For instance, in the United Kingdom, a Health Department study noted: "It is thus clear that fluoridation does not prevent or reduce tooth decay. Instead, it merely postpones the appearance of caries by about 1.2 years. Fluoridated children develop the same amount of tooth decay as their non fluoridated counterparts. The only difference is that caries starts developing approximately 1.2 years later in the fluoridated group." (Schatz & Martin, 1972). This is illustrated in Figure 3. The rate of increase in caries with age was the same for fluoridated and unfluoridated towns (U.K. Dept. of Health, 1969).

There is some evidence that this delay might be due, at least in part, to delayed tooth eruption (Sutton, 1980 - cites Feltman & Kosel, 1961, Dr. JW Benfield's clinical observations, New York; Krook and Maylin, 1979). Krook and Maylin (1979) reported a delay in tooth eruption of 1.5-3 years in cattle with crippling skeletal fluorosis. Fluoride toxicity significantly decreased numbers of resorbing osteocytes, which play a key role in the resorption of both the deciduous (first) tooth roots and the supporting bone (ibid.). One recent study of humans confirmed that fluoride may delay tooth

eruption, and that the effect increases with increasing fluoride intake (Szelag, 1990). Apparently, there are few published studies on this issue, making resolution difficult (Sutton, 1996). Further evidence that fluoride ingestion can cause delayed tooth eruption stems from the finding that dft (decayed or filled deciduous teeth) rates were found to reach a maximum later in fluoridated and "partially fluoridated" children, compared with unfluoridated children, in the National Institite of Dental Research study of U.S. children (Yiamouyiannis, 1990). It has been proposed that fluoride may inhibit tooth eruption by inhibiting thyroid function (Sutton, 1996 - cites Baume & Becks, 1954). Several pertinent studies are noted below (under "effects on hormones").

A final possibility is that any initial caries protective effect of fluoride is subsequently diminished or offset in those individuals who develop dental fluorosis. As noted previously, dental fluorosis has been found to result in tooth damage, decay and loss (Kim, 1984; Waldbott, 1978; Weeks et al., 1993; Colquhoun , 1997; Teotia and Teotia, 1994; Diesendorf, 1986; Nunn, 1984; Tinanoff et al., 1999).

Figure 2   Caries trends in New Zealand Children, 1930-1990

Source: Colquhoun, 1997.

Figure 3   **DMFT* rates in U.K. Children from Fluoridated and Unfluoridated Towns**

Source: Diesendorf, 1986.

* DMFT = decayed, missing, filled permanent teeth

The discrepancies noted above, combined with the findings of continued caries decline after cessation of fluoridation in several locations, indicate that the relationship between fluoride exposure and tooth decay is complex and is probably influenced by various factors or conditions which are not universal (see also Angelillo et al., 1999; Moss et al., 1999; Nunn et al, 1994). Fluoridation has been shown to be neither necessary nor sufficient for optimal oral health (Diesendorf, 1986). This is self-evident from the findings that water fluoridation does not prevent caries in all people, and that unfluoridated communities can have lower caries rates than fluoridated ones.

Studies (as opposed to models) have also been unable to demonstrate economic benefits of fluoridation. For example, an early study (reported in the Journal of the American Dental Association in February, 1972 - cited in Hileman, 1988) compared 5 fluoridated cities in Illinois with 5 unfluoridated cities with similar dental treatments and fees. The cost per patient and the average number of visits to the dentist per year were higher in the fluoridated communities (Hileman, 1988).

While the question of fluoride's beneficial health effects is still under study and scientific debate, there is growing agreement even among proponents of fluoride use that a reduction in fluoride intake is warranted, because of the steadily increasing intakes of fluoride, and associated health risks (e.g., Gray, 1987; Limeback, 1993, 1996, 1999, 1999b). As well, since the main benefits of fluoride are deemed to occur during childhood tooth development (Gray, 1987; Limeback, 1999b), fluoride in drinking water would offer small benefits after this stage, yet exposure and fluoride accumulation via drinking water continues for a lifetime in a fluoridated community. The benefit which is ascribed to fluoridated water currently amounts to less than one decayed tooth per person, on average (Diesendorf et al., 1997; Gray, 1987).

Adverse effects and health risks

Fluoride ingestion - even at the low concentrations which may protect teeth - can give rise to a variety of biological effects, some of which can be harmful (Boeckhhaebisch and Oliveira, 1997; Susa, 1999; Krook, 1998; Waldbott, 1998). In this respect fluoride differs from most other trace elements like zinc, manganese and chromium, which exhibit large differences between beneficial and harmful ranges of exposure (Hileman, 1988). The adverse effects of fluoride which have been studied the most include dental and skeletal fluorosis, kidney disease, hypersensitivity reactions, effects on enzymes, genetic mutations, birth defects and cancer (Hileman, 1988). Clinical and epidemiological observations (Grimbergen 1974; Hileman, 1988; Waldbott, 1956, 1962, 1976, 1998; Liu, 1989; Li et al., 1994, 1995; Yang et al., 1994) and more recent animal studies (Zhao et al., 1994; Mullenix et al., 1995; Varner et al., 1993, 1995; 1998; Guan et al., 1998; Zhao and Wu, 1998) strongly suggest that fluoride may have neurotoxic effects which had not been previously recognised.

The fluoride concentrations in water at which adverse health effects have been observed are relatively low - less than 2 parts per million (ppm) (Burgstahler et al., 1998; Susheela et al, 1992, 1993; Desarathy et al., 1996; Waldbott, 1956, 1998; Hileman, 1988). This makes fluoride one of the few drinking water contaminants for which the Maximum Acceptable Concentration (MAC, 1.5 ppm) does not include a margin of safety of one or more orders of magnitude. Because over 90% of the population of Ottawa-Carleton (more than 585,000 persons) consumes fluoridated drinking water, potential adverse

health effects of this practice could affect significant numbers of people, even if only a small proportion were affected.

The following sections refer to several types of studies, each of which have strengths and weaknesses.

Epidemiological (ecological) studies focus on large, usually human, populations. They can provide valuable information on associations between variables and outcomes of interest in human populations, but cannot establish causality. Clinical studies or case reports provide more detailed information and can better control for many variables, but typically cannot study large numbers of individuals, thereby diminishing their ability to recognise the usually wide range of sensitivities in large populations[17].

Animal studies and models can ensure even better control of variables, enable the use of dosages and techniques which could not be administered to human subjects, and permit the evaluation of longer term or intergenerational effects, which would not be feasible given the long life span of most people. However, questions tend to arise about the extrapolation of findings from animal models to humans. As well, given practical and financial constraints, the numbers of animals in most studies must be limited, thereby reducing statistical power and the ability to detect outcomes which may affect a very small proportion of a population. Toxicological assessments using animal models typically involve administration of higher doses of a toxicant than a human population would be exposed to, in order to compensate for the reduced numbers of test subjects, and with the view that safety factors are required to allow for intra-individual and inter-species differences in toxicity. It is generally recognised that there are wide variations in the sensitivity of individuals to most toxic substances in any given population (e.g. ATSDR, 1997). For most toxic substances, safety factors (the difference between exposure level and expected toxic level) of at least two orders of magnitude are considered necessary, especially for chronic exposures (Hodge, 1963; examples in ATSDR, 1997).

Another common method of studying the effects of chemical substances involves the use of cell lines of specific cell types (for example, liver cells or fibroblasts). This method can help to elucidate the effects of the chemical on specific biochemical pathways or cell components, and requires substantially fewer resources than other approaches (e.g., animals and their care). It also obviates many practical and ethical issues in animal and human experimentation. However, as with animal models (discussed below), it can be a challenge to extrapolate findings from such studies to a whole-animal model, where many more systems and processes interact.

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[17] This is often seen when new drugs are introduced, where controlled clinical trials fail to detect important side-effects that become apparent only when large numbers of people are exposed to the drug.

Acute toxicity and Hypersensitivity

Case reports of acute and progressive illness due to fluoride in drinking water have been reported (Hileman, 1988; Waldbott, 1998, Susheela et al., 1992). In the Netherlands, double-blind experiments on patients who became ill after water fluoridation was introduced showed that the symptoms were caused by fluoride (Grimbergen, 1974; Petraborg, 1974, 1977; Hileman, 1988). (For this reason, drinking water fluoridation was banned in the Netherlands in the 1970s). Hundreds of similar cases (including double-blind studies) have been documented in North America (Waldbott, 1998). Afflicted individuals are more sensitive to fluoride that the general population. The symptoms in such cases, typically encompassing digestive, dermal, neurological and neuromuscular symptoms[18], disappear when nonfluoridated water is used for drinking and cooking (described in detail in Waldbott, 1956, 1998; Spittle, 1993; and Roholm, 1937). Respiratory, gastro-intestinal and dermal symptoms have been reported following the use of fluoride supplements and fluoridated toothpastes (Waldbott, 1998; Shea et al., 1967). In addition to its toxicity, it has been suggested that fluoride may act as a sensitizer; two other halogens, iodine and bromine, are recognised as sources of allergic symptoms (Shea et al., 1967), as well as a producing symptoms of toxicity similar to those of fluoride[19] at low levels (Woolf end Shannon, 1999).

The Canadian Compendium of Pharmaceuticals and Specialties (1989 edition) lists examples of adverse effects of the administration of 0.55-2.21 mg sodium fluoride, equivalent to 0.25 - 1 mg fluoride ion; these include skin rash, gastrointestinal upsets and headache. The symptoms usually disappear when fluoride administration is discontinued. Such symptoms have also been observed in persons ingesting food contaminated by airborne fluoride deposition (Waldbott, 1998). The proportion of the population which may be hypersensitive to fluoride is unknown, as no epidemiological studies of this phenomenon have been conducted.

A number of studies have reported gastric irritation as a consequence of fluoride ingestion (Waldbott, 1998, Spittle, 1993; Muller et al, 1992; Roholm, 1937). This effect is probably due to the generation of hydrofluoric acid (HF), a powerful irritant, and seems to be influenced by the type of fluoride compound (type of counter-ion) (Waldbott, 1998; Muller et al., 1992). For example, in a randomised double-blind study of healthy adults, gastric mucosal lesions were observed in subjects treated for 7 days with NaF and with sodium monofluorophosphate (MFP) tablets (Muller et al., 1992). The differences in lesion scores were statistically significant (p<0.0015) for both groups, which had

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[18] The symptoms reported included: stomach pain, nausea, bowel spasticity, polydipsia, polyuria, arthritic pains- especially lower back, migraine-like headaches, paresthesia in arms and legs with loss of muscle power, loss of memory and ability to concentrate, and extreme exhaustion as a hallmark symptom.

[19] The bromine exposure in a swimming pool produced symptoms such as skin rashes, fatigue, headache, gastrointestinal disturbances and myalgias at 8.2 $\mu g/L$ (twice the recommended concentration). In some individuals, the symptoms persisted or recurred for weeks to months after the one-time exposure.

similar serum fluoride levels (ibid.). However, lesions were much more common in the NaF-treated group (ibid.). Possible adverse drug reactions were reported in 4/10 of the NaF-treated subjects, and in 1/10 of the MFP subjects (ibid). These findings have implications for fluoride supplementation in children.

Research conducted in Japan has reported the minimum dose causing slight or incipient fluoride intoxication in humans as ranging from 0.08-0.2 mg/kg body weight (80-200 μg/kg bw) (Murakami, 1998; Akiniwa, 1997). In the U.S., the Nonlethal Toxic Dose for fluoride has recently been listed as 0.04-3.9 mg/kg bw - i.e., as low as 40 μg/kg bw (Calabrese et al., 1999 (1997).

Adverse effects on teeth

Fluoride intakes, from the various sources noted above, are currently much higher (10 times or more) than they were when fluoridation was first introduced in the 1940s and 1950s (Burgstahler et al., 1998; Nosal, 1998, Gray, 1987). This is borne out by the documented and continuing rise in the earliest detectable sign of fluoride toxicity: dental fluorosis in children (Gray, 1987; Limeback, 1993; Clark, 1993; Colquhoun, 1997).

Dental fluorosis is a disturbance in the formation of tooth enamel by ameloblasts, the tooth-forming cells in the jaw, during tooth development (i.e., in children). It is a progressive effect: the mildest forms are a barely noticeable mottling of tooth surfaces, while more severe cases result in staining and pitting of the teeth. Several fluoride-induced effects may be involved in the etiology of fluorosis, but current evidence suggests that inhibition of enzymatic degradation of amelogenins, causing delay their removal from the developing enamel and impair crystal growth, may be the primary mechanism (Whitford, 1997).

Even mild cases of dental fluorosis can result in dark stains on the teeth in adulthood (Waldbott, 1978). In addition, fluorosed teeth become brittle and are often difficult or impossible to repair when caries sets in because they cannot hold fillings, resulting in a higher rate of tooth loss due to fluorosis in adulthood in comparison with normal teeth (Waldbott, 1978, ch. 12, cites Smith and Smith, 1940). A leading Canadian dental practitioner and researcher has stated that "we are now spending more treating dental fluorosis than we would spend treating cavities if water were not fluoridated" (Limeback, 1999b). As seen in the previous section, it is not at all certain that caries rates would rise if fluoridation were stopped, since caries rates have declined after cessation of water fluoridation in numerous countries.

