SUBJECT: Water Fluoridation BOH08024 (City Wide)

RECOMMENDATION:

(a) That this report, requested at the October 2007 Board of Health meeting, be received as information.

(b) That the Board of Health support the formation of an Ontario Fluoridation Office, funded by the province, through correspondence to the Ministers of Environment, Health and Long-Term Care, Health Promotion and the Chief Medical Officer of Health for Ontario.

(c) That the item related to Water Fluoridation be removed from the outstanding business list.

Elizabeth Richardson, MD, MHSc, FRCPC
Medical Officer of Health
Public Health Services

EXECUTIVE SUMMARY:

At the November 2007 Board of Health meeting, staff were directed to report back on the following:
Options to water fluoridation such as good nutrition, preventive dental care and targeted topical applications, including an evaluation of the effectiveness of water fluoridation vs topical applications;

Costs for alternative programs should water fluoridation be discontinued;

An objective review of data, (i.e. National Research Council (NRC), 2006 National Academy of Science (NAS), 2006) on this issue including experience from other municipalities;

Comparison of topical v.s. water fluoridation options in an evaluation of chemical composition and any potential toxicity concerns.

The purpose of this report is to report back, as directed.

Fluoridation of drinking water provides important oral health benefits. Credible scientific evidence supports the view that at 0.5-0.8ppm, the benefits of fluoridation outweigh any risks. The fluoridation of water continues to be supported by the World Health Organization, the Canadian Dental Association and many other highly recognized organizations. The balance of scientific evidence of fluoridating water shows that water fluoridation is a safe, economical and effective oral health preventive measure that has resulted in improved oral health for the population.

In cases where the fluoridation of water has been discontinued, increased rates of dental decay have been observed unless extensive preventive programs have been put in place. If municipal water fluoridation were to be discontinued, alternate forms of fluoridation, such as topical fluoride application, would be required. Costs for such programs are described in the report; they would be significantly higher per person than the costs of fluoridating municipal water, and would reach a smaller number of people.

Of particular concern are families living in poverty, who may be less able to afford preventive dental care. Water fluoridation is available to everyone using municipal water. This issue is of particular importance in cities such Hamilton, with high rates of poverty.

Since some individuals have expressed concern about water fluoridation, it is recommended that the Board of Health support the establishment of an Ontario Fluoridation Office by the provincial government. This Office would constantly assemble and review current scientific evidence on fluoride and water fluoridation, and would result in the best advice to Ontarians regarding water fluoridation, with consideration of any new evidence that may become available in upcoming years.

**BACKGROUND:**

Fluoride is a naturally occurring substance in ground water. Generally, surface water sources such as lakes, rivers and streams have very low concentrations of fluoride. The naturally occurring level of fluoride in Lake Ontario is 0.15 p.p.m.

In the 1920’s and 1930’s it was discovered that there was a correlation between fluoride levels in the water and reduced incidence of dental decay. In 1940, four community wide studies were conducted to examine the effect on dental health of adding sodium fluoride to fluoride deficient water supplies. The studies were conducted in Grand Rapids.
Michigan, Newburgh, New York, Brantford, Ontario and Evanston, Illinois. The results of these studies confirmed that fluoridation is a practical and safe public health measure to prevent tooth decay.

Water fluoridation, the practice of adjusting the level of fluoride in potable drinking water to maximize its benefit to oral health, has been ongoing for over 60 years. Fluoride has been, and continues to be, extensively studied worldwide to examine the possibility of any adverse health effects from low-level consumption. Some adverse health effects have been attributed to fluoride. However, the body of credible scientific evidence worldwide attests to the fact that fluoridation of community water supplies is safe and effective. It is estimated that over 405 million people worldwide are the beneficiaries of water fluoridation.

In keeping with Canadian water standards, the level of fluoride in Hamilton’s water ranges between 0.5-0.8 p.p.m. which is a decrease from the 1999 standard of 1.0-1.2 p.p.m. This reduction was implemented to comply with the revised Ministry of the Environment target fluoride concentration for water fluoridation and to address concerns that there was an increase in the number of children exhibiting the mild form of fluorosis. Dental fluorosis is a change in the appearance of teeth caused when higher than optimal amounts of fluoride are ingested in early childhood during the time that the teeth are forming. Although mild fluorosis is cosmetic, it is undesirable, and the fluoride levels were reduced to avoid the occurrence of fluorosis.

Some community members have expressed concerns about water fluoridation, and have requested that Council consider discontinuing the practice of fluoridating water. At the October 2008 Board of Health meeting, both staff and delegations against water fluoridation made presentations.

Staff were directed to report back on the following:

(a) Options to water fluoridation such as good nutrition, preventive dental care and targeted topical applications, including an evaluation of the effectiveness of water fluoridation vs topical applications;

(b) Costs for alternative programs should water fluoridation be discontinued;

(c) An objective review of data (i.e. National Research Council (NRC), 2006 National Academy of Science (NAS), 2006) on this issue including experience from other municipalities;

(d) Comparison of topical vs water fluoridation options in an evaluation of chemical composition and any potential toxicity concerns.

ANALYSIS/RATIONALE:

Information is provided, below, regarding each of the four requested areas:
(a) **Options to water fluoridation such as good nutrition, preventive dental care and targeted topical applications, including an evaluation of the effectiveness of water fluoridation vs topical applications**

While adequate nutrition is important to dental health, no clear evidence has been identified showing the improvement of nutritional status to be a viable approach to improving the dental health of a population.

Preventive dental care, including targeted topical applications, can definitely lead to improvements in dental health. Topical applications of fluoride are effective, with the advantage that it is possible to target applications based on dental history and individual needs.

Fluoride works to prevent tooth decay by increasing the resistance of tooth enamel to decay, both topically and systemically. The systemic application of fluoride occurs when fluoride is ingested during the formation of teeth, and also throughout life. The fluoride becomes incorporated into the tooth structures. The ingested fluoride is also deposited throughout the entire tooth surface and provides long lasting protection against tooth decay. The major source of systemic fluoride is fluoridated water. In some countries (i.e. Germany), fluoride is given systemically by adding it to salt or giving it as supplements.

Topical fluorides are applied after the teeth are already present in the mouth. The fluoride is incorporated into the surface of the teeth making them more resistant to tooth decay. The major sources of topical fluoride are toothpaste, professionally applied fluoride foams, gels and varnishes.

It should be noted that systemic fluoride also provides protection topically. After ingestion of sources such as water fluoridation, low levels of fluoride present in saliva and in plaque, a film covering the teeth, can also prevent and reverse the process of dental decay. The maximum protection from tooth decay is realised when fluoride is available both systemically and topically. Water fluoridation provides both types of exposure.

A communication campaign could be used to encourage families to improve their nutritional status and seek out preventive dental care, including topical fluoride, but it is not anticipated that such an approach would be effective, particularly among families with financial challenges.

(b) **Costs for alternative programs should water fluoridation be discontinued**

In order to offset the discontinuation of water fluoridation, topical fluoride applications would be required to avoid increases in dental decay. For a program to be considered an equivalent alternative, it must be made available at no additional cost beyond the current cost to families. This would involve additional costs for the City.

The most practical approach for providing topical fluoride would be through schools, although this approach would have the significant drawback of being limited to school students rather than all residents of Hamilton. Both the Hamilton-Wentworth District
School Board and the Hamilton-Wentworth Catholic District School Board have advised Public Health Services that space for delivering such a service is not available in schools. This means that in order to provide topical fluoridation services, another approach would be required. The most practical alternatives would be the introduction of another Community Health Bus or land based clinic. Alternatively, private dentists could be reimbursed for the application of topical fluoride.

These alternatives are high in cost, and limited in the number of people they would reach, when compared to the fluoridation of municipal water:

<table>
<thead>
<tr>
<th>Approach</th>
<th>Estimated Number Reached Annually</th>
<th>Estimated Capital Budget</th>
<th>Estimated Annual Operating Budget</th>
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<tbody>
<tr>
<td>Additional Community Health Bus</td>
<td>4,000 (based on 15 clients per day)</td>
<td>$350,000 every ten years</td>
<td>$250,000</td>
</tr>
<tr>
<td>Additional Clinic</td>
<td>12,000 (based on 15 clients per day/per chair)</td>
<td>$975,000 one time set-up</td>
<td>$500,000</td>
</tr>
<tr>
<td>Private Dentist Reimbursement</td>
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<tr>
<td>Water Fluoridation</td>
<td>475,000</td>
<td>$2,100,000*</td>
<td>$350,000**</td>
</tr>
</tbody>
</table>

* Based on 2007 budget estimates

** This is based on the chemical cost of $380 per tonne, which has substantially increased to $800 per tonne, starting in June 2008, and will increase to $1,020 per tonne in June 2009. Based on the current market conditions and supply, it is anticipated that the price of chemical will continue to increase in future years due to fertiliser manufacturing moving offshore.

(c) An objective review of data, (i.e. National Research Council (NRC), 2006 National Academy of Science (NAS), 2006) on this issue including experience from other municipalities;

Health Canada has stated that they plan to release a report reviewing available evidence related to water fluoridation in 2009/10. The reference documents being used for this report have been reviewed, and they indicate that water fluoridation will be fully supported by Health Canada, and still recommended as the method of tooth decay prevention for the general population. Although individual studies have suggested some specific concerns, the balance of scientific evidence continues to support water fluoridation.

The Health Canada review will update the recommendations for the Maximum Allowable Concentration (MAC) for water fluoridation and the optimum level of fluoride. When these new guidelines are issued, Hamilton Public Health will review the current concentration of fluoride in Hamilton’s drinking water to ensure that the total exposure to fluoride strikes a balance between providing maximum dental benefits while minimizing dental fluorosis.

Documents released by the National Research Council and the National Academy of Science are among the materials that are reviewed by Health Canada and other national
and international organizations when making their conclusive statements about the safety and efficacy of water fluoridation.

Current scientific evidence shows mixed results on the oral health status of populations when water fluoridation is discontinued. In Antigo, Wisconsin, after five and one-half years of cessation of water fluoridation, second graders had over 200% more decay, fourth graders 70% and sixth graders 91% more decay. Similar results were observed in Wick, Scotland and Stranraer, Scotland.

There are several studies that report no increase in dental decay following the discontinuance of fluoridation. However, in these cases, implementation of other preventive measures were introduced at the time that water fluoridation was discontinued. For example in La Salud, Cuba a study showed that the rate of dental decay did not increase after water fluoridation was stopped. However, when water fluoridation was stopped, children received regular fluoride mouth rinses, and fluoride varnish was placed on teeth in a comprehensive school preventive dental program. Similarly in Finland a longitudinal study of Kuopio (fluoridated from 1959 to 1992) and Jyvasskyla (low levels of natural fluoridation) showed little difference in decay rates. However, Finland has a government-sponsored free, comprehensive dental program where Finnish children are exposed to a regimented, professionally supervised preventive dental program of topical fluorides and dental sealants. In East Germany when water fluoridation was discontinued, salt fluoridation and other preventive measures were introduced and there was no significant increase in dental decay.

The study that gives the greatest indication of what might happen in Hamilton if water fluoridation is discontinued was conducted in British Columbia, Canada. This study found that removing water fluoridation led to an increase in dental caries compared to the fluoridated control site. This relationship was determined for both the prevalence and incidence of caries when other risks factors were controlled. Over the three years of the study, 14 year olds from the site where fluoride was discontinued acquired 2.29 cavities compared to 1.82 in the site that was still fluoridated.

Some local municipalities do not have municipal water fluoridation. It would be valuable to consider the rates of dental decay across local municipalities with different approaches to water fluoridation, but this information is not available in a format that allows accurate comparisons. For example, the municipalities may report on dental decay in different age groups, have varying rates of naturally occurring fluoride, offer different levels of dental care to residents, and have widely varying sociodemographic compositions.

(d) Comparison of topical vs. water fluoridation options in an evaluation of chemical composition and any potential toxicity concerns.

A toxicity report is attached (Appendix A) and there are no contra-indications forthcoming from Health Canada.

While exposure to low levels of fluoride has beneficial effects for teeth, exposure to too much fluoride can cause adverse health effects. Various effects of fluoride on health have been studied including impacts on blood, bone, kidney, liver, lungs and
reproduction. Concern has also been expressed about the possible carcinogenic effect of fluoride. One credible scientific study has indicated a potential link between exposure to fluoride and osteosarcoma in young males. However, the researchers caution that this is the only study that has shown this association, that there are limitations to the study and that the study should be repeated in other jurisdictions to confirm the findings. The overall assessment of the scientific evidence is that fluoride is not a likely cause of cancer at levels optimal for oral health. Of all the potential adverse effects, dental and skeletal fluorosis - impact on teeth and bone structure – are the most documented. These effects are most common in areas where natural levels of fluoride in water are very high and in excess of the concentrations used when the level of fluoride in potable water is adjusted.

Health Canada established a tolerable daily dose of 0.122mg/kg-day for fluoride, based on protection from moderate dental fluorosis. The US EPA has established a reference dose of 0.06mg/kg-day, level at which no adverse impacts would be expected.

Protection from dental fluorosis also protects from other potential adverse effects that may occur at higher levels of exposure. The level of fluoridation of Hamilton's water is within these limits.

**Contaminants in Hydrofluosilic Acid (HFS)**

Hydrofluosilic Acid (HFS) is added at in the water treatment process to provide fluoride residual in the treated water. Some community members expressed concerns with the presence of trace contaminants, such as lead and arsenic, in the chemicals that are added to the water. For every load of chemical delivered to the treatment facility, the City mandates the chemical supplier provides a Certificate of Analysis for the chemical, which is audited per the ISO standards and confirmation that the chemical meets the American Water Works Association (AWWA) Standard B703-00 and American National Standards Institute (ANSI) / NSF Standard 60 requirement. These standards ensure that the chemical is safe from health perspective for use in water treatment and production. The chemical analysis for the last few shipments of HSF were reviewed and the Arsenic and Lead concentrations averaged 6.62 mg/L and <1.0 mg/L respectively. For further verifications, a sample of the chemical was tested for lead and arsenic at an accredited laboratory and the arsenic concentration was 5 mg/L and lead concentration was less than 1 mg/L.

The HFS is dosed at less than 3 mg/L of treated water resulting in Arsenic and Lead addition of approximately 0.00002 mg/L and 0.000003 mg/L of treated water respectively. These are not only well below the Maximum Acceptable Concentration (MAC) of 0.025 mg/L and 0.01 mg/L for Arsenic and Lead respectively, but also below the Minimum Detection Limits (MDL) of 0.001 mg/L for both the parameters. The contaminants in the HSF are therefore not considered an issue.

**Environmental Pollution**

The Woodward WTP produces approximately 350 million litters of water per day and adds HFS to reach a level about 0.7 mg/L of fluoride. Less than 0.1% of this water is consumed for drinking water and given that the primary target of water fluoridation are
children, the water fluoridation therefore potentially serves only less than 0.5% of the total water produced. Most of the water provided to the community returns back in the form of sewage. The wastewater has high concentration of fluoride compared with potable water because of the fluoride added due to toothpaste use and some industrial discharges. A sample of the wastewater was tested for fluoride and the concentration was approximately 1.2 mg/L. Very little fluoride is removed in the wastewater treatment process and effluent had fluoride concentration of 1.05 mg/L.

There are many studies about the impacts of fluoride in aquatic environment and generally there is a consensus that fluoride concentration of about 0.6-0.7 mg/L has detrimental impact on aquatic life. If water fluoridation is stopped, it may be possible that the wastewater fluoride concentration would drop by 0.5-0.6 mg/L, which will be beneficial to aquatic environment.

The Canadian Council for Ministers of the Environment (CCME) is currently in the process of finalizing a Canada-wide Strategy for the Management of Municipal Wastewater. The draft strategy was posted for public consultation and comments. The draft strategy proposed a compliance requirement of end of the pipe toxicity testing for the wastewater treatment plants. It is anticipated that this requirement will be included in the Certificate of Approval for the Woodward WWTP upgrades. The fluorides in wastewater can’t be removed in the treatment processes and as such the toxicity associated with it will be a significant challenge, if source controls are not implemented. Failure to achieve toxicity targets will require substantial future investments in the wastewater treatment systems.

Operator Health and Safety

Hydrofluosilic Acid (HFS) is an extremely hazardous chemical and poses significant health and safety risk to City’s staff. Though significant safeguards have been in place at the water treatment plant, the risk of any chemical spill can’t be completely overruled. There are significant risks during delivery and filling operations and any chemical spill would require extensive resources to manage and control the damage.

Ontario public health dentists have convened to discuss the topic from an Ontario perspective, and have recommended the establishment of an Ontario Fluoridation Office by the provincial government. This Office, if formed, would:

- constantly assemble and review current scientific evidence on fluoride and water fluoridation
- monitor and maintain an inventory of the fluoridation status and fluoride challenges in Ontario and keep track of the concerns presented and outcomes
- evaluate Ontario data for evidence of the effectiveness of water fluoridation

This Office would result in the best advice to Ontarians regarding water fluoridation, with consideration of any new evidence that may become available in upcoming years.
ALTERNATIVES FOR CONSIDERATION:

Council could choose to consider the discontinuation of water fluoridation, with the option of including a referendum in the next municipal election.

The Region of Niagara has decided to not re-institute water fluoridation but is investigating the possibilities of alternate methods of delivery, including topical applications and health promotion. Their report is scheduled for submission this summer.

Waterloo Region has decided to hold a referendum at the next Municipal election.

The Fluoridation Act specifies that a referendum would be required if a petition with 10% of the population is presented. This would require a petition with approximately 50,000 signatures from the City of Hamilton. This is an avenue that could be pursued by those in the community who would prefer that water fluoridation be discontinued. In the absence of a petition, no referendum would be required and Council may choose to discontinue water fluoridation.

If the Board of Health were to pursue the discontinuation of water fluoridation, it would be an obligation of the Board to ensure an alternate approach is put in place to avoid increases in dental decay. In such an event, the Board of Health would further need to decide to whom the alternative services would be made available: children, seniors, those living in poverty, or the entire community.

FINANCIAL/STAFFING/LEGAL IMPLICATIONS:

Financial
There are no financial implications related to the recommendations of the report.

The existing fluoride dosage system is past its useful life and requires upgrading. The upgrade of the fluoride dosage system was estimated at $2.1 million in 2007. Given the increase in construction costs, it is anticipated that the cost of the upgrade will increase by 15-20% over the original anticipated budget.