In Canada, dental fluorosis typically affects between 35% - 60% of children in communities with fluoridated water, and 15% - 45% in communities with nonfluoridated water, depending on the extent of water fluoridation, proximity to water fluoridation and consistency of the recommended concentration of fluoride in water systems (Clark,

1993). Similar ranges have been reported in other countries (Angelillo et al., 1999). Fluorosis has been known to develop in populations consuming fluoridated water below 1 ppm, even below 0.3 ppm (Waldbott, 1962; Weeks et al., 1993). The U.S. EPA reported in May, 1985 that severe dental fluorosis was found to occur at 0.8 mg/L (the newly established level in Ottawa-Carleton) (Carton and Hirzy, 1998). The extent, severity and rate of increase of dental fluorosis in Ottawa-Carleton children have not been assessed (Cutter, 1998), but are consistent with national trends (A. Burry, Ottawa-Carleton Health Dept, 1999, pers. comm.).

The majority of dental fluorosis cases in Canada are mild, but the prevalence of moderate to severe fluorosis also appears to be rising (Clark, 1993). A 1998 survey of Ontario children reported up to 30% incidence of moderate fluorosis, and up to 10% of severe fluorosis (Cutter, 1998). Despite the contention by Dental Associations that dental fluorosis is only a "cosmetic" effect (e.g., Cutter, 1998), some experts and agencies consider the condition, and particularly moderate and severe dental fluorosis, to be adverse health effects (Kim, 1984; Health Canada, 1996 - based on the TDI, discussed under 'Guidelines and related observations"). For example, the former Director of the Office of Drinking Water, U.S. EPA, wrote: "It is difficult to conclude *a priori* that teeth which spontaneously pit are stronger teeth. Further, data suggest that the effects of fluorosis are not merely discoloration and pitting, but fracturing, caries and tooth loss as well...it is difficult to conclude... that such effects are not adverse" (Kim, 1984).

It has been found that dental fluorosis rates are typically about two times higher in fluoridated areas than in areas where water is not fluoridated (Clark, 1993; Weeks et al., 1993). These findings clearly indicate that current intakes of fluoride are often excessive, that this can result in adverse health effects, and that the risks are increasing. Fluoride in drinking water contributes significantly to daily intakes (see Table II), and thus to associated fluorosis risks. For instance, a study comparing the prevalence of developmental defects of enamel in the deciduous dentition of 4- to 5-year-old children residing in fluoridated (1 ppm F) and non-fluoridated (less than 0.2 ppm F) communities in Cheshire, UK, reported significantly higher prevalence of developmental defects of enamel in fluoridated Nantwich (29%), than in non-fluoridated Northwich (14%) (Weeks et al., 1993). It was also determined that after controlling for the age at which parents claimed toothbrushing commenced, the children in fluoridated Nantwich still had significantly more diffuse defects than the children in Northwich (ibid.).

For children, swallowing toothpaste and other dental products such as rinses is a significant additional risk factor for excessive fluoride intake. As children tend to ingest a large proportion of toothpaste used for daily brushing, a significant number exceed the "optimal daily requirement"[20] (1 mg/day, as recommended by dental associations - e.g.,

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[20] Quotation marks are used because, as noted, Health Canada states that the essentiality of fluoride has never been demonstrated.

Clark, 1993) by this route alone (Clark, 1993). However, because the alleged benefits of fluoride are topical, the ingestion of this substance is unnecessary; therefore, the term "optimal daily requirement" is questionable. As well, there is no unequivocal scientific evidence to support the claim that an optimum level of intake exists (for example, see Angelillo et al., 1999; Nunn et al., 1994; Hartshorne et al, 1994; Ziegelbecker and Ziegelbecker, 1993).

As can be seen from Tables I and II above, the "optimal daily requirement" can also be exceeded in a significant proportion of children through their consumption of fluoridated drinking water alone, a fact also recognised by Health Canada (1996d). Fluoride from drinking water contributes 35%-65% of daily fluoride intake in children in fluoridated communities (Health Canada, 1996). Because fluoride is also present in various foods and beverages, many children are consuming fluoride at levels far in excess of 1 mg/day. This has recently been confirmed by Lewis and Limeback (1996), who determined that "for formula-fed infants and all other age groups using fluoridated water, the estimates of actual [fluoride] intake greatly exceed the recommended intake, especially for the seven month to four years age group".

There have been numerous reports of adverse effects of fluoride ingestion on teeth in addition to fluorosis. In some instances, more tooth defects were found in fluoridated areas than in unfluoridated areas; in some regions, caries rates have been found to increase with increasing fluoride levels in water (both naturally and artificially fluoridated)[21] (Colquhoun, 1997; Rugg-Gunn et al., 1997; Nunn, 1994; Teotia and Teotia, 1994; Ziegelbecker and Ziegelbecker, 1993; Hileman, 1988; Gray, 1987; Diesendorf, 1986). Tooth enamel defects have in some cases been associated with increased dental caries (Tinanoff et al., 1999). Published data clearly do not support the claim that a universal "optimal" fluoride concentration in drinking water exists which protects teeth from decay. Moreover, because of the significantly increasing and highly variable fluoride intakes from all sources (as noted above), it is very unlikely that an "optimum" concentration in water can be set which will not result in excess fluoride intake by some proportion of a population (apart from the problem of hypersensitivity noted above).

Skeletal effects: fluorosis

Ingested fluoride is quickly and nearly completely absorbed from the digestive tract Health Canada, 1996), although the amount absorbed from food may be lower than that absorbed from water (Minister of Supply and Services Canada, 1993; Waldbott, 1962, 1978). Between 50% and 90% of absorbed fluoride is deposited at a nearly constant rate

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[21] For example, in Japan, children with the lowest rates of caries lived in areas with 0.3-0.4 ppm fluoride in the water; both above and below this range, caries prevalence was much higher (Hileman, 1988). In England and Sri Lanka, more tooth defects were reported in fluoridated (1 ppm) than unfluoridated (0.1 ppm) areas (Nunn et al., 1994).

in calcified tissues, mostly the teeth (during development only), bones and aorta (Varner et al., 1998; Hileman, 1988; Waldbott, 1978). As fluoride is excreted mainly by the kidneys, retention is greater in individuals with impaired kidney function (Waldbott, 1998; Health Canada, 1996).

Skeletal fluorosis is a complex, multi-stage illness caused by the accumulation of excessive fluoride in bones. The development of skeletal fluorosis is modified by other factors such as nutritional status (Teotia and Teotia, 1994; Hileman, 1988). The stages of skeletal fluorosis, and attendant bone ash fluoride concentrations, are depicted in Table III. The first two stages, termed "preclinical", involve biochemical abnormalities in the blood and bone, and histological changes in bone structure (Hileman, 1988). At this stage, no overt health symptoms are observed, but the changes are precursors to more serious disease (ibid.). The early clinical stage of skeletal fluorosis includes arthritis-like bone and joint pains, paresthesia (burning, tingling and numbness in limbs), muscle weakness, chronic fatigue, gastrointestinal disturbances and reduced appetite (Hileman, 1988; Desarathy, 1996). These symptoms are accompanied by observable changes in bone structure of the pelvis and the spinal column (Hileman, 1988).

Later clinical stages involve constant bone and joint pain and calcification of ligaments, and may be accompanied by osteoporosis and osteosclerosis (gross bone abnormalities) and the formation of bone spurs (ibid.). In the final stage, crippling skeletal fluorosis occurs. This stage is marked by weakened extremities and partially fused vertebrae (ibid.). The U.S. National Research Council reports cases of crippling skeletal fluorosis at intakes of 10-20 mg fluoride for 10 years (Whitford, 1996, p. 138, cited in Nosal, 1998). With this clinical condition, fluoride concentrations in bone ash "generally exceed 9,000 ppm Calcification of ligaments often precludes joint mobility and numerous exostoses may be present. These effects may be associated with muscle wasting and neurological complications due to spinal cord compression" (ibid.). The preclinical and early clinical stages of skeletal fluorosis are known to occur at fluoride doses as low as 2-5 mg/day (ibid.), well within the range consumed by Canadians using fluoridated drinking water (Nosal, 1998, citing U.S. Dept. of Health and Human Services, 1991). In some far- and middle-eastern countries, cases of skeletal fluorosis have been observed in communities with water naturally fluoridated below 1 ppm (Hileman, 1988; Waldbott, 1978).

Table III   Stages of skeletal fluorosis

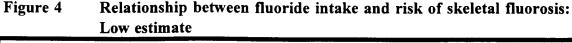| Osteosclerotic phase	Bone ash fluoride concentration mg F/kg	Health symptoms
Normal bone	500-1000	none
Preclinical phase	3,500-5,500	asymptomatic; slight radiographically-detectable increases in bone mass; histological evidence of bone cell toxicity in cattle at fluoride levels of 4733 +/-67 mg/kg (Krook et al, 1998)
Clinical Phase I	6,000-7,000	sporadic pain; stiffness of joints; osteosclerosis of pelvis and vertebral column; intermittent gastrointestinal pain
Clinical Phase II	7,500-9,000	chronic joint pain; arthritic symptoms; slight calcification of ligaments; increased osteosclerosis/cancellous bones, with/without osteoporosis of long bones
Phase II: Crippling fluorosis	>8,400	Limitation of joint movement; calcification of ligaments in neck/vertebral column; crippling deformities of spine and major joints; muscle wasting; neurological defects; compression of spinal cord

Source: U.S. Public Health Service. 1991. "Review of Fluoride, Benefits and Risks" -
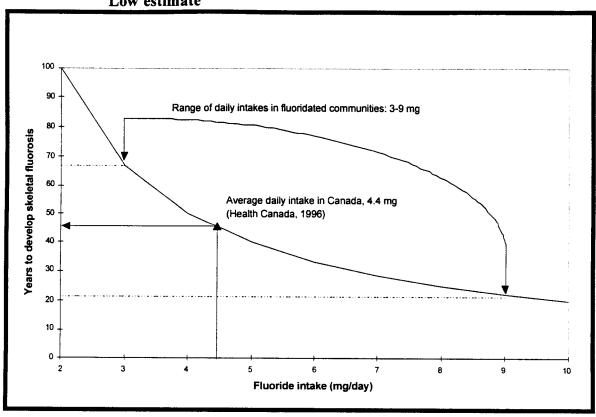adapted from *Fluoride*, 1997, 30:4.

Because fluoride bioaccumulates in bones, long-term exposure to low concentrations may have effects similar to those resulting from shorter term exposure to higher concentrations (Nosal, 1998). Given that the range of fluoride intakes in Canadian municipalities fluoridating water to 1 ppm is currently estimated at 3-9 mg/day (U. S. Department of Health and Human Services, cited in Nosal, 1998), it can be concluded that "total fluoride intake represents a potential risk of mild to moderate skeletal fluorosis in adult populations drinking water fluoridated at 1 ppm over long periods of time" (Nosal, 1998).

This is illustrated by the following calculation: for a young adult, assuming 50% retention of ingested fluoride (Health Canada, 1996), an absorbed intake of 10 mg/day results in an annual accumulation of 1.8 grams, or over 50 grams after 30 years (excluding amounts accumulated during childhood and adolescence, when deposition rates are higher (ibid.)). Bone fluoride levels of this magnitude have been associated with debilitating skeletal fluorosis in Roholm's often-cited classical studies of cryolite workers (Burgstahler et al., 1997; Roholm, 1937). This condition would be preceded by pre-skeletal phases of

fluoride intoxication, particularly in combination with other conditions such as poor nutrition (ibid.). On the basis of Roholm's studies, it has been estimated that a daily fluoride intake of 3.5-6.0 mg/day could result in "recognizable sclerosis" after 37 years, and "severe sclerosis" around age 84 (Foulkes, 1997). The average daily intake in Canada is listed as 4.4 mg/day (Health Canada, 1996).

The relationship between daily fluoride intake and the risk of developing skeletal fluorosis is shown below in Figure 4. The graph is based on the established direct (approximately linear) relationship between fluoride intake and its deposition in bones (e.g., Health Canada, 1996, Minister of Supply and Services, 1993), and Whitford's[22] findings that "Most estimates indicate that crippling skeletal fluorosis occurs when 10-20 mg of fluoride have been ingested on a daily basis for at least ten years" (Whitford, 1996, p. 138; Nosal, 1998; Burgstahler, 1998). The graph uses the more conservative estimate, i.e., 10 mg/day over 20 years. It is therefore possible that the time required for fluorosis to develop could be half that indicated on the graph below.

Figure 4   Relationship between fluoride intake and risk of skeletal fluorosis: Low estimate

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[22] Professor Gary M. Whitford of the Medical College of Georgia is considered one of the most distinguished and long-time experts on fluoride. He was expert member of the Panel on Calcium and Related Nutrients, U.S. National Academy of Sciences, Food and Nutrition Board of the Institute of Medicine.

In the 1970s, the U.S. Surgeon General's panel estimated that 3% of a population whose water contains 1 ppm fluoride would be likely to ingest sufficient fluoride, 5 mg/day, to incur detectable changes in bone structure corresponding to early stages of skeletal fluorosis (Hileman, 1988). In terms of today's population, that would correspond to over 17,000 persons. However, fluoride intakes have since increased to the point where *average* daily intakes in fluoridated Canadian municipalities are currently estimated at 4.4 mg/day (Health Canada, 1996). Therefore, a substantial proportion of the population of Ottawa-Carleton (likely in the tens of thousands) is now consuming fluoride at or above the level which is expected to result in detectable changes in bones (i.e., preclinical and clinical stage skeletal fluorosis). The Panel did not indicate what proportion of such populations would experience clinical manifestations - e.g., arthritis-like symptoms (Hileman, 1988).

In the city Kuopio, the only fluoridated municipality in Finland, it was found that some people accumulated high levels of fluoride (typically 900-2,300 ppm) in their bones after 10 years of fluoridation (Alhava et al., 1980; Arnala et al., 1985). Levels were highest (as high as 3890 ppm) in people with impaired kidney function (ibid.). These levels are as high as those reported in patients who had been given fluoride therapy for osteoporosis (Baud et al., 1978). (Fluoridation was stopped shortly thereafter, with no observed increase in caries rates, despite a concomitant "sharp" decline in numbers of fluoride varnish and sealant applications (Seppa et al., 1998)).

The incidence of skeletal fluorosis in North America is unknown, as there have been virtually no studies of this condition (Hileman, 1988). Most reported cases in the U.S. have occurred at fluoride concentrations in water greater than 4 ppm, but also at lower levels when other modifying conditions such kidney impairment or diabetes were present (Hileman, 1988). Due to the wide range of fluoride intakes from water and other sources, intakes at the higher end of the intake range can reach or exceed levels known to cause preclinical and early clinical stage fluorosis (Hileman, 1988). It is currently not known what proportion of such people would experience clinical effects such as joint pain, which have been documented in some patients with stage 1 clinical fluorosis (ibid.). It has recently been observed that "in the absence of sufficient numbers of contemporary biopsy and necropsy bone fluoride analyses, it is very unwise to assume that little or none of the extensive middle and old-age osteoarthritis that plagues so many people in the United States is not an undiagnosed manifestation of various stages of skeletal fluorosis" (Burgstahler et al, 1997; Burgstahler, 1998).

In addition, the effects of fluoride at various levels on bone development in children, and modifying factors such as nutrition, have not been well studied and are poorly understood

(U.S. Surgeon General's Committee, cited in Hileman, 1988). Populations which are likely to be most vulnerable to developing skeletal fluorosis have been excluded from study (ibid.). Some recent findings are discussed in the next section.

Effects on childhood bone development

Recent analyses of bone structure in children with endemic dental fluorosis (drinking water F⁻ level of 2.7 mg/L) suggest that fluoride can affect early bone development. Bones of children with dental fluorosis exhibited statistically significant ($p<0.05$) greater trabecular height and area, compared with controls (drinking water F⁻ level of <0.1 mg/L) (Chlebna-Sokol and Czerwinski, 1993). Sex and age-dependent differences were reported, with the greatest effects in younger children (average age 11.8 years) and in boys (ibid.). When age and gender sub-groups were compared, the differences were statistically significant in young boys ($p<0.05$) (ibid.)[23]. It was noted that at this age, boys are at an earlier stage of development than girls (ibid.).

Fluoride can exert effects during the period of fast growth and during continuous remodelling of bone structure (ibid.). Statistically significant correlation coefficients were reported for the group with dental fluorosis where the increases in trabecular height and area correlated with lower serum calcium and higher alkaline phosphatase activity levels (ibid.). Paradoxically, mean serum calcium levels were higher, but magnesium and alkaline phosphatase activity reduced in the fluorosis group relative to the controls (ibid.). It has been shown that increased trabecular height is indicative of an increase in bone mineral content (ibid.). A proposed mechanism explaining increased bone mineralization involves retarded bone resorption due to formation of less soluble fluorapatite and osteoclastic activity inhibition by fluoride (ibid.). The reduced alkaline phosphatase activity in children with dental fluorosis was interpreted as indicative of lower metabolic turn-over in the bone and its growth retardation (ibid.). This study demonstrates that there is an apparent relationship between dental fluorosis and bone development abnormalities, that fluoride may affect bone development in young children at relatively low levels, and that these effects appear to be more pronounced in young boys.

In a previous study, cortical defects in bone X-rays were reported in 13.5% of children from fluoridated Newburgh, USA, compared with 7.5% in unfluoridated Kingston after 11 years of fluoridation (Yiamouyiannis, 1993, cites Schlesinger et al., 1956). The difference was statistically significant and substantive (ibid.). It was noted previously that the bone defects closely resembled osteogenic sarcoma (osteosarcoma) (ibid., cites Caffey, 1955) (refer to section on mutagenicity and cancer below). It was noted that "while progression of cortical defects to malignancies has not been observed clinically, it would be important to have direct evidence that osteogenic sarcoma rates in males under

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[23] This might be due to the small sample size, 43 in each group.

30 have not increased with fluoridation" (ibid., *cites Drinking Water and Health,* National Academy of Sciences, 1977).

In studies of children from endemic fluorosis areas, dose-dependent changes in collagen metabolism in children exhibiting dental fluorosis (DFIII degree) have been reported (Shen et al., 1992). Children with fluorosis also showed altered zinc metabolism and smaller average height compared with children from the same area but without fluorosis (ibid). Although preliminary, these findings suggest that fluoride might affect collagen metabolism in children with less severe dental fluorosis as well. Collagen is a major structural component of skin, bones, teeth, ligaments, tendons, muscles and cartilage.

Effects on connective tissue and collagen

Glycosaminoglycans (GAG) and collagen are the main building blocks of connective tissues. As well, the viscosity of joint synovial fluid is dependent mainly on hyaluronic acid, a long polysaccharide chain composed of N-acetyl glucosamine and glucuronic acid subunits (Shahid, 1998). In early stages of joint disease, changes occur in the chain length and pattern of sulfation of glycosaminoglycans, indicative of cellular (e.g., chondrocyte) response to damage to the articular cartilage matrix (Hardingham, 1998; Plaas et al., 1998). Fluoride compounds have been found to cause changes in collagen (Pawlowska et al., 1998, cite: Ammintzbool et al., 1988; Grucka-Mamczar et al., 1992; Veron and Couble, 1992), and glycosaminoglycan metabolism (Susheela and Kharb, 1990; Pawlowska et al., 1998, cite: Ammintzbool et al., 1988; Grucka-Mamczar et al., 1992) in animal and in tissue culture experiments. In the primary fibroblast tissue culture model of Pawlowska et al. (1998), low levels of fluoride were observed to inhibit growth. These findings were consistent with those of previous studies (Pawlowska et al., 1998, cite: Sato et al., 1986; Oguro et al., 1990; Veron et al, 1993). The presence of F$^-$ ions caused changes in the size and shape of cultured fibroblasts; fibroblast area and spherical volume decreased by 20-30% (ibid.). Sato et al. (1986) had previously reported folding of the surfaces of fibroblasts cultured in the presence of fluoride (this may be related to the effects of fluoride on membranes, discussed below)).

The effect of fluoride on the incorporation of ^{35}S was studied in three culture fractions: fibroblast, pericellular substance, and medium (Pawlowska et al., 1998). In cultures containing fluoride (as NaF) at low concentration, GAG permeation from fibroblasts to pericellular substance was inhibited by 50% (ibid.). Qualitative and quantitative analysis of sulphated GAG from the 3 individual fibroblast fractions indicated fluoride interference, "probably not only in the diffusion process of individual sulphated GAG synthesized in the fibroblasts in pericellular substance, but also in synthesis and metabolism of the GAG" (ibid.). Changes were also observed in the degree of sulphation of dermatan, chondroitin and heparan sulphates present in fibroblasts (ibid.). Fluoride

was also found to cause changes in the biosynthesis and/or sulphation of GAGs, with an increase in dermatan sulphate relative to chondroitin and heparan sulfates (ibid.).

The authors concluded that even at low concentrations, fluoride may be toxic to fibroblasts, as evidenced by their growth inhibition, size reduction and shape alterations; that fluoride significantly modifies sulphur incorporation into GAGs and into dermatan, heparan and chondroitin sulphates in fibroblasts; and that there may be concomitant changes in GAG diffusion outside the cell to pericellular substance (ibid.). These findings are of significance because of the numerous reports of arthritic symptoms and joint problems due to fluoride ingestion (e.g., Waldbott, 1956, 1962, 1978, 1998; Hileman, 1988; Limeback, 1999).

Nutritional supplements of glucosamine sulphate and chondroitin sulphate (building blocks of polysaccharide chains which make up GAGs) have been reported to alleviate symptoms of arthritis by medical practitioners (Burton Goldberg Group, 1995, p. 533) and in recent clinical trials (Conrozier, 1998, 1998b; 1998c; Shankland, 1998; Uebelhart et al., 1998; Bourgeois et al., 1998; Leffeler et al., 1999). Improvement or disappearance of arthritic symptoms and joint problems has been reported after cessation of the ingestion of fluoridated water (Waldbott, 1956, 1962, 1978, 1998; Limeback, 1999).

Bone brittleness and fractures

Several ecological studies have found that accumulation of high levels of fluoride in bones is associated with increased risk of bone brittleness and breakage in older persons (Health Canada, 1996b; major publications reviewed by Nosal, 1998 - cites Danielson, 1992; Jacobsen 1990/92; Jacqmin-Gadda, 1995; Sowers, 1986, 1991; Suarez-Almazor, 1993). Health and Environment Canada state that the weight of evidence in ecological studies indicates that there may be an association between the consumption of "fluoridated" drinking water and an increased incidence of hip fracture (based on hospitalization rates) particularly among the elderly (Minister of Supply and Services Canada, 1993 - additional studies cited include Cooper et al, 1991, Keller, 1991, and May and Wilson, 1991, both cited in Gordon and Corbin, 1992). The differences observed in the better-designed studies were found to be statistically significant. It is deemed important that several of the studies finding statistically significant associations between water fluoridation and hip fractures controlled for various other known risk factors for osteoporosis, in one case (Sowers, 1986) on an individual basis. Ecological studies have limitations because they cannot fully address all important variables, making it difficult to establish the nature of associations with certainty. They cannot establish causality, but can provide statistically significant information about relations between factors and variables of interest (Nosal, 1998).

Clinical trials where fluoride was administered in an effort to treat osteoporosis established that, while fluoride increases bone density via remodelling, at least some bones (e.g., hip) become more brittle and fracture more easily (Health Canada, 1996; also reviewed by Nosal, 1998 - cites Fratzl et al., 1994; Hedlund, 1989; Riggs, 1990, 1994; Sogaard, 1994). Fluoride treatment for osteoporosis results in greater amounts of trabecular bone and a decline in compact bone (Dambacher et al., 1978). Fratzl (1994) found that bone biopsies taken from osteoporosis patients before and after fluoride treatment had increased density with no biomechanical improvement. Recent reviews have concluded that fluoride administration resulted in no demonstrable benefits in the treatment of osteoporosis, and caused adverse effects such as limb pain (Meunier, 1999; Prescrire Int., 1998).

When significant amounts of fluoride are present, old normal bone is replaced by new pathological bone (ibid.). A proposed biochemical mechanism for the increased amount of trabecular bone accumulation in patients treated with fluoride has been presented by Krook and Minor. (1998). Increased bone density probably results from pathological bone formation by osteoblasts injured by fluoride, and decreased bone resorption by resorbing osteocytes and osteoclasts (ibid.).

Together with the ecological studies of the relationship between fluoridation and bone fractures, the studies of fluoride "therapy" for osteoporosis indicate that short- and long-term fluoride ingestion may be detrimental to bone quality. A precise threshold level (or concentration range) of fluoride ingested, or accumulated, for these effects to occur has not been determined. However, such effects have been observed in animals at levels very similar to those found in some people exposed to fluoridated drinking water over extended periods of time (e.g., in the Finnish city of Kuopio; cited in Colquhoun, 1997; Krook and Minor, 1998). Studies of cattle showed clear histological evidence of toxicity to bone matrix and cells (osteocytes) at bone fluoride levels of 4733 +/-67 ppm (Krook et al, 1998).

In the ecological studies cited above, increased risk of hip fracture was found at estimated daily fluoride intakes of 72 µg/kg bw/day (Sowers et al., 1986, 1991), which is within the range of intakes for some segments of populations drinking fluoridated water (nearly all young children and an undetermined proportion of adults), and very close to the upper end of average intakes of adults in fluoridated communities (Health Canada, 1996; Nosal, 1998) (refer to Tables I and II). Intakes of 200 µg/kg bw/day are predicted to result in bone levels of fluoride associated with skeletal fluorosis (Minister of Supply and Services Canada, 1993); this amount is within the upper range of daily intakes, which can be as high as 9 mg/day (Whitford, 1996). The relationship between fluoride intake and risk of skeletal fluorosis is illustrated above in Figure 4.

Fluoride has long been recognised as a bone anabolic agent (Susa, 1999; Health Canada, 1996). Recent studies have begun to elucidate the induction of signal transduction pathways in bone (osteoblastic) cells. Fluoride combines with traces of aluminum to form a complex termed fluoroaluminate, which stimulates cellular heterotrimeric G proteins (guanine nucleotide-binding proteins - discussed below) (Susa, 1999). Fluoroaluminate can form in food, in drinking water and in living organisms after the administration of sodium fluoride (ibid.). The fluoroaluminate complex can cross the cell membrane and binds (beside GDP - guanosine diphosphate) to membrane-associated inactive G_α protein subunits (ibid.) This results in the formation of a G_a-GDP-AlF$_4$ complex, which changes conformation (i.e., 3-dimensional structure) to an active state which resembles that of G_a-GTP (guanosine triphosphate).

The effects of fluoroaluminate complexes on bone were investigated in a recent study, where rabbits were treated with various concentrations of aluminum and fluoride in drinking water, alone and in combination (Ahn et al., 1995). The accumulation of fluoride in plasma, urine, incisor teeth and tibia increased proportionally with the concentration of fluoride (0, 1, 4 or 50 ppm F as NaF) in drinking water for any constant concentration of aluminum. However, fluoride accumulation was highest in rabbits treated with the lowest dose of aluminum (100 ppm), and decreased with increasing aluminum levels (similar findings reported by Varner et al, 1993). Another important finding was that aluminum levels in tibia were increased significantly by the addition of fluoride to the drinking water, even in animals not treated with aluminum. It was proposed that some of the osteotoxicity which seems to be associated with high levels of fluoride in bone may be due to accumulation of aluminum or an aluminum-fluoride complex. These studies are notable because fluoride is present in the Regional drinking water supply at a concentration which resulted in aluminum accumulation in bone, and because inorganic aluminum is present in Regional water (as well as in our food) at low concentrations (see section 4.8.5.1 of the Drinking Water Quality section of the Health Department's State of the Environment Report, 1999). The presence of fluoride enhanced aluminum accumulation in a dose-dependent manner. Furthermore, the findings are significant because similar observations have been made in studies on the effects of aluminum and fluoride on the nervous system (discussed below).