The cost of the chemical went up from $380 per tonne to $800 per tonne, starting in June 2008, and will increase to $1,020 per tonne in June 2009. Based on the current market conditions and supply, it is anticipated that the price of chemical will continue to remain at this level or increase in future years due to fertiliser manufacturing moving offshore. Recently many municipalities were faced with the chemical shortages resulting in disruptions in water fluoridation. Due to this volatility, it is not possible to predict the future operational costs associated with water fluoridation.

Staffing
There are no staffing implications related to the recommendations of the report.

Legal
Where a municipality owns and operates a waterworks system, the process of fluoridating and the discontinuance of adding fluoride to its water is governed by the Fluoridation Act R.S.O 1990, Chapter F.22.Section 3.
POLICIES AFFECTING PROPOSAL:

The report recommendations do not affect any City policies.

RELEVANT CONSULTATION:

Public Works – Water and Wastewater
Legal Services
Health Canada - Chief Dental Officer for Canada
Health Canada, Healthy Environment and Consumer Branch
Public Health Agency of Canada – Health Products and Food Branch
Ontario Association of Public Health Dentistry
Ontario Dental Association
Canadian Dental Association
Royal College of Dentistry, Ontario

CITY STRATEGIC COMMITMENT:

By evaluating the “Triple Bottom Line”, (community, environment, economic implications) we can make choices that create value across all three bottom lines, moving us closer to our vision for a sustainable community, and Provincial interests.

Community Well-Being is enhanced. ☑ Yes ☐ No
Ongoing review of emerging evidence related to water fluoridation will help to ensure that services and programs are delivered in an equitable manner, coordinated, efficient, effective and easily accessible to all citizens.

Environmental Well-Being is enhanced. ☑ Yes ☐ No
Ongoing review will help to ensure that human health and safety are protected.

Economic Well-Being is enhanced. ☑ Yes ☐ No
Ongoing review of evidence related to water fluoridation will assist in the selection of the most economical approaches are used.

Does the option you are recommending create value across all three bottom lines? ☑ Yes ☐ No

Do the options you are recommending make Hamilton a City of choice for high performance public servants? ☐ Yes ☑ No
TOXICOLOGY
OF
FLUORIDE

Scientific Review of Human and Animal Toxicological Data

By

Robert Tardif, MSc, PhD
Université de Montréal
Toxicologist

Presented to Health Canada
March 2006
Montréal, Canada
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Preface

This report presents a review of the data that appear in papers that have addressed the toxicological effects of fluoride since the last revision of the CMA for fluoride by Health Canada in 1997. Part 1 presents a review of the main data produced in animal studies, whereas Part 2 refers to toxicological effects reported in humans. A special emphasis is put on the effects that are associated with the exposure to fluoride in drinking water. Only papers published during and after 1995 were considered.
PART 1

Animal Data

1.1 MUSCULOSKELETAL EFFECTS

The influence of fluoride intake on bone strength was investigated in four groups of rats fed chronically with a low-fluoride diet ad libitum received 0, 5, 15, or 50 ppm of fluoride in their drinking water. Mechanical strength of the right femur was measured by three-point bending after 3, 6, 12, or 18 months of treatment. Femoral failure load was not significantly decreased in rats treated for 3 and 6 months, but was decreased as much as 23% in rats treated 12 and 18 months at 50 ppm fluoride. The decreased strength resulting from fluoride intake was significant in older rats only and was not associated with a decrease in bone density or mineralization defects. Fluoride intake at high levels resulted in slight increases in trabecular bone volume and trabecular thickness, but these effects could not be demonstrated consistently (Turner et al, 1995).

In another chronic study by the same group (Turner et al, 1996), skeletal fragility and mineralization were evaluated in control and in rats with renal deficiency (4/5 nephrectomy) chronically exposed to fluoridated water at concentrations of 0, 5, 15, and 50 ppm for a period of 6 months. Plasma fluoride levels were equivalent to those in humans consuming fluoridated water levels of 0, 1, 3, and 10 ppm, respectively and were greatly increased by renal deficiency in all animals consuming fluoridated water. Importantly, rats with renal deficiency drank more after (approx. + 60%) and excreted more water (approx. + 85%). There was a strong positive, nonlinear relationship between plasma fluoride and bone fluoride levels, suggesting nonlinear binding characteristics of fluoride to bone. The amount of unmineralized osteoid in the vertebral bone was related to the plasma fluoride levels. Vertebral osteoid volume was increased over 20-fold only in animals with renal deficiency that received 15 or 50 ppm fluoride, suggesting osteomalacia. Vertebral strength and femoral bone strength were reduced more importantly in renal-deficient rats given 50 ppm fluoride.

Two chronic studies investigated whether changes in fluoride metabolism in nutritionally deficient rats resulted in manifestation of any extraskeletal toxic. Whereas the first study monitored the effect of calcium deficiency on the effects of chronic fluoride exposure, the second one observed fluoride effects in rats that were deficient either in protein or in energy and total nutrient intake. Control and experimental rats received drinking water containing 0, 5, 15 or 50 mg/L for 16 or 48 wk. Control rats were fed optimal diets and experimental rats were fed diets deficient in calcium (Study 1) or protein (Study 2). An additional group of experimental rats (Study 2) was provided with a restricted amount of diet (deficient in energy and total nutrient intake). There were significant differences among fluoride treatment groups in fluoride excretion and retention that resulted in significantly greater fluoride levels in tissues of the experimental rats. However, fluoride treatment did not result in any harmful, extraskeletal biochemical, physiologic or genetic effects in the nutritionally deficient rats (Dunipace et al, 1998a).

In a two-stage investigation, adult male Wistar rats were exposed to fluoride for 6 months by inhalation (to HF for 2 hr/day at a concentration of 8.7 ± 5.7 mg/m³) and through drinking water (NaF:
20 mg F/L). The fluoride content in urine, vertebrae L-2 - L-4, tibia, and incisors was significantly increased in the exposed animals. Bones of control and exposed animals were of similar mechanical resistance. No differences in bone mineral density (BMD) were found between exposed and control animals. The results indicate that even a threefold increase in bone fluoride does not cause a distinct change in metabolism, mineral density, and mechanical resistance of bones of exposed animals (Urbanska et al, 2001).

Effect of fluoride in drinking water on compressive cancellous bone strength in the appendicular skeleton was assessed in twenty eight 6-week-old female Wistar rats. One group received distilled water and three groups were exposed to fluoridated water (8, 30 and 60 mg F/L) during six weeks (sub-chronic). The results showed that the level at 8 mg F/L in drinking water fluoride increases compressive strength of cancellous bone in the appendicular skeleton in growing rat. (Bohatyrewicz et al, 2001) (4).

Results from studies looking at the potential musculoskeletal effects of fluoride indicate that it is plausible that fluoride could affect the bone when present at high concentrations in water. However, few chronic or sub-chronic animal studies have been completed since 1995. In these studies fluoride was administered in drinking water to normal rats, to nutritionally deficient rats or to rats with renal deficiency. Although some effects were reported they were always associated with high doses of fluoride in drinking water (lowest fluoride concentration being 5 mg/l or 5 ppm). Results from human studies should be more relevant in terms of doses encountered by subjects and risk associated with fluoride exposure from drinking water. According to NRC (2006), «...more research is needed to address the uncertainties associated with extrapolating data on bone strength and fractures from animals to humans ».

1.2 GENOTOXICITY

Ribiero et al (2004) looked at the potential DNA damage associated with exposure to fluoride using the single cell gel (comet) assay in peripheral blood, oral mucosa and brain cells in vivo. Male Wistar rats were exposed to sodium fluoride (NaF) at a 0, 7 and 100 ppm dose for drinking water during 6 weeks (33). In another study the potential DNA damage was assessed by the single cell gel (comet) assay in vitro on Chinese hamster ovary cells exposed to sodium fluoride (NaF) at final concentration ranging from 7 to 100 ppm for 3 h, at 37°C. Both studies, the results showed that NaF did not contribute to the DNA damage in all cellular types evaluated.
1.3 NEUROTOXICITY AND NEUROBEHAVIORAL EFFECTS

Mullenix et al (1995) compared the behavior, body weight, and plasma and brain F levels after sodium fluoride (NaF) exposures in Sprague-Dawley rats during late gestation, at weaning or in adults. Prenatal exposures of dams consisted of injections (SC) of 0.13 mg/kg NaF or saline on gestational days 14-18 or 17-19. Weanlings received drinking water containing 0, 75, 100, 125 ppm F for 6 or 20 weeks, and 3 month-old adults received water containing 100 ppm F for 6 weeks. Behavior evaluation consisted of recording activity of rats placed in plexiglass chamber with video cameras. Two groups of females received a high level (175 ppm) at weaning which resulted in high mortality. Fluoride exposures caused sex- and dose-specific behavioral deficits with a common pattern. Males were most sensitive to prenatal day 17-19 exposure, whereas females were more sensitive to weanling and adult exposures. The severity of the effect on behavior increased directly with plasma F levels and F concentrations in specific brain regions. Plasma levels in this rat model (0.059 to 0.640 ppm F) are similar to those reported in humans exposed to high levels of fluoride. While this study provided evidence that exposure to fluoride might have affected the behaviour of rats, various aspects of this study regarding particularly the design of the study (e.g., configuration of apparatus, computer program, difficulty to replicate the study) make the results difficult to interpret as pointed out in NRC (2006). For instance, according to NRC (2006), 1) increase in activity observed in rats treated on days 17-19 of gestation was attributed to increased instances of grooming and head turning and not to locomotor movement, 2) plasma levels were comparable to those of controls and, 3) brain fluoride was not measured in this group.