Fluoride interaction with G proteins

G proteins are a key component of a ubiquitous biological second messenger system. They act as signal transducers for a vast array of over 1,000 proteins, hormones, neurotransmitters, chemokines, local mediators and sensory stimuli which exert their effects on cellular and physiological responses via G protein-coupled receptors (Hamm, 1998; Farfel et al., 1999). There are four main classes of G proteins, which share a common structural core (Sprang, 1997). G proteins have an inactive (GDP-bound) and an active (GTP-bound) form (Hamm, 1998).

The activation of G proteins sets off a cascade of chemical reactions beginning with adenylate cyclase, cAMP (cyclic adenosine monophosphate), and activation of mitogen activated protein kinases involved in the regulation of gene transcription and protein synthesis (Susa, 1999; Varner et al., 1998). This results in the phosphorylation of various substrates. G proteins play roles in the regulation of ion channels, metabolism, gene expression, and cytoskeletal structures via second messenger systems (ibid.).

Fluoroaluminate (AlF_4^- or $AlF_3(OH)^-$) can activate certain G proteins[24] which then activate several cytoplasmic protein tyrosine kinases (Susa, 1999). The effects can include osteoblast proliferation and differentiation, and modulation of the adhesion properties of osteoblasts (ibid.). Osteoblast adhesion can in turn affect cellular differentiation, migration and apoptosis (programmed cellular death) (ibid.). It is noteworthy that activation of kinases and protein phosphorylation are key steps in the activation of some oncogenes. Also, increased cellular proliferation, altered cell differentiation, and changes in cell adhesion and migration properties are important features of carcinogenic processes.

Of particular interest is that the Ras family of oncogenes is a member of the G protein superfamily (i.e., Ras is a G protein) (Sprang, 1997). Ras performs key roles in regulating cell proliferation, and "...is an essential component of signal transduction pathways..." (Sprang, 1997, p. 643). Ras homologs regulate many vital cellular processes in cytoskeletal remodelling, differentiation and vesicle transport (ibid.). Mutations activating the function and expression of the Ras proto-oncogene, and disruption of Ras signalling pathways, are among the most common changes involved in various types of human cancers, including lung, pancreas and colon cancers (Ferrante et al., 1999; Fahraeus et al., 1999; Agapova et al., 1999; Davidson et al., 1999; Beaupre and Kurzrock, 1999). Ras mutations are thought to be implicated in 25% of human cancers (Bourne, 1997). Ras mutation frequency varies from 95% in pancreatic cancer to 30% in acute myeloid leukemia and 5% in breast cancer (Weijzen et al., 1999).

Ras is believed to activate mechanisms which favour tumour growth and metastatic capability, and modulate tumour-specific immune responses (Weijzen et al., 1999). The oncogenic activity of Ras seems to be due mainly to permanent stimulation of cellular proliferation and morphogenic changes[25] (Agapova et al., 1999; Weijzen et al., 1999). For instance, mutations which replace amino acid 12 (glycine) in Ras give rise to mitogenic signals which cannot be turned off (Bourne, 1997). Ras activation can additionally

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[24] Pertussis toxin-insensitive proteins, probably from Ga 12 class (Susa, 1999).

[25] Ras is also involved in downregulation of major histocompatibility complex molecules, upregulation of certain cytokines, growth factors and degradative enzymes (Weijzen et al., 1999).

contribute to carcinogenesis by increasing genetic (chromosomal) instability (Agapova et al., 1999).

Together, these factors may be relevant to, and provide evidence for biological plausibility of, the potential carcinogenic properties of fluoride (discussed below). Because G proteins have similar structures and binding domains, it is likely that if a general G protein activator such as fluoroaluminate can bind to and activate "normal" Ras, it can also activate at least some mutated Ras proteins[26], enhancing oncogenic effects. Aluminum fluoride (as AlF_4^- or $AlF_3(OH)^-$) is a strong activator of G_α subunits (Sprang, 1997). AlF_4^- binds tightly to the Ras-GDP-Mg++-GAP[27] complex (ibid.).[28]

Because G proteins are involved in the regulation of such a wide array of physiological processes, there is a strong possibility that their interaction with fluoroaluminate can play a role in many health conditions and symptoms. In addition, it has been determined that some proteins interact differently with G proteins, depending on whether the nucleotide-binding site contains AlF_4^- or a GTP analogue; this could either enhance or reduce signal transmission (Bourne, 1997). Both of these outcomes (i.e., increased and decreased G protein signal transmission) have been linked to human diseases (Farfel et al., 1999).

Additional studies on the interaction of fluoride with aluminum are discussed below (under neurotoxicity).

Mutagenicity and cancer

Fluoride is a recognised mutagen (Health Canada, 1996; Minister of Supply and Services Canada, 1993; Yiamouyiannis, 1993). Mutagens are, in general, considered potentially carcinogenic. Fluoride can induce the transformation of fibroblasts to fibrosarcomas (Yiamouyiannis, 1993, cites Tsutsui et al., 1984). In experiments on rats, fluoride (at high levels) had mitogenic effects on osteoblasts and stimulated their activity when administered in vivo, but not in vitro (Chavassieux et al., 1993). It was suggested that fluoride may act on osteoprogenitor cells or through an indirect mechanism mediated by a cofactor (ibid.). As discussed above, fluoride and fluoroaluminate complexes can interact with key biochemical regulatory mechanisms in bone tissues, for instance via G protein activation (Susa, 1999).

It is suspected that fluoride can affect DNA by interfering with its hydrogen bonds (Health Canada, 1996; Hileman, 1988). Experiments have shown that levels as low as 1 ppm caused changes in human leukocyte chromosomes in vitro (Hileman, 1988, cites

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[26] Site-specific mutagenesis studies have demonstrated that certain mutations selectively reduce the affinity of $Gi\alpha1$ for GDP-AlF4-Mg++ (Sprang, 1997).

[27] GAP = GTPase-activating proteins.

[28] The affinity of Ras GAP for the mutant Ras (glycine-12) is unchanged (Bourne, 1997).

Jachimczak, 1978). Fluoride can accumulate in some tissues like bone at levels several thousand times higher than this (see Table III). Fluoride is considered capable of inducing chromosomal aberrations, micronuclei, sister chromatid exchanges *in vitro* in mammalian cells, but the effect is dependent on several factors (Minister of Supply and Services Canada, 1993). Health Canada states that fluoride can damage chromosomes at a level of 10 µg/L (10 parts per billion) (Health Canada, 1996). This clastogenic activity is believed to be consistent with a mechanism involving inhibition of DNA synthesis and/or repair (Minister of Supply and Services Canada, 1993). Fluoride inhibits several enzymes involved in DNA processing (see section on enzymes, Figure 5).

The research assessing the ability of fluoride to cause or promote cancer is considerable but controversial. Some earlier epidemiological studies found correlations between water fluoridation and cancer incidence (various sites) and deaths, but they have been criticised on grounds such as inadequate exposure assessment and questionable statistical analysis (Yiamouyiannis, 1993; Hileman, 1988). A very important, more recent study by the U.S. National Toxicology Program (NTP) reported statistically-significant elevated rates of osteosarcoma, a rare bone cancer, in male rats after long-term fluoride ingestion, with a dose-response trend (NTP, 1990). Health and Environment Canada state that "such a trend associated with the occurrence of a rare tumour in the tissue in animals and humans in which fluoride is known to accumulate cannot be easily dismissed. Moreover, the level of fluoride in bones of the high-dose group of male rats in the NTP carcinogenicity biassay, in which a non-significant increase in osteosarcomas was observed, is similar to that measured in humans with skeletal fluorosis" (Minister of Supply and Services Canada, 1993, p. 46). Not all experts agree that the observed increase in the NTP assay was "non-significant" (Marcus, 1990; Calabrese, 1991).

It has been pointed out that the historical control animals in the NTP study were exposed to 0.7-1.2 mg fluoride/kg/day in the feed (28-47 ppm F) (Hirzy, 1998; Marcus, 1990). This level of exposure, equivalent to 200 µg/kg bw/day, falls between the low- and mid-dose exposed animals in the experiment (ibid.). Plotting this level of exposure on the same scale used for the NTP bioassay and the data from that study shows the incidence of osteosarcomas in the historical control group aligns precisely with the regression line for the NTP data (ibid.). This finding is deemed significant, due to the large number of control animals in the group (approx. 6000) (ibid.).

Increasing rates of osteosarcoma have been reported in young males in several fluoridated communities; for instance, the New Jersey Dept. of Health determined that male osteosarcoma rates were 3-8 times higher in fluoridated areas (Yiamouyiannis, 1993; New Jersey Dept. of Health, 1992). Others have used a different analytical technique, in which females are used as a "control" population in assessing the effect of fluoride on osteosarcoma in males (because this particular cancer seems to be linked with fluoride

ingestion in males only). It was reported that:1) the bone cancer incidence rate was as much as 0.95 cases/year per 100,000 population higher in males under age 20 living in fluoridated areas; 2) the osteosarcoma incidence rate was 0.85 new cases a year per 100,000 population higher in males under age 20 living in fluoridated areas; and 3) for males of all ages, the bone cancer death rate and bone cancer incidence rate was as much as 0.23 and 0.44 cases higher per 100,000 population, respectively, in fluoridated areas in the U.S. (Yiamouyiannis, 1993). Net bone cancer incidence rates (males minus females) in fluoridated areas and nonfluoridated areas were compared for the U.S., Canada, New York State and the U.K. Significant differences were reported in the U.S., New York and Canada (0.31-0.54/100,000) (ibid.). (The estimated fluoride consumption in the U.K. from tea was 1-2 mg/day (ibid.)). While this analysis did not determine fluoride intakes and did not examine potential confounders, unless such confounders were closely linked to fluoridation in all locations, the net effect would tend to diminish, not increase, the observed differences. Because dental fluorosis rates are twice as high in fluoridated areas as in unfluoridated areas in Canada, it seems reasonable to assume that fluoride intakes are considerably higher in most, if not all, fluoridated communities. This is substantiated in the estimates of daily intakes by Health Canada (1996d), listed in Table II. It is also noteworthy that fluoride has been linked with bone and testosterone abnormalities in males in other studies (Chlebna-Sokol and Czerwinski, 1993; Susheela and Jethanandani, 1996; Kranwar et al., 1983).

An even more significant finding was a reported 30%-60% increase in oral cancers (oral cavity and pharynx), also reported (but downplayed) in studies by the National Cancer Institute (Yiamouyiannis, 1993; also cites Persing, 1989). Because oral cancers are much more common than osteosarcoma, an increase of this magnitude would affect many more people. Another cancer for which there is substantial evidence of a link to fluoridated drinking water is a very rare form of liver cancer, hepatocholangiosarcoma (ibid; cites Toft, 1960; Marcus, 1990; Hirzy, 1998).

The Yiamouyiannis (1993) analysis estimated an increase of 10.3 fluoridation-linked cancer deaths in the U.S. per 100,000 population per year over the period 1953-1968, and 7.1 excess deaths/100,000 per year using census figures around the years 1950 and 1970 (ibid.). This would be equivalent to an excess of 9,000-13,000 fluoridation-linked cancer deaths in the U.S. each year (ibid.). Similar estimates have been reported by others, including Canadian researchers (Hileman, 1988).

A number of other studies have reported an association between fluoridation and cancers at various sites (reviewed in Health Canada, 1996), but the findings were deemed inconclusive or inconsistent by regulatory agencies (ibid.). There is considerable disagreement among experts with respect to the interpretation of the studies which have been conducted on fluoride's carcinogenic properties. The interpretation by the NTP that the evidence for fluoride's carcinogenicity is "equivocal" has been rigorously reviewed and

critiqued, and found to be in disagreement with the generally accepted definition of "equivocal" (Calabrese, 1991). It has been pointed out that in the NTP experiments, the high-dose animals (in which a significant dose-response trend was observed for osteosarcoma in male rats) had *the same or lower* levels of fluoride in bones than those found in some people, which is highly unusual in a toxicological assessment of the carcinogenic potential of chemicals (Marcus, 1990; Calabrese, 1991; Hirzy, 1998). The effects of prenatal exposure to fluoride and cancer development later in life have also not been investigated. There is a dearth of research addressing this unresolved issue, which is surprising given that fluoridation has been practised in North America for 50 years.

It has been widely accepted that carcinogenicity is a "non-threshold" effect - i.e., there is little evidence to support a "high-dose only" phenomenon in carcinogenesis (Fan et al., 1995; Bucher, 1999).

Effects on enzymes

Fluoride is a potent inhibitor of many enzyme systems (Friedman, 1983; Hileman, 1988; Connett, 1998; Krook & Minor, 1998). The effects are due to several of fluoride's chemical properties. Fluoride can affect enzyme conformation (3-dimensional structure) by binding to sites within the enzyme or in close proximity to it (Susa, 1999), and by altering the physical and chemical properties of molecules due to its ability to form strong hydrogen bonds with amide groups (Hileman, 1988).