In a sub-chronic study, Paul et al 1998 tested the spontaneous motor activity and motor coordination in adult female Wistar rats after daily oral administration (gastric intubation) with high doses of NaF (20 or 40 mg/kg dissolved in saline) for 60 days. Total protein concentrations were determined in skeletal muscle, liver and serum of similarly treated animals. Food intake and gain weight were lower in fluoride treated rats. The activities of total cholinesterase and acetylcholinesterase were determined in blood and brain regions, respectively. Whereas NaF suppressed spontaneous motor activity there was no change in the motor coordination of these animals. Tissue and serum protein concentrations were decreased. Cholinesterase activity was decreased in the blood in a dose-related fashion but not in brain regions. NRC (2006) pointed out that «...a reduction in spontaneous motor activity was based on results from an apparatus that recorded every type of movement, bodily adjustment, or twitch» and not on the detection locomotor movements as measured in a large arena. Finally, no significant changes were observed on the rota-rod motor coordination test.

Zhao et al (1998a) investigated the effect of NaF on acetylcholinesterase (AChE) activities in the cerebral synaptic membranes (SPM) and the peripheral red blood cells (RBC) of rats in vivo and in vitro experiments. In the in vivo study, pregnant rats ingested ad libitum fluoridated drinking water (5, 15, 50 ppm F-) during their gestation and lactation. The AChE activities of the SPM and peripheral RBCs in maternal rats exposed receiving 5-50 ppm F- for 60 days were elevated significantly by 30.0-67.6% and 12.5-31.9% in a dose-dependent manner, respectively whereas the AChE activities of their offspring 80 days after birth were also increased (8.7-28.7% for SPM and 20.6-32.4% for RBC). In contrast, the AChE activities of SPM in vitro were inhibited by 5.0-50.0 mmol F-/L treatment in a
time- and dose-dependent manner. Results suggested that the enzyme substrate kinetics is consistent with a mixed type of inhibition.

Long et al (2002) investigated the neuronal nicotinic acetylcholine receptors (nAChRs) in the brain in rats receiving either 30 or 100 ppm fluoride in their drinking water for 7 months with employing ligand binding and Western blotting. A significant reduction in the number of [3H]epibatidine binding sites in the brain of rats exposed 100 ppm of fluoride, was observed; however, no alteration was noted after exposure to 30 ppm. The number of [125I]alpha-BTX binding sites was significantly decreased in the brains of rats exposed to both levels of fluoride and the level of the nAChR alpha4 subunit protein in the brains of rats was significantly lowered by exposure to 100 ppm, but not 30 ppm fluoride; whereas the expression of the alpha7 subunit protein was significantly decreased by both levels of exposure. There was no significant change in the level of the beta2 subunit protein in the brains of rats administered fluoride.

Varner et al (1998) analysed the alterations in the nervous system resulting from chronic administration of the fluoroaluminum complex (AlF3) or equivalent levels of fluoride (F) in the form of NaF. Adult male Long-Evans rats were administered one of three treatments for 52 weeks: control group with double distilled deionized drinking water (ddw); aluminum-treated group ddw with 0.5 ppm AlF3; NaF group with 2.1 ppm NaF in ddw. Aluminum (Al) levels of brain, liver and kidney and its distribution were assessed. No differences were found between the body weights of rats in the different treatment groups although more rats died in the AlF3 group than in the control group. The Al levels in samples of brain and kidney were higher in both the AlF3 and NaF groups relative to controls. The effects of the two treatments on cerebrovascular and neuronal integrity were qualitatively and quantitatively different. These alterations were greater in animals in the AlF3 group than in the NaF group and greater in the NaF group than in controls.

Fluoride intoxicated animals performed poorly in motor co-ordination tests and maze tests. Inability to perform well increased with higher fluoride concentration in drinking water (30, 60 and 120 ppm for 30 days) (Bathnagar et al, 2002).

The possibility that chronic oral ingestion of fluoride water could modify peripheral pain sensitivity was studied in two strains of adult male rats which were given NaF in drinking water (Sprague-Dawley 75 and 150 ppm for 15 weeks; Lou rats 150 ppm for 27 weeks). Using classical behavioural evaluation methods of pain symptoms, only slight tendencies to a thermal hyperalgesia and a mechanical allodynia were observed in Sprague-Dawley rats (Balayssac et al, 2002).

Several neurohistopathological effects were observed in brain tissues at autopsy from albino rabbits that had been subcutaneously injected for 15 weeks with 0, 5, 10, 20, and 50 mg of NaF BW/day. No effect was noted at the lower dose. These neurotoxic changes in the brain suggested that there was a direct action of fluoride upon the nerve tissue which was responsible for central nervous system problems such as tremors, seizures, and paralysis indicating brain dysfunction seen at the two highest doses (Shashi, 2003a).

The effects of oral administration of NaF and/or arsenic trioxide (As$_2$O$_3$) at 5 and 0.5 mg/kg body weight doses, respectively, for 30 days on the physiology and histology of brain (cerebral hemisphere) of adult mice (Mus musculus) were examined. Recovery after 30 additional days by some antidotes
(vitamins C and E and calcium phosphate) was also reported. The observed significant decline in levels of DNA and RNA and acetylcholinesterase activity in brain of mice treated with NaF, As$_2$O$_3$ and NaF + As$_2$O$_3$ is related to its altered histology. The combined antidote treatment was conducive for recovery of this fluoride and arsenic induced toxicity in the brain (Shah et al 2004).

The same group (Chinoy and Shah 2004a) looked at the biochemical effects on the brain (cerebral hemisphere) associated with NaF and arsenic trioxide As$_2$O$_3$, singly or combined, at doses of 5 and 0.5 mg/kg body weight, respectively, administered orally to mice for 30 days. The effects of withdrawal of the treatment and ingestion of vitamin C, vitamin E, and calcium (as phosphate) were also investigated. Levels of dehydroascorbic acid and lipid peroxides increased, but the activities of the antioxidant enzymes superoxide dismutase, catalase, and glutathione peroxidase, as well as the levels of glutathione, total ascorbic acid, and reduced ascorbic acid decreased. The data suggest that metabolic changes associated with the treatments could be the result of free radical toxicity rendering the brain more susceptible to injury. Withdrawal of the NaF + As$_2$O$_3$ treatment resulted in incomplete recovery after 30 days. However, administration of the antidotes alone or in combination during the withdrawal period provided almost complete recovery, possibly due to their antioxidant properties and/or synergistic action.

Overall, the various neurohistopathological, neurochemical or biochemical changes reported in animal studies following fluoride administration involved of high doses of fluoride. For instance, the lowest dose administered through drinking water was 5 ppm as NAF. Some effects were observed after administration of fluoride via subcutaneous route in rabbits. In that study the lowest dose of NaF administered (5 mg/kg) to rabbits is approximately 30 times larger than the dose corresponding to the estimated dose absorbed via the ingestion drinking water (100ml/kg) containing 1.5 ppm NaF. Results obtained after administration by subcutaneous route are not relevant for assessing hazard or toxicological risk associated with fluoride exposure through oral ingestion.

Therefore, these results are difficult to interpret as whether these observed effects might translate in brain dysfunctions and occur after oral route. Moreover research is needed in other to better understand the impact of chemical speciation of fluoride on this type of effect.

1.4 REPRODUCTIVE AND DEVELOPMENTAL EFFECTS

REPRODUCTIVE TOXICITY

MALES

Kumar and Susheela (1995) investigated the effect of chronic fluoride (20 and 30 months) on the structure of the ductus epididymis, testis and spermatozoa in male rabbits in treated (orally) with 10 mg NaF/kg (F$^-$ = 4.5 mg) (in distilled water) body weight/day. Fluoride levels were significantly increased in the sera. Loss of stereocilia, significant decrease (P < 0.001) in the height of the pseudostratified columnar epithelium and significant increase in the diameter of both the caput and cauda ductus epididymis were observed only in the 23-month fluoride treated rabbits. The decreases in the epithelial cell height (P < 0.01) and the tubular diameter (P < 0.001) of the testis were significant only in 23-month treated animals. Spermatozoa in the caput and cauda ductus epididymis of 23-month
treated animals were fragmented. In the 23-month fluoride treated rabbits, the weights of the caput and cauda epididymis were significantly reduced and there was also a reduction in the number of secretory granules in these organs. The structural changes observed in the caput and cauda ductus epididymis might adversely affect the maturation of spermatozoa.

Effects of sodium fluoride (NaF) (30 mg/kg body weight) and the impact of ascorbic acid ingestion were assessed in guinea pig treated for 30 days by gavage. Structural and metabolic alterations of the cauda epididymal spermatozoa were affected by NaF treatment which led 1) to marked decreases in their motility, live:dead ratio and sperm mitochondrial activity index. In contrast increases in sperm abnormalities and alterations in sperm membrane phospholipids, particularly phosphatidylinositol and phosphatidyl serine were observed. The activities of ATPase and succinate dehydrogenase and glutathione levels were decreased in testis by sodium fluoride treatment. Ingestion of ascorbic acid led to the enhancement in the levels of these parameters almost to control levels. Chinoy at al (1997).

Sprando at al 1997 did not observed any effect of sodium fluoride (NaF), administered to P generation male and female in drinking water (25, 100, 175, 250 ppm) for 10 weeks, on spermatogenesis and endocrine function in P and F1 generation male rats. Reproductive tissues were collected from P generation male rats after approximately 14 wk of treatment. Pregnant females (P) were exposed to sodium fluoride via their drinking water through gestation and lactation. F1 generation weanling male rats remained within the same treatment groups as their parents. F1 generation male rats were exposed to sodium fluoride in their drinking water for 14 wk, at which time reproductive tissues were collected. Dose-related effects were not observed within the P and F1 treatment groups in testis weights, prostate/seminal vesicle weights, non-reproductive organ weights, testicular spermatid counts, sperm production per gram of testis per day, sperm production per gram of testis, LH, FSH or serum testosterone concentrations. Histological changes were not observed in testicular tissues from either the P or F1 generation.

Another study by the same group (Sprando et al 1998) looked at the potential effect of sodium fluoride (NaF) on the testes of F1 generation male rats exposed in utero and during lactation to NaF (25, 100, 175, 250 ppm). At weaning, the F1 generation males were exposed to NaF in their drinking water for 14 weeks. No differences between control and NaF-treated rats were observed with respect to absolute volume of the seminiferous tubules, interstitial space, Leydig cells, blood vessels boundary layer, lymphatic space, macrophages, tubular lumen or absolute tubular length and absolute tubular surface area, mean Sertoli cell nucleoli number per tubular cross-section, mean seminiferous tubule diameter and the mean height of the seminiferous epithelium. A statistically significant decrease in the absolute volume and volume percent of the lymphatic endothelium was observed in the 175 and 250 ppm NaF-treated groups and in the testicular capsule in the 100 ppm NaF-treated groups.

The effect of sodium fluoride treatment (20mg/kg/day for 29 days by oral gavage) on testicular steroidogenic and gametogenic activities in relation to testicular oxidative stress in rats. Sodium fluoride treatment significantly reduced the relative wet weight of the testis, prostate, and seminal vesicle without alteration in the body weight gain. Testicular delta(5),3beta-hydroxysteroid dehydrogenase (HSD) and 17beta-HSD activities, epididymal sperm count and levels of testosterone in plasma were decreased significantly in the fluoride-exposed group. Fewer mature luminal spermatozoa in comparison to the control. The seminiferous tubules were dilated in treated animals. Fluoride treatment was associated with oxidative stress as indicated by an increased level of
conjugated dienes in the testis, epididymis, and epididymal sperm pellet with respect to control.
Peroxidase and catalase activities in the sperm pellet were decreased significantly. According to the
authors «…The results of this experiment indicate that fluoride at dose encountered in drinking water
in contaminated areas exerts an adverse effect on the male reproductive system and this effect is
associated with indicators of oxidative stress» (Ghosh et al 2002).

**FEMALE (gestation) - multi-generation**

Minta et al (1998) did not observe any teratogenic effects in three groups of female rats (n=25)
treated with NaF solution containing 0, 20 and 40 mg/l in drinking water 3 to 5 weeks before
pregnancy and 3 weeks after insemination. Highest dose produced foetus abnormalities in a few cases.

Heindel et al (1996) administered NaF ad libitum in deionized/filtered drinking water to Sprague-
Dawley-derived rats (26/group) on Gestation Days (GD) 6 through 15 at levels of 0, 50, 150, or 300
ppm and New Zealand White rabbits (26/group) on GD 6 through 19 at levels of 0, 100, 200, or 400
ppm. Animals were killed on GD 20 (rats) or 30 (rabbits) and examined for implant status, fetal
weight, sex, and morphological development. An initial decreased maternal body weight gain which
recovered over time group of both studies receiving the highest dose. No clear clinical signs of toxicity
were observed. Exposure to NaF during organogenesis did not significantly affect the frequency of
postimplantation loss, mean fetal body weight/litter, or external, visceral or skeletal malformations in
either the rat or the rabbit. The NOAEL for maternal toxicity was 150 ppm NaF in drinking water
(approximately 18 mg/kg/day) for rats, and 200 ppm (approximately 18/mg/kg/day rabbits. The
NOAEL for developmental toxicity was ≥ 300 ppm (approximately 27 mg/kg/day) for rats and ≥ 400
ppm (approximately 29 mg/kg/day) for rabbits administered during organogenesis in drinking water.

Collins et al (1996) conducted a study to determine the effects of NaF on foetal development. Sperm-
positive female rats were given 0, 10, 25, 100, 175 or 250 ppm NaF daily throughout gestation. Fluid
consumption was significantly less in females receiving the high dose (175- and 250-ppm).No dose-
related behavioural changes or maternal clinical signs were noted. Daily amount of NaF ingested were
0, 1.4, 3.9, 15.6, 24.7 and 25.1 mg/kg body weight. Feed consumption decreased significantly at 250
ppm, and body weights of pregnant females reflected feed consumption trends. Exposure to NaF did
not modify the mean number of viable foetuses per female. A significant decrease in the mean
number of implants per litter in the 250-ppm group was observed. There was no effect on the
occurrence of in utero deaths and on foetal growth. There was no dose-related increase in the number
of external anomalies in foetuses due to NaF ingestion. At the doses given, NaF had no effect on the
development of specific bones, including sternebrae. A significant increase was seen in the average
number of foetuses with three or more skeletal variations in the 250-ppm group (25.1 mg/kg body
weight).

When exposed at 60 days of age to 100, 200 and 300 ppm (NaF) in their drinking water during 10
weeks sexually mature male Swiss mice showed significantly reduced fertility after breeding with
untreated female mice. 4-week exposure did not reduce fertility. The number of implantation sites and
viable fetuses was significantly reduced in females mated with males at a concentration of 200 ppm
for 10 weeks. Relative weights of seminal vesicles and preputial glands were significantly increased in
mice exposed to 200 and 300 ppm NaF for 4 weeks but not in mice exposed for 10 weeks (Elbetieha et al 2000).

Al-Hiyasat et al (2000) investigated the toxic effects on the reproductive system of adult female Sprague-Dawley rats (10/group) treated with NaF at 200 ppm (22.58 mg/kg-day), 400 ppm (18.35 mg/kg-day) and 600 ppm (28.03 mg/kg-day) administered in drinking water for 30 days. Several rats receiving the highest dose did not survive (10/10 at 28.03 mg/kg-day and 7/10 at 18.35 mg/kg-day). Ingestion of 200 ppm NaF significantly reduced the number of viable fetuses. Furthermore, the pregnant rats with resorptions and the total number of resorptions increased in the NaF-treated group. There was also a significant increase in maternal organ weights. Rats which had ingested NaF showed increases in both the absolute and relative weights of the ovaries and in the relative weights of the uterus and kidney. Clinical signs of toxicity were noted in all treated groups.

Finally, Darmani et al (2001) showed that exposure to NaF for 12 weeks resulted in a significant reduction in the percentage of pregnancies at all concentrations used. Mice exposed to 200 and 300 ppm NaF showed a significant increase in relative ovary weights and a decrease in the number of viable fetuses. Exposure to 300 ppm NaF for 12 weeks resulted in a significant decrease in number of implantations. Exposure to NaF for 4 weeks did not have much effect on fertility, although there was a significant increase in the relative ovary weights and a decrease in the embryo weights in mice exposed to 300 ppm NaF.

Oral administration of sodium fluoride (NaF; 40 mg/kg body weight) daily from day 6 of gestation to day 21 of lactation caused significant reductions in body weight and feed consumption and decreased the concentration of glucose and protein in the serum of P- and F(1)-generation rats. Sodium and potassium concentrations in the serum were significantly higher. Vitamins C, D or a combination of vitamins C+D+E along with NaF produced significant amelioration in body weight, feed consumption, glucose, protein, sodium and potassium concentrations in the serum of P- and F(1)-generation rats. Withdrawal of NaF treatment during lactation resulted in significant amelioration in feed consumption (days 15-21 only), sodium, potassium, glucose and protein concentrations in the serum of both P- and F(1)-generation rats. Co-administration of vitamin E produced a significant amelioration in body weight (days 15 and 20 of gestation only), sodium, potassium, glucose (only in P-generation females) and protein (only in P-generation female) concentrations in the serum of rats than in NaF-treated rats alone (Verma and Sherlin 2002a).

**DEVELOPMENTAL TOXICITY**

In a multigeneration study Collins et al (2001a) investigated the developmental toxicity of NaF (0, 25, 100, 175 or 250 ppm in drinking water) in rats exposed continuously during three generations. Parental (F0) generation rats were treated for 10 weeks and mated within groups. Decreased fluid consumption for F0 and F1 dams at 175 and 250 ppm was reported. No dose-related effects in feed consumption or mean body weight gain were observed in either F0 or F1 females. Numbers of corpora lutea, implants, viable fetuses and fetal morphological development were similar in all groups. No dose-related anomalies in internal organs were observed in F2 fetuses. Ossification of the hyoid bone of F2 fetuses was significantly decreased at 250 ppm. Regarding this effect (developmental toxicity), 250 ppm may be considered as the LOAEL level, whereas the NOAEL would be 175 ppm. On the other hand, results from the same study did not disclose any cumulative effects in the three generations. Mating, fertility
and survival indices were not affected. Organ-to-body-weight ratios and organ-to-brain weight ratios were not affected. Authors concluded that reproduction in rats was not affected by NaF up to 250 ppm (Collins et al 2001b).