Fluoride is capable of interacting with many metal and nonmetal cations (Jolly et al., 1980). Metals are important co-factors in many enzymatic reactions; therefore, fluoride-induced interactions with metals could affect enzyme kinetics. It has been demonstrated that enzyme inhibition can result when fluoride combines with phosphate to form the bivalent cation FPO_3^{2-} (Peters et al., 1964; Slater and Bonner, 1951). Fluoride can also replace hydroxyl groups in molecules (Health Canada, 1996), and may competitively occupy active sites on enzymes such as cytochrome oxidase (Machoy-Mokrzynska and Machoy, 1992 - cites Reiman, 1988). In addition to G protein interactions, these properties suggest other possible mechanisms for the wide range of physiological effects which fluoride can exert (Hileman, 1988).

Enzymes inhibited by fluoride include many phosphatases, kinases and ATP-ases, glucose-6-phosphate dehydrogenase, enolase, succinic dehydrogenase, catalase and cytochrome oxidase (Miller, 1997; Baykov et al., 1992; Machoy-Mokrzynska and Machoy, 1992 -cite Strochkova and Zhavoronkov, 1983). Examples are shown in Figure 5. These enzymes are associated with vital cellular processes, including energy generation and glycolysis. For instance, fluoride inhibits glycolysis by inhibiting enolase (ATSDR, 1993, cite: Guminska and Serkowicz, 1975, Peters et al., 1964). Energy generation is

inhibited by fluoride due to its blocking the entry of pyruvate and fatty acids into the tricarboxylic acid (TCA) cycle, and by its inhibition of succinate dehydrogenase (ATSDR, 1993, cite Slater and Bonner, 1952). Several of the enzymes are inhibited at millimolar or lower fluoride concentrations - i.e., at physiological levels[29] (Miller, 1997; Baykov et al., 1992; Waldbott, 1978). The fluoride concentration of concern in enzyme inhibition would be the level present in the tissue or organ of interest, not the level of fluoride in drinking water. However, there has been relatively little research on the effects of typical levels of fluoride intake, or water fluoridation, on enzyme activity in people (Hileman, 1988). As noted previously, individuals exhibit tremendous variation in their ability to absorb, excrete and sequester fluoride in various tissues (Wadbott, 1962, 1978). This may also help to explain the wide range of biological and health effects observed at different fluoride levels in water.

For example, kinetic parameters obtained in studies of fluoride inhibition of rat liver inorganic pyrophosphatase determined that "appreciable inactivation of pyrophosphatase can occur at fluoride concentrations found in human plasma. This effect may therefore be one of the major factors contributing to fluoride toxicity" (Baykov et al., 1992). Activities of total, Na(+)-K(+)-, Mg(2+)- and Ca(2+)-ATPases were found to be significantly reduced in red cell ghosts of patients with chronic fluoride toxicity (Kumari and Rao, 1991). In at least one study, a transient decrease in human serum enzyme activity was associated with the advent of water fluoridation (Hileman, 1988). Fluoride has been shown to affect pseudocholinesterase activity (Kambam et al., 1990).

On the other hand, some enzymes, such as adenylate cyclase (which generates the second messenger molecule cyclic AMP, important in many cellular processes), can be activated by fluoride (Machoy-Mokrzynska and Machoy, 1992). Recent studies have reported that fluoride affects G protein structure and function, and can activate several protein kinases (Susa, 1999) and acetylcholinesterase (Zhao and Wu, 1998)[30]. Fluoride was found to significantly inhibit the activity of $Ca^{2+}Mg(^{2+})$-ATPase in synaptic membranes in rat brain, demonstrating non-competitive type inhibition (Zhao et al., 1994).[31] The inhibition of this enzyme was also noted in the offspring of the fluoride-treated female rats (ibid.).

It is noteworthy that drinking water is the single major source of fluoride intake in fluoridated areas, and that its bioavailability can be greater from water than from food (Minister of Supply and Services Canada, 1993).

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[29] As shown by the accumulation of HF across membranes of organelles at different pH values (Miller, 1997).

[30] In the latter case, ingestion by maternal rats of 5-50 ppm F for 60 days resulted in dose-dependent increases in AChE activity. Moreover, the AChE activities of their offspring were also significantly increased 80 days after birth (Zhao and Wu, 1998).

[31] Administration in drinking water, 5-50 mg/L fluoride during gestation and lactation.

Figure 5   Examples of enzyme inhibition by fluoride

Source: Waldbott, 1962.

Taken together, these findings are of concern because most major bodily processes are regulated, at least in part, by enzyme systems and cellular messengers. Recent studies on the administration of low levels (0.8 to 2.2 ppm) of fluoride in drinking water to rats found altered systemic biochemical homeostasis mechanisms and ion levels (Ca^{++}, Na^+, Mg^{++}, Zn^{++}) in several organs after 6 months, even though no clinical signs of fluorosis were evident (Boeckhhaebisch & Oliveira, 1997). Fluoride had previously been shown to be capable of altering the calcium homeostatic mechanism, including calcium absorption and excretion, thereby affecting calcium metabolism (Das and Susheela, 1993).

Effects on hormones

The ability of fluoride to interfere with hormones has not been studied as extensively as its effects on enzymes; however, there have been reports that it can alter levels and activities of several hormones, including androgens (Colquhoun, 1997), and possibly estrogens and pineal gland activity (W. Hirzy, U.S. EPA, 1999, pers. comm.). It has been reported that fluoride can accumulate in the human pineal gland at levels equal to or greater than those in teeth and bones, and that the gland's melatonin biosynthesis pathway is inhibited as a result (Luke, 1994; Mullenix, 1998). Melatonin is involved in important physiological processes, such as sleep patterns and carcinogenesis, but the mechanisms are not well-understood at this time.

Animal studies have determined that changes in cyclic AMP-dependent protein kinase activity are a feature of prepubertal development in females (Hunzicker-Dunn et al., 1989). Fluoride stimulates cAMP production, including mediation by G protein mechanisms; exposure to fluoride has been implicated in female precocious puberty in animal experiments (W. Hirzy, U.S. EPA, 1999, pers. comm).

Androgens

Fluoride has been shown to depress testosterone synthesis *in vitro* at 1 ppm (Kranwar et al., 1983). In human studies, circulating serum testosterones were significantly lower ($p<0.01$) in patients with skeletal fluorosis than those of 2 groups of controls: healthy males consuming water containing less than 1 ppm fluoride, and individuals consuming the same highly fluoridated water, but with no clinical signs of skeletal fluorosis (Susheela and Jethanandani, 1996). Testosterone levels in the second control group were lower than those in the first group ($p<0.05$) (ibid.). The findings suggest that fluoride in drinking water may bring about, or contribute to, changes in circulating testosterone levels in males.

It has been demonstrated that fluoride can react to form fluoride-substituted steroids in the testosterone and nortestosterone series; some of these substituted hormones display altered binding affinity for their own receptors and receptors for other, structurally similar androgen hormones (Liu et al., 1992; Brandes and Katzenellebogen, 1987)

Thyroid and glycoprotein hormones and the immune system

There have been various reports that fluoride can affect thyroid hormone levels and thyroid function (Hileman, 1988; Waldbott, 1962). The evidence, although not conclusive, is suggestive of various clinical and subclinical effects. Studies of ^{131}I (^{32}) uptake found that fluoride inhibits the iodine concentration mechanism in the thyroid gland (Waldbott, 1962). Significant changes in serum iodine levels, suggestive of thyroid

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[32] Radioactive iodine used as a tracer.

function alterations, have been reported in two areas with fluoride levels in drinking water at 1 - 2.1 ppm (Waldbott, 1962). In China, thyroid enlargement prevalence rates were elevated in regions with high fluorine levels, particularly in children (Yang et al., 1994). The affected children had significantly higher urine fluoride levels relative to controls, and a markedly reduced thyroid uptake of ^{131}I (ibid.).

Studies in workers continuously exposed to fluorine found alterations in the immune system and hypothyrosis (tri-iodothyronine reduced in 51% of cases) (Balabolkin et al., 1995). Workers with subclinical hypothyrosis had a higher degree of immune system abnormalities, such as higher T-lymhocyte count with a concomitant decline in functional activity (ibid.). Workers with euthyroid condition had increased number of B-lymphocytes and IgA, interpreted as "immune disorders with an allergic tendency" (ibid.).

One possible mechanism for such effects entails the inhibition of hormone-receptor interaction. An investigation of this effect found that several compounds, including phenylmethylsulfonyl fluoride were capable of interfering with thyroid hormone T3 binding to its receptor, apparently by interfering with a nucleophilic site at or close to the hormone binding domain of the receptor (Brtko et al., 1993). Fluoride can behave as a nucleophile, and may thus be capable of competing with the hormone for the nucleophilic receptor site.

The growth and function of thyroid cells is regulated by several hormones and growth factors which bind to cell surface receptors coupled via G proteins (Gs and Gq) to adenylyl cyclase and phospholipase C stimulation, respectively (Laglia et al., 1996). As has been discussed previously, fluoride and aluminum-fluoride complexes can interfere with G protein and adenylyl cyclase activation.

A number of hormones exert their effects on target cells by stimulating the enzyme phospholipase-C, which catalyzes the hydrolysis of phosphoinositides to the second messenger molecules diacylglycerol and inositol phosphates (Quirk and Reichert, 1988). Aluminum-fluoride (AlF$_4^-$) was shown to induce a 4 to 5-fold increase in IP, IP2 and IP3 (inositol phosphates) *in vitro* (ibid.).

Fluoride can activate the phosphatidylinositol-Ca2+ cascade (PiP2 cascade), one of two major known regulatory pathways in human thyrocytes (Corvilain et al., 1994). This pathway inhibits thyroid-stimulating hormone (TSH)-stimulated secretion of thyroid hormone (ibid.)[33]. These cascades also regulate in tandem the activity of the pentose phosphate pathway (ibid.), a major metabolic process in virtually all living cells.

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[33] Sodium fluoride is capable of inhibiting inositol phosphate production *in vitro* (Laglia et al., 1996).

Fluoride (as hydrogen fluoride) has also been observed to be capable of destroying N-linked oligosaccharides on glycoprotein hormones, with resultant reduction of biological activity (Cole et al., 1987).

The net effect of such perturbances to vital hormonal and messenger systems is unclear, but it appears that fluoride can interfere with the production of thyroid hormones and with hormone-receptor interactions, as well as with many other coupled metabolic processes.

Effects on membranes

Fluoride can liberate phosphates from membranes (Machoy-Mokrzynska and Machoy, 1992). This has potential implications not only for enzymatic processes, but also for other cellular properties, such as membrane permeability and stability. The main structural component of cell membranes is a phospholipid bilayer. Fluoride-induced changes in membrane phosphorylation would be consistent with, and suggest one possible mechanism for, the observed decline in the integrity of the blood-brain barrier in rats after the administration of 1 ppm F⁻ in drinking water, reported by Varner et al. (1998), discussed below under "neurotoxic effects".

Another recent rat study found that long-term (7- month) ingestion of relatively low (30 and 100 ppm)[34] levels of fluoride resulted in a dose-dependent decrease in total brain phospholipid content, and alterations in ubiquinone (Guan et al., 1998). The main types of phospholipid influenced by fluorosis included phosphatidyl ethanolamine, phosphatidylcholine, and phosphatildylserine (ibid.). It was noted that these changes to brain membranes could play important roles in the pathogenesis of fluorosis (ibid.).

Studies of red blood cells from people chronically exposed to high levels of fluoride in drinking water showed changes in membrane protein electrophoretic profiles and significant increases in lipid peroxidation and membranous cholesterol and phospholipids (Kumari and Rao, 1991).

In vivo studies of rabbits consuming relatively low[35] levels of fluoride (10 mg NaF/kg bw, about 4.5 mg/kg bw of fluoride) for 18-29 months detected substantial changes in the epithelial lining of the male reproductive system; after only 18 months, cell boundaries were unclear, and membranes "appeared to be peeled off" (Susheela and Kumar, 1991).

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[34] It has been established that rats are significantly more resistant to fluoride than humans (Mullenis et al., 1995; Mullenix, 1998).

[35] In animal models, these levels of fluoride are considered to be low by researchers in this field of study.

The findings were interpreted as suggestive of structural changes in the blood-testis barrier (ibid.).

Chronic fluoride ingestion at relatively low levels was also found to result in significantly reduced calcium uptake by rat kidney membranes (Borke and Whitford, 1999). Fluoride ingestion in all treated groups was associated with significantly lower activities in both the plasma membrane and the endoplasmic reticulum calcium pumps ($p < 0.05$) (ibid.). The plasma F levels in this study ranged from <0.4 to 35 micromol/L. Both of these pumps are very important in the regulation of calcium homeostasis (ibid.), and are membrane-associated proteins.

Effects on kidney metabolic processes

As noted above, fluoride excretion is impaired in people (and animals) with reduced kidney function. Some studies suggest that fluoride may influence the characteristics and severity of related health disorders. For instance, a recent study of diabetic rats found that fluoride intake gradually increased, hyperglycemia was more severe, and renal hypertrophy was less pronounced in rats consuming fluoridated water (10 ppm) compared with controls (consuming deionized water) (Boros et al., 1998). The femoral fluoride concentration was found to increase proportionally with fluoride intake, but concentrations of fluoride in bone compared with plasma were higher in the fluoridated group (ibid.). It was concluded that fluoride intake from drinking water may enhance the severity of diabetes, and that, due to metabolic and functional imbalance, fluoride metabolism may also change (ibid.). In another study, the presence of ammonium ion, calcium, magnesium and phosphorus in urinary calculi were reported as key determinants of bound fluorine (Machoy-Mokrzynska and Machoy, 1992).