Several studies reported reproductive effects in various species (rats, mice, guinea pigs and rabbits) following ingestion (drinking water) or oral administration of fluoride (NaF). However, the doses or water concentrations producing toxicity are very high and involved various time durations. In several cases fluoride exposure was associated with other type of toxicities especially observed in mothers. Importantly, clinical signs of toxicity were reported at 200 ppm (Al-Hiyasat et al, 2000). Therefore care should be exercised in interpreting toxicity data on reproductive or developmental effects (NOAEL, LOAEL) observed in mothers exposed to levels close to 200 ppm NaF or higher levels.

According to NRC (2006) most of these studies were conducted for the purpose of hazard identification except the multigeneration study by Collins and co-workers (2001) which can be used for risk evaluation regarding a developmental toxicity end-point. In that case the LOAEL and NOAEL reported are 250 ppm and 175 ppm, respectively.

1.5 SYSTEMIC EFFECTS

Endocrine Organs (general)

Shashi et al (2003b) investigated the effect of sodium fluoride (NaF) on adrenal gland function in male and female albino rabbits administered with NaF subcutaneously (5, 10, 20, and 50 mg/kg bw/day) for 15 weeks. Slight to large decreases in body weight gain were noted in the NaF exposed animals. A significant decline in the DNA and RNA content of the adrenal gland of fluoridated animals of both sexes was reported. Significantly enhanced levels of free amino acids, accumulation of glycogen and a significant decrease in acidic, basic, and total proteins in test animals were also observed as well as hyperlipidemia and hypertriglyceridemia in the adrenal gland. In males, phospholipids exhibited significant declines in groups II and III, but an increase in group IV. In females, the amount of phospholipids was significantly reduced in groups II, III, and IV. In group V, the level of phospholipids in both sexes returned to the control values. An inhibitory effect of fluoride was also observed on the levels of adrenal free fatty acids and cholesterol.

Gharzouli et al (2000) investigated the effects of NaF administration on gastric secretion and mucosal barrier by filling the rat stomach for 1 h with very high concentrated solution of NaF (5 and 20 mmol/L, i.e., 210 and 840 mg/kg). This treatment induced an increase in fluid, fucose and galactose output which was accompanied by a marked pi-I-dependent reduction of titratable acidity of the lumen. The amount of Alcian blue bound to adherent mucus was reduced in a pH-independent manner by sodium fluoride. The absence of a correlation between soluble glycoproteins and adherent mucus suggests that sodium fluoride does not stimulate mucus secretion, but rather acts as a barrier-breaking agent by its accumulation, together with acid, into the mucosa.
**Parathyroid**

In order to examine the potential of NaF to affect serum cations in the parent (P) and F1 generation rats, Verma et al (2002b) administered sperm-positive pregnant experimental Wistar female rats with 40 mg NaF/kg (18 mg/kg/day F-) from day 6 of gestation either up to 21 days of lactation or only up to gestation followed by withdrawal of the treatment during lactation. Whereas sodium and potassium in the serum of P and F1 generation rats increased significantly in the NaF-treated group, calcium and phosphorus concentrations were significantly lower. Withdrawal of NaF treatment during lactation caused significant recovery in sodium, potassium and phosphorus concentrations in P and F1 generation rats as compared with NaF-treated animals. The calcium concentration in P and F1 generation rats was comparatively higher on withdrawal of NaF treatment than in the NaF-treated group. Exposure of 40 mg NaF/kg body weight in pregnant female rats caused significant alterations in cationic concentration which recovered significantly (except calcium) on withdrawal of the treatment.

Various effects on thyroid function and bone maturation were observed in suckling Wistar mice resulting from ingestion by their mothers of very high concentration of NaF (600 ppm) in their drinking water from the 15(th) day of pregnancy until the 14(th) day after delivery: 15% decrease in body weight, reduction in plasma free T-4 (15%) and T-3 (6%); a 10% and 3% increase was observed in the fluoride content of bone and urine, but not in the plasma. Calcium and phosphate levels in bone decreased by 30% and 27%, respectively. Calcium in plasma increased by 34%, the phosphorus concentration in plasma decreased by 26%, urinary levels of calcium decreased by 25% and those of phosphate increased by 28%. Results suggest that fluoride accelerated bone resorption activity. Biochemical markers such as total tartrate-resistant acid phosphatase (ACP) and total alkaline phosphatase (ALP) increased significantly by 46% and 35%, respectively (Bouaziz et al, 2004).

In order to elucidate the effect of various concentrations of iodine and fluorine on the pathogenesis of goiter and fluorosis in mice Zhao et al (1998) feed a total of 288 Kunmim mice divided into 9 groups (32 animals group) which received one of the following iodide/fluoride mixtures: ID+FD, ID+FN, ID+FE, IN+FD, IN+FN, IN+FE, IE+FD, IE+FN, IE+FE. The concentrations of iodide were: iodine deficiency (ID): 0.0; iodine normal (IN): 20.0; iodine excess (IE) 2500.0 µg/l; and these of fluoride were: fluoride deficiency (FD) 0.0; fluoride normal 0.6; fluoride excess (FE), 30.0 mg/l. One half of the animals of each group were treated for 100 days and the other half for 150 days. Both ID only and IE induced the goiter. FE induced dental fluorosis and increased fluorine content in the bone. Fluorine affected the thyroid changes induced by ID or IE. After 100 days of treatment, fluorine showed some stimulatory effect on the thyroid in ID conditions and inhibitory effect in IE conditions. After 150 days the effects of fluorine on the thyroid reversed as compared with that of 100 days. Difference of iodide intake could also increase the toxic effects of FE on the incisors and bones. The rate and degree of the incisor fluorosis, the fluorine contents in the bone were significantly higher in the ID+FE group than those in the IN+FE and IE+FE groups. Both iodine deficiency and excess induced goiter as well as other functional and histopathological changes in the mouse thyroid. Excessive fluorine caused fluorosis of incisors and limb bones. In addition, iodine and fluorine do have mutually interacting effects on both goiter and fluorosis in the experimental mice.
Renal effects

The effect of chronic ingestion of NaF on ATP-dependent $^{45}$Ca uptake was examined in rat kidney membrane vesicles in female Sprague-Dawley rats raised on low-F- (0.9 mg/L), semi-purified diet with a Ca$^{++}$ concentration of 400 mg/100g diet. Rats were divided into four groups and were fed ad libitum deionized water containing F- at 0, 10, 50, or 150 mg/L (i.e., 22.2, 111 and 333 mg/L as NaF) for 6 wk. ATP-dependent $^{45}$Ca uptake was significantly lower in the 150 mg F-/L exposure group. Studies with thapsigargin showed that the lower uptake was associated with significantly lower activities of both the plasma membrane Ca$^{++}$-pump (50 mg F-/L group versus control) and endoplasmic reticulum Ca$^{++}$-pump (for both the 50 and 150 mg F-/L groups versus control). Slot blot analysis of kidney homogenates with specific Ca$^{++}$-pump antibodies showed less (P < 0.05) endoplasmic reticulum Ca$^{++}$-pump protein and plasma membrane Ca$^{++}$-pump protein in all treatment groups than controls (Borke and Whitford, 1999) (5).

The impact of surgically induced renal insufficiency was examined in rats with. Uremic and sham-operated control rats received 0, 5 (5 mg/L), 15 (15), or 50 µg/ml (50) of fluoride (corresponding to 11, 33 and 110 mg/L as NaF) in their drinking water for 3 or 6 months. Significantly higher levels of fluoride in the tissues of the animals with renal insufficiency were observed. There were no clinically adverse, fluoride-induced, extraskeletal physiological, biochemical, or genetic effects of chronic exposure to common levels of fluoride in these rats (Dunipace et al 1998).

Shashi et al (2002) assessed the renal damage in young albino rabbits injected subcutaneously with 5, 10, 20, and 50 mg NaF/kg body weight/day for 15 weeks and then sacrificed. No significant clinical signs of toxicity were found in animals exposed to the lowest dose. At the higher doses, however, the cytoarchitecture of the kidneys exhibited increasing amounts of cloudy swellings, degeneration of tubular epithelia, tissue necrosis, and extensive vacuolization in renal tubules, hypertrophy and atrophy of glomeruli, exudation, interstitial oedema, and interstitial nephritis.

No significant changes were found in relative kidney weights of fluoride-treated female Wistar mice and their pups that were given 500 ppm NaF (226 ppm F-) in drinking water from the 15(th) day of pregnancy until the 14(th) day after delivery. Urinary fluoride excretion was three times higher in mothers treated with NaF, whereas the rate of fluoride excretion increased by only 3% in their pups. Fluoride administration strongly affected urinary and plasma parameters in 14-day-old mice and their mothers. Daily urine volume in treated groups was higher in the adult mice and their pups than in the controls. Creatinine showed significantly higher plasma and lower in urine in the treated groups. Lipid peroxidation increased in the treated mice while plasma and urinary uric acid levels showed a significant decline. There was also a significant increase in urinary zinc and copper levels in both mothers and pups, whereas the plasma levels decreased (Bouaziz et al 2005).