It has been reported that chronic fluoride exposures can adversely affect several calcium-dependent processes, including kidney glomerular and tubular function (Borke and Whitford, 1999).

There has been relatively little published research assessing the chronic renal toxicity of fluoride in humans (Lantz et al., 1987). One case study reported renal failure in a young patient, associated with long-term ingestion of water with a high (8.5 mg/L) fluoride content (ibid.). It was concluded that the particulars of the case, including the presence of osteosclerosis, "suggest a causal relationship between fluoride intoxication and renal failure" (ibid.). A possible role of fluoride in kidney toxicity was recently reported by Varner et al. (1998). A recent series of experiments detected pathological changes in kidneys (glomerular distortions) and accumulation of fluoroaluminate in kidney (and other) tissues in rats consuming water with 1 ppm fluoride (ibid.). These observations were similar to the findings of Ahn et al. (1995), who reported fluoroaluminate accumulation in bones of rats consuming fluoridated drinking water. Another possible

mechanism for fluoride toxicity to kidneys entails enzyme inhibition. Decreased Na-K-ATPase activity in the thick limb loop of Henle has been shown to impair kidney concentrating ability[36] (Gutsche et al., 1984). Fluoride is a powerful inhibitor of many ATPases, including Na-K-ATPase (Kumari and Rao, 1991; Waldbott, 1962, 1978; Hileman, 1988).

Effects on the heart and other organs

Fluoride can accumulate in heart tissue, most consistently in the aorta (Hileman, 1988; Waldbott, 1962), and can cause ectopic calcification (Susheela and Kharb, 1990). The highest reported fluoride concentration in the aorta was 8400 ppm, measured in 2 persons living in fluoridated Grand Rapids, Michigan (Waldbott, 1978, p. 152, cites Geever et al., 1971) There have been reports of direct fluoride toxicity to heart muscle tissue (Burgstahler, 1974). Clinical observations of young persons (ages 25-55) with skeletal fluorosis have detected calcification of medium-sized arteries (Waldbott, 1962). Susheela and Kharb (1990) found that administration of relatively low levels of fluoride (10 mg NaF/kg bw [equivalent to 4.5 mg/kg bw F⁻]) to rabbits daily for 17 and 42 months resulted in degeneration of smooth muscle fibres in the tunica media of the aorta, as well as other effects such as the presence of electron-dense granules in mitochondria and on the inner surface of plasma membranes of smooth muscle cells, increased glycosaminoglycan and reduced dermatan sulphate content. The findings of enhanced calcium content and Ca/P ratio were interpreted as suggestive of aortic mineralization (ibid.).

The biochemical mechanism(s) involved in the calcification process are not well understood (ibid.). However, it is noteworthy that abnormal G protein expression/signalling has recently been found to cause several cardiac disorders, including abnormal calcium-channel regulation, cardiac hypertrophy and congestive heart failure (Farfel et al., 1999). Phospholipase C gamma 1 (PLCgamma1) is a widely expressed enzyme involved in regulating cell growth (Hodson et al., 1999). It appears that factors such as G proteins may be required for PLCgamma1 activation in some cells (ibid.). PLCgamma1 activity in bovine aorta appears to be associated with and activated by G proteins, and is significantly enhanced by sodium fluoride(ibid).

Although the fluoride levels in the above-noted animal experiments are considerably higher than typical adult Canadian intakes, the effects on people of long-term ingestion of lower levels are unknown. Death rates from heart disease 5 years after the introduction of fluoridation in Grand Rapids, MI, reportedly nearly doubled, and were 25%-50% higher than those of Michigan as a whole (Waldbott, 1978, ch.11). Although these findings are preliminary, as several factors such as age structures of the populations were not carefully assessed (ibid.), it is not clear why better-designed follow-up studies were not

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[36] This may explain symptoms such as polyuria and polydipsia, noted previously under acute toxicity.

undertaken.   These findings raise the possibility that fluoride may be implicated in sclerosis of arteries and cardiovascular disease.

Various reports have documented that chronic experimental fluorosis causes damage to the gastrointestinal, buccal and oral mucosa, to stomach, liver, pancreas, spleen, lungs, adrenals, thyroid, salivary glands, pituitary gland, brain, heart, retina, testes and ovaries (discussed in Waldbott, 1962, 1978).

Neurotoxicity

Although clinicians have for many years been documenting that exposures to low levels of fluoride can trigger neurological symptoms in hypersensitive individuals (Waldbott, 1956, 1978, 1998; Grimbergen, 1974), this is not generally acknowledged in the medical community. It is currently widely believed that bones are the most sensitive to the toxic effects of fluoride (Minister of Supply and Services Canada, 1993; Health Canada, 1996; ATSDR, 1997). The findings of recent research bring this assumption under question.

In 1995, a study reported sex- and dose-specific behavioural deficits in rats exposed to fluoride *in utero* and from drinking water after birth (Mullenix et al., 1995). The study found that severe behavioural disruption increased as plasma fluoride levels increased. Animals exposed as adults became hypoactive.   Such effects had previously been reported in workers occupationally exposed to fluoride (Spittle, 1994). The severity of the effect increased in proportion to plasma F levels and F concentrations in specific brain regions (ibid.).   This finding was deemed of physiological significance in humans, as plasma levels in the rat model (0.059-0.640 ppm F) were similar to or lower than those in humans exposed to high levels of fluoride; for example, serum levels in the rats were similar to those in humans exposed to 5 ppm in drinking water, and the levels in the rat study are exceeded 10-fold in children immediately after they have some types of fluoride dental treatments (ibid.).   No threshold level for these effects has yet been established (it was not the intent of the study to establish a threshold dose.   It is important to note that the rat model used in this study used a well-established computer pattern recognition system for the objective quantification of behaviour, previously tested and applied in the assessment of neurotoxic agents such as childhood leukemia treatments.

Similar findings had been reported previously in pups of rats given NaF in drinking water during gestation (Liu, 1989).   The pups had abnormal behavioural responses, and exhibited mild degeneration of nerve cell organelles and higher nerve cell density than controls (ibid.).   Excessive fluoride intake in rats has been associated with increased brain norepinephrine, and reduced 5-hydroxyl-indole acetic acid (Li et al., 1994).   Animal experiments therefore provide evidence that fluoride is a developmental neurotoxicant. Effects of fluoride on human behaviour have been reviewed by Spittle (1994).

In human populations, associations have been reported between exposure to elevated fluoride levels (via water or airborne sources) and (i) dental fluorosis, and (ii) IQ deficits in Chinese children (Zhao et al., 1996 - drinking water; Li et al., 1995 - coal burning exposures; Li et al., 1994). IQ scores were reduced in children with high fluoride exposures (compared with controls), particularly at the lower end of the IQ spectrum (Li et al., 1995; Yang et al., 1994). The results of these studies are presented in Tables IV and V. Li et al (1995) reported that the development of children's intelligence appeared to be more adversely affected by fluoride in areas of medium or severe prevalence of fluorosis, and to a lesser extent in areas with a "slight prevalence of fluorosis". It was proposed that this neurotoxic effect may occur at early stages of embryonic and infant development when brain nerve cells are undergoing rapid differentiation and development (ibid.). The studies by Li et al (1994) also noted alterations in zinc metabolism in children living in an endemic fluorosis area, as has been reported in other studies of the effects of fluoride on homeostatic mechanisms and bone abnormalities (e.g., Chlebna-Sokol and Czerwinski, 1993; Boeckhabisch and Oliveira, 1997).

In a comparison of children in two similar towns, Zhao et al. (1996) reported a statistically significant ($p<0.02$) association between reduced IQ and endemic fluorosis following *in utero* exposures to fluoride in water. IQ scores were shifted downwards at both ends of the spectrum (i.e., low and high); as well, IQ scores increased more slowly with age in the high-exposure group (ibid.). The study considered socio-economic variables such as parental occupation, living standards and social customs (ibid.). Children's IQ scores correlated with the education level of parents, as was expected (ibid.). Although the two study populations were exposed to fluoride concentrations of 4.12 (which is a shade over the U.S EPA Maximum Contaminant Level of 4.0 ppm) and 0.91 ppm, no threshold level for the effects has been established. No effects threshold exists for some neurotoxins like lead. Fluoride is considered to be more toxic than lead (Clinical Toxicology of Commercial Products, 1984, 5th edition).

Table IV   Mean IQ Scores for Chinese Children in Areas with Varying Prevalence of Dental Fluorosis

Degree of Fluorosis	None	Slight	Moderate	Severe
Number of children	226	227	224	230
Dental Fluorosis Index	<0.4	0.8	2.5	3.2
Urinary F (mg/L)	1.02	1.81	2.01	2.69
IQ (mean +/- SD)	89.9 +/-10.4	89.7 +/-12.7	79.7 +/-12.7	80.3 +/- 12.9

Source: Li et al., 1995.

Table V   Distribution of Children's IQ Scores from Areas with Differing Fluorosis Prevalence

	IQ Range						
Fluorosis status	**<70**	**70-79**	**80-89**	**90-109**	**110-119**	**120-129**	**>129**
none	2.6%	9.7%	37.1%	46.8%	3.9%	0.8%	0
slight	3.1%	15.9%	29.1%	47.1%	3.1%	1.3%	<0.4%
moderate	25.4%	23.7%	29.9%	20.5%	0.4%	0	0
severe	20.9%	26.6%	26.9%	25.2%	0.4%	0	0

Source: Li et al., 1995.

The studies by Mullenix et al. (1995) predicted that certain areas of the brain (hippocampus) would be found to be particularly vulnerable to fluoride toxicity. This prediction has been supported by the recent studies by Varner et al. (1998), also discussed above.

As noted previously, recent work has established that fluoride forms complexes with aluminum, and that such molecules have biological activity (see also Varner et al., 1995, 1993). Isaacson (1997), in an experiment using NaF alone, found that fluoride in drinking water at 1.0 ppm (the level recommended for drinking water) increased the level of aluminum in the brain of rats by almost 75%. It was suggested that the fluoride complexed with the aluminum in the feed forming AlF3 which then penetrated into the brain. Neuronal abnormalities were observed in NaF-treated animals (ibid.). Cells were distorted and cell losses in different regions of the brain were noted. A previous study on AlF_3 noted that both AlF_3-treated animals and NaF-treated animals were found to have a "general impairment in the immune capacities of the treated subjects" (Varner et al., 1993).

A 1998 study (a follow-up to previous work studying the role of drinking water aluminum and fluoride in aging-related neurological impairments) described alterations in the nervous systems of rats after chronic administration of the fluroaluminum complex (AlF_3 at 0.5 ppm) or equivalent levels of fluoride in drinking water (as NaF containing 1 ppm fluoride) (Varner et al., 1998, 1995). The results of the 1995 study were replicated in 1998. The levels of aluminum in brain and kidney tissues were higher in rats drinking water with either AlF_3 or NaF, relative to controls. Both treated groups showed pathological changes in kidneys (glomerular distortions) and brain tissues. Brain aluminum levels in both treated groups were elevated: double the control level in the NaF group, and even higher in the AlF_3 group. There was clear evidence of neuronal injury in both treated groups. IgM was found at increased levels in the right hemisphere of the brain cortex in both treated groups. This finding was deemed unusual, since such

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antibodies are typically excluded from neuronal tissue by the blood-brain barrier (ibid.). Treated groups also exhibited increased vascular β-amyloid in the lateral posterior thalamus, which "may be causally related to the neuronal degeneration found in the area to which it is principally connected, the superior parietal cortex" (ibid.). The authors noted that "striking parallels were seen between aluminum-induced alterations in cerebrovasculature and those associated with Alzheimer's disease and other forms of dementia..." (ibid.). While the experiments do not prove a causal role, they show that ingested aluminum reaches the brain, and that this seems to result in neural injury. These effects (i.e., aluminum penetration into and accumulation in brain tissues, and subsequent toxic effects) appear to be enhanced by fluoride at levels as low as the 1 ppm added to the Regional drinking water supply.

As the authors note, "While the small amount of aluminum [0.5 ppm AlF$_3$] in the drinking water of rats required for neurotoxic effects is surprising, perhaps even more surprising are the neurotoxic results of NaF at the dose given in the present study (2.1 ppm, or about 1 ppm fluoride)" (ibid., p.296). These studies also underscore some of the limitations of current methods for assessing the potential toxic effects of contaminants. When a chemical is evaluated individually, major effects and synergies due to its interactions with other substances can be missed. The apparent link between dental fluorosis and nervous system damage is of particular concern due to the already substantial and increasing prevalence of dental fluorosis - including moderate and severe fluorosis - in children in Ottawa-Carleton and elsewhere[37] (e.g., Clark, 1993, Cutter, 1998; Angelillo et al., 1999).

Fluoride has also been shown to affect axonal transport (AT) in the spinal cord and vagal and hypoglossal nerves: small doses of fluoride accelerated AT, correlating with an increase in the second messenger cyclic AMP (Frolkis et al., 1997). Hydrocortisone and testosterone were transported along axons, reached skeletal muscle fibres, and hyperpolarised the plasma membrane (inid.). High doses of fluoride and castration decelerated AT more significantly in old rats (ibid.). It was suggested that changes in axonal transport may be an important mechanism of disordering the growth of neurons and innervated cells in old age (ibid.). This experiment seems to show that fluoride may be able to affect the axonal transport process, which is sensitive to uncoupling of oxidative phosphorylation, inhibition of glycolysis and hypoxia in old rats compared with younger adults (ibid.). As discussed above, fluoride can interfere with these latter metabolic processes by inhibiting, activating, or changing levels of many enzymes and hormones, including testosterone.