Brain and Gastrocnemius muscle

Guan et al (1998) analyzed the brain membrane lipid in rats after being fed either 30 or 100 ppm fluoride for 3, 5, and 7 months. The protein content of brain with fluorosis decreased, whereas the DNA content remained stable during the entire period of investigation. After 7 months of fluoride treatment, the total brain phospholipid content decreased by 10% and 20% in the 30 and 100 ppm fluoride groups, respectively. The main species of phospholipid influenced by fluorosis were
phosphatidylethanolamine, phosphatidylcholine, and phosphatidylserine. The fatty acid and aldehyde compositions of individual phospholipid classes were unchanged. No modifications could be detected in the amounts of cholesterol and dolichol. After 3 months of fluoride treatment, ubiquinone contents in brain were lower; however, at 7 months they were obviously increased in both groups of fluoride treatment.

Vani and Reddy (2000) examined the activities of enzymes involved in free-radical metabolism and membrane function in whole brain and gastrocnemius muscle of female mice treated with NaF (20 mg/kg/body weight) for 14 days were examined. The body weight and somatic index were decreased, whereas fluoride levels were significantly increased in both brain and gastrocnemius muscle. The enzymes SOD, GST, and catalase decreased significantly while XOD activity moderately increased. SDH, LDH, AlAT, AAT, and CPK activities and membrane-bound enzymes, viz Na+-K+, Mg++ and Ca(++)ATPase and AChE were decreased significantly in both brain and gastrocnemius muscle. The effect of fluoride on enzymes of muscle was comparatively larger, which corroborates the greater accumulation of fluoride in muscle than brain.

NaF (5 mg/kg bw) and arsenic trioxide (AS$_2$O$_3$, 0.05 mg/kg bw), individually or in combination, were administered orally for 30 days to Swiss strain adult female mice (Mus musculus). A decline in total protein levels and acetylcholinesterase activity was observed in the gastrocnemius muscle which was accompanied with a significant increase in levels of glycogen and a concomitant decrease in phosphorylase activity. Withdrawal of the NaF and/or AS$_2$O$_3$ treatment for 30 days produced incomplete recovery. Supplementation with ascorbic acid, calcium, and vitamin E, alone or in combination, during the withdrawal period, was beneficial for recovery of the muscle parameters. (Chinoy et al, 2004a).

**Hepatic effect**

Shashi (2003c) reported a significant reduction in acidic proteins, basic proteins, total proteins, RNA, and cholesterol in the liver of albino rabbits (both sexes) treated with 5, 10, 20, and 50 mg NaF/kg-bw/day (subcutaneously) for 15 weeks. Parameters of hepatotoxicity were proteins, DNA, RNA, free amino acids, and cholesterol. Hepatic free amino acids were highly elevated in the exposed rabbits whereas hepatic DNA synthesis increased at 5 mg NaF/kg-bw/day and then decreased at higher doses.

Sondhi et al (1995) treated adult Swiss Albino mice (6-7 weeks old) with drinking water (ad libitum) containing NaF (100 ppm) during 30 days. The organo-somatic index decreased significantly on day 7 and 15; the total protein and cholesterol values declined significantly, whereas those of glycogen and acid, and alkaline phosphatase activities, increased significantly on day 7 and to day 30. The crypt cells (intestines) exhibited cytoplasmic degranulation and vacuolation. Hydropic degeneration in lamina propria and muscular tissue, increase in the number of goblet cells, broken tips of villi, nuclear pyknosis, and abnormal mitoses were observed.

Wang et al (2000) analysed the cellular membrane lipids of the liver after a long term fluoride treatment in Wistar rats supplied with drinking water containing either 30 or 100 ppm fluoride (NaF) for seven months. Total liver phospholipid content decreased in the rats treated with high dose of fluoride due to a lower content of phosphatidylethanolamine (PE), phosphatidylcholine (PC) and phosphatidylserine (PS). Among the fatty acid compositions of PE and PC in the livers of fluoride
poisoned animals, the proportion of polyunsaturated fatty acids (20:4 and 22:6) decreased, whereas saturated fatty acids (16:0 and 18:0) increased. No changes could be detected in the amounts of liver cholesterol and dolichol. Total ubiquinone contents in rat liver were reduced by 11% in the group treated with 30 ppm fluoride and by 42% in the group treated with 100 ppm fluoride. In the subclasses of ubiquinone, both ubiquinone-9 and ubiquinoine-10 amounts decreased after fluoride treatment. According to the authors these modifications of membrane lipids might be induced by oxidative stress, which might be an important factor in the pathogenesis of chronic fluorosis.

Shivashankara et al (2002) investigated the extent of lipid peroxidation and response of liver antioxidant systems in litters (Wistar) of rats exposed to 30 and 100 ppm fluoride (NaF) from weaning up to 10 weeks of age. Three group of pregnant rats (2nd pregnancy to weaning) received water of one of the following concentrations: 0.5, 30 or 100 ppm fluoride. Litters received the same treatment for 10 weeks after weaning. Evaluation of the oxidative stress in the liver showed that both 30 and 100 ppm increased MDA (malonaldehyde) levels in liver homogenates. Whereas liver GSH was reduced, GST and GSH-Px activities were induced by fluoride exposure. Ascorbic acid levels were decreased.

Cardiac effect

Wistar strain female and male rats were reared until the second generation of rats obtained, during which they were given 1, 10, 50 and 100 mg/L NaF in drinking water. 28 male (F2) rats were divided into four groups and had the same treatment for 6 months. All F2 rats were sacrificed and autopsied at the end of the 6 months. Significant histopathological changes were found in the myocardial tissue of rats treated with 50 and 100 mg/L NaF. These were myocardial cell necrosis, extensive cytoplasmic vacuole formation, nucleus dissolution in myoits, swollen and clumped myocardial fibers, fibrillolysis, interstitial oedema, small hemorrhagic areas and hyperaemic vessels. Additionally, the increased activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), catalase (CAT) and thiobarbituric acid-reactive substance (TBARS) levels were observed in the myocardial tissues of rats treated with 10 and 50 mg/L NaF. Activities of SOD, GSH-Px, and CAT decreased, while the TBARS levels increased in the myocardial tissues of rats treated with 100 mg/L (Cicek et al 2005).

Haematopoietic organs

Machalinska et al (2002) studied the early-response morphological effects of sodium fluoride (NaF) on hematopoietic organs in mature Balb C mice which were given three intravenous injections of NaF in their tail veins on alternate days at total doses of 0, 10, and 50 mg NaF/kg bw. In the mice receiving NaF, morphological abnormalities were seen in the spleen as increased lymphocyte nodules, decreased white pulp, and increased red pulp infiltrated by lymphocytes. The changes were greater at the higher dose and, in other exposed mice, were still evident after three weeks. Te liver, kidneys, and bone marrow showed little alteration.

Blood

Wistar albino rats were exposed to 0.5 (control), 30 or 100 ppm fluoride in drinking water during their fetal, weanling and post-weaning stages of life up to puberty. Rats treated with 100 ppm fluoride showed enhanced lipid peroxidation as evidenced by elevated malondialdehyde (MDA) levels in red blood cells but, 30 ppm fluoride did not cause any appreciable change in RBC MDA level. 30 ppm
fluoride-intake resulted in increased levels of total and reduced glutathione in red blood cells and ascorbic acid in plasma while 100 ppm fluoride resulted in decreases in these levels. The activity of RBC glutathione peroxidase was elevated in both the fluoride-treated groups. Reduced to total glutathione ratio in RBC and uric acid levels in plasma decreased in both groups. RBC superoxide dismutase activity decreased significantly on high-fluoride treatment. (Shivarajashankara et al, 2003).

Overall the animal studies that looked at potential systemic effects, associated with fluoride exposure, showed that exposure to sufficiently high fluoride concentrations/doses (much higher than those reported or expected in humans) and sometimes by routes (intravenous or subcutaneous) different to oral route produced several adverse effects in various organs or systems. However, most of these studies do not provide sufficient information that would allow identifying with a reasonable level of certainty the risk associated with lower exposure of safe levels (i.e., LOAEL, NOAEL). These effects are unlikely to pose a risk to human health even at relatively high levels of fluoride (i.e., 4 ppm, according to NRC, 2006), except in certain sensitive individuals who may accumulate more fluoride, such as those with renal impairment.

1.6 REFERENCES


Chinoy N.J. and Shah V.C. Biochemical effects of sodium fluoride and arsenic trioxide toxicity and their reversal in the brain of mice, Fluoride - Quarterly Reports. 2004a; 37(2) 80-87.


1.7 Appendix

This appendix presents some important figures useful to interpret appropriately the toxicological data produced in the animal studies identified in the above section:

NaF molecular weight: 42 g/mol; 1 mmol/L = 42 mg/L or 42 ppm
F atomic number: 19
Na atomic number: 22
F/NaF ratio = 0.45 ; NaF/F ratio = 2.22

Therefore, 1 ppm (1 mg/L) NaF represents 0.45 ppm (0.45 mg/L) F.