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[37] Cutter (1998) survey of Ontario health units noted the following fluorosis rates: mild: 1-29%; moderate: 0.2-31%; severe: 0.1-10.2%. Specific data for Ottawa-Carleton were unavailable.

The experiments of Zhao et al. (1994, 1998) show that fluoride can alter levels of important enzymes like acetylcholinesterase and Ca2+Mg(2+)-ATPase in brain synaptic membranes. Chronic toxicity studies of fluoride have not included extensive histological characterization of injury to the brain (Varner et al., 1998). These findings indicate that more study is needed to clarify the neuropathological effects of fluoride on the central nervous system, the immune system and in the aging process.

It has recently been observed that "For some neurotoxic chemicals, neurobehavioral effects are now considered to be among the most sensitive end points yet detected, particularly if exposures occur during critical windows of vulnerability. Chemically induced problems with perception and cognitive ability in children can be hard to identify; teasing them out of a host of genetic and sociocultural influences is a difficult task. Today, most data on environmentally relevant neurobehavioral effects in children are concentrated in three chemicals: lead, methylmercury, and polychlorinated biphenyls. But mounting evidence of the neurobehavioral effects of chemicals along with growing public concern over pediatric mental health problems such as attention deficit/hyperactivity disorder dictates that scientists and legislators improve test methods, explore mechanisms, and develop appropriate strategies for risk assessment and policy making" (Schmidt, 1999).

Reproductive effects

Studies conducted since the early 1960s reported no solid evidence of adverse effects of fluoride on reproductive outcomes (Health Canada, 1996). Some past and recent research has reported associations between fluoride exposure and birth defects, in particular, Down Syndrome (see below). As noted above, brain damage and behavioural deficits were observed in the offspring of rats which were administered fluoride during gestation (Mullenix et al., 1995; Liu , 1989). In the Liu study, morphological examination of the pup brains showed mild degeneration of nerve cell organelles and higher nerve cell density in the high-dose group (ibid.).

High levels of fluoride have been associated with reduced fertility in most animal species studied (Freni, 1994). A study of rats administered relatively low fluoride levels (4.5 mg/kg bw/day) found slightly lower activities of intermediary enzymes in androgenesis[38], and declining circulating testosterone levels after 50 days, indicating that fluoride may interfere with steroidogenesis in short term exposures (Narayana and Chinoy, 1994). Male rabbits and mice fed 20 or 40 mg NaF/kg bw/day developed reproductive effects including sperm abnormalities, lower sperm motility, sperm counts and fertility rates (Health Canada, 1996).

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[38] 3β- and 17β-hydroxysteroid dehydrogenase.

Adverse effects on reproductive function in male and female rodents have been observed after short-term administration of fluoride (Minister of Supply and Services Canada, 1993). Histopathological changes on rodent testes and ovaries have also been observed following both short and long-term fluoride administration (ibid.). As noted above, high levels of fluoride have been found to cause profound damage to the male reproductive system (Susheela and Kumar, 1991).

Freni (1994) conducted a population analysis of human fertility rates using an existing database of drinking water systems to determine fluoride levels in drinking water for U.S. counties. Two different measures of exposure were defined, and the annual total fertility rate (TFR) for 10-49-year-old females was calculated for the period 1970-1988 (ibid.). For each region, the annual TFR was regressed on the fluoride exposure measure and on sociodemographic covariables (ibid.). In most regions, an association of decreasing TFR with increasing fluoride levels was found (ibid.). Meta-analysis of the region-specific results again found a negative TFR/fluoride association with a consensus combined p value of 0.002-0.004 (ibid.). There was reportedly no evidence of influences due to selection bias, inaccurate data, or improper analytical methods (ibid.). Although ecological studies cannot determine cause-and-effect relationships, these findings indicate that the potential role of fluoride exposures in infertility and other reproductive problems warrants further investigation.

Possible mechanisms whereby fluoride may affect fertility include effects on the myometrium and attendant contractions. In animal studies, fluoride (as aluminum fluoride, AlF_4^-) stimulates phasic myometrial contractions (Philippe and Basa, 1997; Philippe, 1995). Uterine and myometrial processes are partially regulated by adenylate cyclase activity, which can in turn be regulated by the presence of the embryo (Boulet et al, 1988; Bekairi et al., 1984); as discussed elsewhere, fluoride stimulates adenylate cyclase. Adenylate cyclase is an enzyme embedded in the cell membrane; as discussed above, fluoride may affect membrane structure by altering phosphorylation, thereby interfering with the activity of associated enzymes. Activation of G protein by fluoroaluminate also stimulates phosphoinositide hydrolysis and prostaglandin F2 alpha secretion at all stages of estrus and early days of pregnancy (Ludwig et al., 1998). It appears that fluoride can interfere with biochemical control mechanisms and pathways in endometrial responsiveness to oxytocin, where control is exerted at multiple levels which involve G proteins and phospholipase C as well as other secretory pathways (Ludwig et al., 1998).

Birth defects

In the late 1950s and early 1960s, epidemiological studies reported a correlation between fluoridated water and the incidence of Down syndrome, a chromosomal aberration (Rapaport, 1956, 1959, 1963). Later reports dismissed these findings as statistically insignificant (Erickson et al., 1976; Needleman et al., 1974). More recently, a reanalysis

of the data has again indicated a strong correlation between fluoridated water and Down syndrome, after correction for maternal age (Takahashi, 1998). The analysis also found that there is no threshold level for the effect, indicating that, after maternal age, fluoride may be a major risk factor for Down syndrome.

Fluoride is considered clastogenic at a level of 10 ppb (μg/L) (Health Canada, 1996). Several of the clinical and biochemical characteristics of Down syndrome are strikingly similar to those of fluoride poisoning (Burgstahler, 1975). Examples include an abnormally high frequency of cataracts, delayed eruption of teeth, staining of tooth enamel, elevated serum alkaline phosphatase (characteristic of skeletal fluorosis), anomalous tryptophan metabolism, calcification of soft-tissue organs and premature aging (ibid.). In addition, women with thyroid disorders have a significantly higher risk for Down syndrome births (ibid.). As seen above, fluoride may affect thyroid function. It appears that more research will be needed to clarify this issue.

Effects on the environment

It is estimated that drinking water fluoridation releases about 2,000 tonnes of fluoride into Canadian waterways annually (Foulkes, 1995). This makes water fluoridation the second largest source of environmental fluoride pollution, after phosphate fertilizer manufacture, but ahead of chemical production (ibid.). Inorganic fluoride has been classified as a toxic substance under the Canadian Environmental Protection Act (CEPA). A 1993 CEPA assessment determined that i) fluoride is currently entering the Canadian environment in quantities or under conditions that may be harmful to the environment; ii) there is insufficient information to conclude whether sulphur hexafluoride is entering the environment in quantities or under conditions that may constitute a danger to the environment on which human life depends (i.e., global climate change); and iii) that inorganic fluorides are not entering the environment in quantities or under conditions that may constitute a danger to human life or health (Minister of Supply and Services Canada, 1993). In the 1993 review, fluorides released to the environment as a consequence of municipal drinking water treatment were not assessed. This was noted as a significant information gap in the review (ibid.).

In Ottawa-Carleton, about 595 metric tonnes of hydrofluorosilicic acid, which contains 20% fluoride by weight (i.e., 119 tonnes of fluoride), are added to the drinking water supply each year (I. Douglas, Region of Ottawa Carleton Dept. of Environment and Transportation, 1999, pers. comm.). The Region of Ottawa-Carleton therefore contributes about 6% of total municipal (drinking water) fluoride emissions in Canada. Fluoride is not significantly removed during wastewater treatment, resulting in an effluent concentration of 0.8 mg/L released to the Ottawa River (K. Middlebrook, Region of Ottawa Carleton Dept. of Environment and Transportation, 1999, pers. comm.). The

toxicity threshold for fluoride in freshwater systems is 0.28 mg/L (Minister of Supply and Services Canada, 1993). The dispersal of fluoride from Ottawa-Carleton's wastewater effluent, and its environmental effects and bioaccumulative characteristics in flora and fauna in the Ottawa River, have not been studied (M. Trudeau, Region of Ottawa Carleton Dept. of Environment and Transportation, 1999, pers. comm.).

Risk factors for fluoride toxicity

Fluoride toxicity is influenced by several variables, such as the intake of certain vitamins and minerals, as well as individual sensitivity and hypersensitivity. Such individuals are at elevated risk for a range of adverse health effects, described previously.

Fluoride accumulation can be higher in cases of nutritional deficiency (ATSDR, 1993; Minister of Supply and Services Canada, 1993 - cites U.S. DDHS, 1991; Susheela et al., 1992). According to the ATSDR the elderly, people with a deficiency of calcium, magnesium and/or Vitamin C, and people with cardiovascular and kidney problems are at higher risk of increased fluoride accumulation and associated health risks (ATSDR, 1993). In people with reduced kidney function, fluoride clearance is impaired. The health risks for people with other health problems or conditions, and those taking various medications, have not been evaluated. Certain medications (and products such as some pesticides) contain fluoride, which may increase total fluoride intakes and toxicity. Fluoride excretion is also strongly influenced by urinary pH (Whitford, 1997); therefore, factors which increase acidity (metabolic acidosis) also raise the risks of fluoride toxicity (ibid.). For example, residence at high altitudes, high protein diets and certain metabolic and respiratory disorders are risk factors for development of dental fluorosis (ibid.).

Bone and plasma fluoride levels increase as kidney function declines (U.S. Surgeon General's committee, cited in Hileman, 1988). The U.S. National Kidney Foundation recommends fluoride-free water for dialysis treatment (Hileman, 1988). Studies indicate that children with moderate kidney impairment, such as those with diabetes insipidus, are at elevated risk of skeletal changes from consuming water fluoridated at 1 ppm (Hileman, 1988). It has not been determined, however, what proportion of persons with kidney impairment develops clinical stage fluorosis (ibid.). Animal studies have shown that fluoride ingestion can effect biochemical changes in kidneys (Hileman, 1988; Varner et al., 1998), although the effects of such changes on kidney function are unknown (ibid.). Areas of endemic skeletal fluorosis report higher levels of impaired kidney function (Hileman, 1988).

Adequate dietary calcium intakes (>800 mg/day) may have a protective effect against the development of dental fluorosis and tooth decay (Teotia and Teotia, 1994). Dietary intakes of less than 300 mg/day of calcium may enhance fluoride toxicity (ibid.). It has

been reported that even relatively low intakes of fluoride (>2.5 mg/day) continuously for more than 6 months in calcium-deficient children may cause severe dental fluorosis and caries (ibid.). Poor nutrition is a risk factor for the incidence and severity of dental and skeletal fluorosis, a factor which should be considered when claims are made that water fluoridation is of particular benefit to persons in lower socioeconomic strata.

Human breast milk is naturally very low in fluoride (Health Canada, 1996), indicating a negligible biological need for this substance during infancy. Table I demonstrates that infants who drink fluoridated water (e.g., in reconstituted formula) consume a considerable amount of fluoride; in some cases, the TDI level is ingested (or even exceeded) via the consumption of drinking water alone. Additional fluoride intake from sources such as juices or other beverages, and foods, may result in an exceedance of the TDI. A similar effect is possible for older children.

Young children appear to be at especially higher risk of fluoride toxicity, especially to developing teeth, bones and the nervous system, based on human and animal studies. Children with dental fluorosis may be at higher risk of adverse effects on the central nervous system, including effects such as behavioural deficits and reduced intelligence. Young males (but apparently not females) may be at higher risk of fluoride-induced bone cancer (osteosarcoma) due to differences in the regulation of bone development in males and females (Yiamouyiannis, 1993). Bone development in boys (but not in girls) is regulated by testosterone.

Recent studies on fluoride-aluminum interactions indicate that fluoride and aluminum, when present together even at low concentrations, can cause biological changes and enhanced toxic effects in bone, kidney, brain tissues and the thyroid gland. Inorganic (free) aluminum is present in the regional drinking water supply at relatively low levels.

Because certain foods and beverages contain relatively high concentrations of fluoride, consumers of these foods, which include certain marine fish, meats, eggs and many teas, may also be at elevated risk (Health Canada, 1996; Nosal, 1998). For instance, it has been estimated that 6 cups of tea can contribute 1-2 mg of fluoride to the diet (Waldbott, 1978). It is not currently known whether consumption of foods high in aluminum has synergistic or antagonistic effects on fluoride toxicity in humans.

People who consume large quantities of water or beverages made with fluoridated water, such as persons with certain diseases, athletes, and workers in occupations involving heavy physical exertion, are at elevated risk of excessive fluoride intake. Clinical case studies have reported intakes of 4-6 litres of fluid/day (e.g., South. Med. J. 91(11):1079-1082, 1998).

For children, ingestion of fluoride from toothpaste and other dental treatments and medications is an important risk factor for excessive fluoride intake. A lethal dose of fluoride for children can be less than 5 mg/kg[39] (Health Canada, 1996); consequently, ingesting a tube of toothpaste (which contains 1000-1500 ppm fluoride) can kill a small child.[40] Children in rural areas may be ingesting fluoride supplements on the recommendation of physicians or dentists (see below). Because such children may be exposed to fluoridated drinking water from the regional supply, for example, while at a daycare or school or while staying with relatives serviced by fluoridated water, such children are also at higher risk of excessive fluoride ingestion (A. Burry, Ottawa-Carleton Health Dept., 1999, pers. comm.; Clark, 1993). This highlights the difficulty in controlling the dose of medication such as fluoride administered via a drinking water supply.

Guidelines and related observations

The MAC for fluoride is 1.5 mg/L. This guideline was recently reviewed by Health Canada and maintained at its current level (Health Canada, 1996b). The "optimal level" for fluoride in drinking water was recently lowered by 20% to 0.8 - 1.0 mg/L for most community supplies (Health Canada, 1998). The average fluoride concentration in the Regional piped water supply has been 1 mg/L; in June, 1999, the level was reduced to 0.8 mg/L. (The province of Ontario had not reduced the recommended range in the Ontario Drinking Water Objectives at the time of writing of this report - June, 1999). As can be observed from the above tables comparing fluoride intakes to toxicity criteria, a 20% reduction in fluoride levels in drinking water will reduce total daily intakes by about 10% or less, and therefore will not influence associated health risk levels in a significant way.

This recommended "optimal level", and the current estimated daily intakes listed above in Tables I and II, can be compared to the value for fluoride listed in the U.S. Agency for Toxic Substances and Disease Registry (ATSDR) Minimal Risk Levels for Hazardous Substances[41] (ATSDR, 1997). The MRL for chronic (>364 days) exposure to fluoride in water is 50 μg/kg bw/day (ATSDR, 1997). This compares with levels on the order of 90-160 μg/kg bw/day that children in fluoridated communities are consuming for at least the first 4 years of life. It must be noted that the ATSDR MRL is based on a

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[39] The U.S. EPA value for the acute human lethal dose of fluoride is 4 mg/kg bw (Calabrese et al., 1999 (1997)).

[40] In the U.S., but not in Canada, poison warnings are now required on fluoridated toothpastes, pursuant to U.S. FDA legislation promulgated in April, 1997 (Fluoride 30(3):141; 1997).

[41] MRLs are estimates of "the daily human exposure to a hazardous substance that is likely to be without appreciable risk of adverse noncancer health effects over a specified duration of exposure". They are intended to serve as screening levels for use in the identification of contaminants and potential health effects of concern at hazardous waste sites. Most MRLs contain some degree of uncertainty because of a lack of precise toxicological information on the people who might be most sensitive to the effects of hazardous substances.

musculoskeletal toxicity endpoint; the possibility that the nervous system may be more vulnerable to fluoride toxicity was not considered.

The Nonlethal Toxic Dose for fluoride has recently been listed as 0.04-3.9 mg/kg bw - i.e., as low as 40 µg/kg bw (based on a body weight of 35 kg, Calabrese et al., 1999 (1997)). Average fluoride intakes in children living in fluoridated communities exceed the lower bound of this range by 200% to 400% (i.e., two to four times - see Tables I and II). Intakes by many adults also exceed this value. The lower bound (i.e., 40 µg/kg bw/day) is more than two times lower than average intakes by young children from drinking water alone (i.e., an exceedance of >100%). Corrected for a lower body weight, the exceedances would be even higher for younger children if the lower value is used for estimates; this calculation does not even take into account children's probable higher sensitivity to the toxic effects of fluoride.

The TDI (Tolerable Daily Intake) for fluoride is listed as 122 µg/kg bw/day by Health Canada, on the basis that it is unlikely to produce moderate to severe dental fluorosis in children 22-26 months old (Health Canada, 1996). Because mild dental fluorosis is the result of fluoride toxicity to tooth-forming cells, the TDI level is a *de facto* acceptance of a toxic endpoint for the majority of the population. The difference between mild, moderate and severe dental (and skeletal) fluorosis is only a matter of degree of toxicity (a difference in *degree*, not in *kind*). As is evident from Tables I and II, many children ingest this amount of fluoride (i.e., the TDI level) from drinking water alone, which leaves no margin of safety for the ingestion of fluoride from other sources such as food and dental products. The latter sources also contribute significantly to the total fluoride intake of children. It is therefore not surprising that dental fluorosis in children is increasing, and is twice as common in fluoridated communities as in unfluoridated ones. Moreover, Health Canada acknowledges that some children in fluoridated communities exceed the TDI for fluoride (Health Canada, 1996). This was not deemed to be cause for concern (ibid.).

A comparison the Health Canada TDI, the ATSDR MRL, the Minimal Toxic Dose in the U.S. (Calabrese et al., 1999 (1997)), and levels currently ingested by children and adults in fluoridated Canadian communities is shown below in Table VI. All units are µg/kg bw/day.

Table VI   Comparison of Fluoride Intakes, Guidelines and Minimal Toxic Dose

TDI	ATSDR MRL	MTD	Average daily intake from fluoridated drinking water - children	Average total daily intake - children: 7 mo-4 yr.	Average total daily intake - adults
122	50	40	0-6mo: **107** 7 mo-3 yr: **67** 3-5 yr: **115**	**87-160** (fluoridated) **45-96** (unfluoridated)	**47-58** (fluoridated) **32-36** (unfluoridated)

The TDI and MAC figures (and the ATSDR MRL) do not take into consideration the documented, severe adverse health effects in persons who are hypersensitive to fluoride at much lower concentrations. The scientific publications reviewed in the development of the TDI and MAC (and the ATSDR MRL) did not include recent human and animal studies providing substantial evidence that fluoride is neurotoxic to children and adults at levels added to the drinking water in many municipalities.

Health and Environment Canada state that "average daily intakes [of fluoride] are at least 20% less than the level at which adverse effects upon the skeleton are anticipated" (Health Canada, 1996, p. 13; Minister of Supply and Services Canada, 1993). This raises at least two pertinent questions: 1) what are the anticipated effects for people with above-average intakes?; and 2) since deposition of fluoride in bones (and tissues) is also a function of other factors such as fluoride excretion[42], which is in turn influenced by age, nutritional and health status, is it possible to reach meaningful conclusions about all potential adverse health effects of fluoride on the basis of intake levels alone?

Summary and conclusion

Fluoride has been added to many drinking water supplies, including the drinking water in (*deleted*), for many years in the belief that it prevents dental caries. This belief is currently disputed within the scientific community, with substantial credible evidence on both sides of the issue. A considerable amount of published scientific research demonstrates that fluoridation has not been universally beneficial - and has even been detrimental to caries rates in some fluoridated communities. No well-designed scientific studies of local residents have been conducted showing that fluoride was in the past, or is now, responsible for improving the oral health of the local population. It is therefore not

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[42] Other factors include the extent of fluoride absorption and its chemical reactions with other substances, which seem to be affected by variables such as diet (especially metal and mineral content).

known whether fluoride in drinking water is currently providing any benefits to any of members of our population.

There is considerable evidence that many members of our population - probably the majority - do not currently derive benefits from drinking water fluoridation. For example:

- most young infants do not have erupted teeth;

- recent studies have found that about 50% of persons in unfluoridated areas are now caries-free (the same or slightly higher proportion than in fluoridated communities) - these persons thus would not benefit from fluoride in drinking water;

- if the average benefit of water fluoridation in a population is less than one DMFT (decayed, missing or filled permanent tooth), statistically (assuming a distribution of benefits), there must be many persons for whom the "benefit" is essentially zero;

- the recommended "optimal daily requirement" for fluoride benefits is 1 mg/day, while the current average daily intake is 4.4 mg/day, with 33%-65% from drinking water (Health Canada, 1996, as seen in Table II). Assuming elimination of the maximum estimated 65% contribution from drinking water (for children), the average daily intake would still be 1.54 mg/day, at least 50% above the "optimum daily requirement".

The beneficial effects of fluoride, if they exist, are due to topical application; therefore, fluoride ingestion offers virtually no benefits. Fluoride ingestion indisputably poses health risks. Some individuals are at risk even at current levels in drinking water. Fluoride from drinking water is the single largest source of ingested fluoride in fluoridated communities, particularly for children. The Maximum Acceptable Concentration (MAC) for fluoride in drinking water is only 0.5 parts per million above the level (1 ppm, reduced to 0.8 ppm in June, 1999) added to our drinking water supply. In this respect, the MAC for fluoride is unusual, because it is standard practice to allow a safety margin of at least 10-100 fold for most toxic substances. The MAC for fluoride provides little or no safety margin.

Fluoride is an extremely reactive chemical, with many and varied biological and physiological effects. Although not all of the mechanisms of action have been elucidated, fluoride can interact with and affect, in a number of different ways, virtually every major organ and system in the body, including:

- teeth

- bones

- skin

- enzyme systems

- endocrine systems

- digestive/gastrointestinal system
- heart and cardiovascular system
- kidneys
- liver
- male and female reproductive systems
- neuroimmune system
- membranes
- homeostatic mechanisms.

The earliest visible sign of fluoride toxicity is dental fluorosis in children. The fact that dental fluorosis prevalence in fluoridated communities currently ranges from 35-60% and is increasing is clearly a cause for concern. Given fluoride's ability to affect so many bodily organs, systems and processes in such a myriad of ways, it would be remarkable if its currently documented adverse effects on tooth enamel development in children were the only manifestation of toxicity. The known and expected symptoms of fluoride toxicity are similar or identical to many other diseases and disorders, including digestive upset, arthritis, dementia, and behavioural problems in children. Therefore, a link to fluoride exposure for such conditions could easily be missed in a medical diagnosis.

As can be seen from estimated daily fluoride intakes shown in Tables I and II, and the 3-9 mg/day intake range for a population with drinking water fluoridated at 1 ppm (U. S. Department of Health and Human Services, cited in Nosal, 1998), it probable that daily intakes in fluoridated communities at the higher end of the intake range exceed the maximum level which can be considered safe for chronic consumption. At current fluoride intake levels in fluoridated communities, at least a portion of the adult population is likely to be at risk of mild to moderate skeletal fluorosis over the longer term. Also, the intake data and guideline levels listed in the previous section (Table VI) clearly show that maximum recommended guideline levels for fluoride intake set by government agencies and documented toxicity thresholds are being exceeded by a significant proportion of the population, and especially by children.

Health Canada has recently stated that "The Health Protection Branch [of Health Canada] continues to subscribe to the position that exposure to substances for which the critical effect has no threshold be reduced to the extent possible" (Health Canada, 1996b, p.12). The following are among the types of effects for which no thresholds are deemed to exist: genetic damage (genotoxicity); damage to the reproductive system (reproductive toxicity); damage to the developing embryo/foetus (developmental toxicity); carcinogenicity (Fan et al., 1995).

Fluoride is a recognised mutagen, is capable of damaging chromosomes at parts-per-billion levels, and a reproductive toxicant (Health Canada, 1996; Minister of Supply and Services, 1993). There is also substantial evidence that fluoride is a developmental toxicant (Mullenix et al., 1995; Liu, 1989; Li et al., 1995) and a carcinogen. According to the above criteria specified by the Health Protection Branch (Health Canada, 1996b, p.12), exposure to fluoride should be reduced to the extent possible. The application of the above principle to fluoride in drinking water appears even more appropriate because ingestion of fluoride confers no known benefits, but poses known health risks, as well as health risks for which there is substantial research and epidemiological evidence.

Also, as discussed above, there are many individuals who cannot derive potential benefits from the topical effects of fluoride in drinking water (e.g., young infants and others who do not have teeth). Such individuals are being exposed to health risks with no attendant benefits, which presents a serious ethical problem from a medical and a human rights standpoint.

A range of alternative sources of fluoride (e.g., toothpastes, gels, sealants, supplements) is available which can deliver fluoride medication in a much more controlled and individualised manner than is possible by adding a uniform concentration of fluoride to drinking water. (As well, other treatments such as antimicrobial therapy (Lopez et al., 1999) and options such as nutritional counselling and assistance and vitamin supplementation can provide large benefits in caries reduction along with overall health improvement). Removal of fluoride from drinking water would significantly (by 40-50%, on average) reduce total fluoride intake, and associated known and probable health risks. Some of these risks are non-threshold events - i.e., there is some risk at any level of exposure. The dose of fluoride an individual receives via drinking water is not controlled, because of widely variable differences in water and food consumption, as well as in metabolic processes such as fluoride excretion and turnover. Compared with administration of an uncontrolled dose of fluoride medication via drinking water, consideration of individual total fluoride exposure could reduce health risks from fluoride ingestion, especially for individuals who are at higher risk due to above-average water consumption or diets which are higher than average in fluoride content, or to other factors such as pre-existing health conditions and poor nutrition.

Individuals using groundwater

The Canadian Dental Association and the (*deleted*) Health Department's Dental Program recommend that private wells should be tested for fluoride if children 3 years and older consume the water. Fluoride concentrations 0.3 to 1.3 mg/L are considered adequate by dental and medical associations. If levels are below 0.3 mg/L, fluoride supplementation may be advised by a physician. At a concentration over 1.3 mg/L, it is considered that fluoride may pose a risk of dental fluorosis (no health risks are noted; dental fluorosis is not considered a health condition or symptom by dental and medical associations). The

water may need treatment to reduce fluoride to acceptable levels (see below). Given the finding that fluorosis rates are increasing in communities with water fluoridated at 1 mg/L and even in unfluoridated communities, and that the "optimal daily intake" is currently being achieved in unfluoridated communities (Clark, 1993), both fluoride supplementation and the 1.3 mg/L threshold value are questionable. Ingested fluoride offers virtually no benefits, and may entail adverse drug reactions, including gastric irritation, as discussed previously.

Removal of fluoride from drinking water

The only treatments effective for fluoride removal are reverse osmosis or distillation (discussed in more detail in the Health Department's State of Environment Report: Focus on Drinking Water Quality (1999), section 4.10). Brita and similar carbon filters do not remove fluoride. Bottled waters contain varying amounts of fluoride; in some cases, levels are far higher than those in municipal drinking water. The fluoride content of bottled water is usually listed on the label.

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