Daily volume of water ingested:
Human adult (70 kg): 1.5 L/day
Rat (250 g): approx. 0.025 L
Rabbit: approx. 0.08 – 0.100 L/kg/day

Estimated oral doses of NaF (F) by rats and humans, corresponding to various concentrations of NaF in drinking water (DW):

<table>
<thead>
<tr>
<th>NaF in DW</th>
<th>NaF dose Human (70 kg)</th>
<th>NaF dose Rat (250 g)</th>
<th>F dose Human (70kg)</th>
<th>F dose Rat (250 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.5 ppm (1.5 mg/L)</td>
<td>0.07 mg/Kg</td>
<td>1.6 mg/kg</td>
<td>0.032 mg/kg</td>
<td>0.07 mg/Kg</td>
</tr>
<tr>
<td>5 ppm (5 mg/L)</td>
<td>0.23 mg/Kg</td>
<td>0.5 mg/Kg</td>
<td>0.11 mg/Kg</td>
<td>0.23 mg/Kg</td>
</tr>
</tbody>
</table>
PART 2

Human Data

2.1 MUSCULOSKELETAL EFFECTS

Ecological studies

Lan et al (1995), investigated the effect of fluoride concentration in drinking water on bone mineral density (BMD) of the lumbar spine region in 248 Taiwanese women, including subjects residing in naturally fluoridated and adjacent areas. Women aged 46-65 years living in areas with fluoride levels < 0.6 mg/l (mean = 0.18 mg/l; n = 130) had slightly lower bone densities than women living in areas with levels ≥ 0.6 mg/l (mean 0.98 mg/l, n = 118). Only the age groups 46-50 and 61-65 years were statistically significant. After controlling for age and body mass index, the BMD of those with a dose ≥ 1.0 mg/l was higher than the reference group (≤0.6 mg/l). Fluoride appeared to have no association with bone density in postmenopausal women. The BMD of the subjects from the area with a fluoride dose > 1 mg/l were significantly higher than those from the reference group (fluoride < 0.6 mg/l) for premenopausal.

An ecological study examined whether geographic area or water fluoride [≤ 0.3 mg/l (natural) vs ≥ 0.7 mg/l (natural and artificial)] were related to the occurrence of fractures among the elderly in the United States. Fractures of the hip, proximal humerus, distal forearm, and ankle were identified in a 5% sample of the white U.S. Medicare population, aged 65 to 89 years during the period 1986-1990. RR of hip fracture in fluoridated areas was 1.00 (95% CI: 0.92 – 1.09) for men and 1.01 (95% CI: 0.96 – 1.06) for women. For ankle fracture RR was 1.01 (95% CI: 0.87– 1.16) for men and 1.00 (95% CI: 0.92 – 1.08) for women. Men had modestly higher rates of fractures of the distal forearm and proximal humerus (23%) than did men in nonfluoridated areas; no such differences were observed among women, nor for fractures of the hip or ankle among either men or women (Karagas et al, 1996).

In a Canadian study, bone mineral density (BMD) was measured in 24 healthy women, from Regina (fluoride 0.1 mg/L) and 33 from Saskatoon (fluoride 1.0 mg/L). There were no differences between groups for height, weight, lifestyle or dietary factors. Women exposed to fluoridated water had significantly higher mean BMD at total anterior-posterior lumbar spine (APS) and estimated volumetric 1.3 (VLS), with no difference at total body (TB) or proximal femur (PF). Authors concluded that exposure to water fluoridation during the growing years may have a positive impact on axial spine bone density in young women (Arnold et al, 1997).

Lehmann et al (1998) assessed the osteoporotic hip fractures and bone mineral density (BMD) in two different communities in eastern Germany: in Chemnitz where drinking water was naturally fluoridated (0.77-1.20 mg/L) over a period of 30 years and in Halle where water was not fluoridated (0.08 – 0.36 mg/l). BMD was measured in healthy hospital employees aged 20-60 years (Halle: 214 women, 98 men; Chemnitz: 201 women, 43 men, respectively). Hip fractures in patients ≥ 35 years admitted to the local hospitals in the years 1987-1989 were collected from the clinic registers. Authors did not find any correlation between fluoride exposure and age-adjusted BMD. No significant difference in spinal or femoral BMD between subjects living in Halle and Chemnitz was found. This
study suggests that optimal drinking water fluoridation (1 mg/L), which is advocated for prevention of dental caries, does not influence peak bone density but may reduce the incidence of osteoporotic hip fractures in the very old.

In a cross-sectional study Phipps et al (1998) compared the bone mineral densities (BMD) of older adults exposed to various levels of fluoride from community water systems in adults (353 white non-Hispanic women and 317 white non-Hispanic men, ≥ 60 years) recruited from 3 rural communities with naturally occurring fluoride in their water systems at 0.03, 0.7, and 2.5 mg/L. Subject were eligible if they were ambulatory and had a long-term history (≥ 20 yrs) of ingesting city water. When the data were stratified by city of residence and gender, men and women living in the community with high levels of fluoride in their community water system had significantly higher lumbar spine BMD. Only women in the high-fluoride community had significantly higher proximal femur BMD. Long-term exposure (≥ 20 yrs) to higher levels of fluoride appears to have a positive impact on lumbar spine and proximal femur BMD. Based on the results of this study, exposure to fluoride at levels considered "optimal" for the prevention of dental caries (from 0.7 to 1.2 mg/L) appears to have no significant impact on bone mineral density.

Fabiani et al (1999) investigated the effect natural water fluoride concentrations in residents of two counties [Bracciano county (mean 1.45 mg/l; 0.15 – 3.40) vs Avezzano county (mean 0.05 mg/l; 0.04 – 0.058)] and the incidence of fractures in the years 1990 and 1991 these two areas where population have a similar life style, economic and social level and employment structure. The authors noticed a significantly greater rate of fracture incidence in particular femur fractures (RR for males 4.28 and for females 2.64), in the population of the district of Avezzano (low exposure) than in the population of Bracciano suggesting that the greater concentration of fluoride in waters distributed for human consumption in Bracciano district seems to have the effect of protecting its inhabitants against fractures.

**Prospective cohorts**

A study by Cauley et al (1995) on a prospective cohort comprising 2076 non-black women (aged ≥ 65 years) (Study of Osteoporotic Fractures at the Pittsburgh clinic) showed that women exposed to fluoride (1.0 mg/l artificial; 0.15 mg/l natural) for > 20 years had lower relative risks (yet none statistically significant) of nonspine fractures (RR, = 0.73; 95% confidence interval [CI] 0.48-1.12), osteoporotic fractures, RR = 0.74 (CI 0.46-1.19), and hip fractures, RR = 0.44 (CI 0.10-1.86), compared with women not exposed and that there was no association with wrist or spinal fractures. No evidence was found that exposure to fluoridated drinking water at the optimal level of 1 mg/l had any impact on bone mass.

In a prospective study, Feskanich (1998) measured toenail fluoride in 62641 women (Nurses' Health Study) to assess long-term intake to evaluate the relation between fluoride intake and subsequent risk of hip and distal forearm fractures between 1982 and 1984. A total of 53 proximal femurs and 188 distal forearms were identified and matched controls to cases on year of birth. The odds ratio of hip fracture among women in the highest quartile of toenail fluoride (> 5.50 ppm), compared with those in the lowest quartile (< 2.00 ppm), was 0.8 (95% CI 0.2-4.0), with adjustment for menopausal status,
postmenopausal hormone use, caffeine intake, and alcohol consumption (none statistically significant). The corresponding adjusted odds ratio for forearm fracture was 1.6 (95% CI 0.8-3.1).

In a subsequent multicentre prospective study involving 4 community based centers in the United States, Phipps et al (2000), investigated whether fluoridation influences bone mineral density and fractures in older women (≥ 65 years) by looking at risk factors for osteoporosis and fractures. Bone mineral density (BMD) of the lumbar spine, proximal femur, radius, and calcaneus plus incident fractures (fractures that occurred during the study) of vertebrae, hip, wrist, and humerus were recorded. Women were classified as exposed or not exposed or having unknown exposure to fluoride for each year from 1950 to 1994 (exposure levels not reported). Outcomes were compared in women with continuous exposure to fluoridated water for the past 20 years (n=3218) and women with no exposure during the past 20 years (n=2563). Continuous exposure was associated with mean BMD 2.6% higher at the femoral neck (0.017 g/cm(2), P<0.001), 2.5% higher at the lumbar spine (0.022 g/cm(2), P<0.001), and 1.9% lower at the distal radius (0.007 g/cm(2), P=0.002). In women with continuous exposure the multivariable adjusted risk of hip fracture was slightly reduced (RR: 0.69 (95% CI 0.50 - 0.96, P=0.028) as was the risk of vertebral fracture (RR: 0.73, 0.55 to 0.97, P=0.033). There was a non-significant trend toward an increased risk of wrist fracture (1.32, 1.00 to 1.71, P=0.051) and no difference in risk of humerus fracture (0.85, 0.58 to 1.23, P=0.378). Authors concluded that long term exposure to fluoridated drinking water does not increase the risk of fracture.

Sowers et al (2005) examined the relation between serum fluoride concentrations and BMD and bone fractures. Data are from 1300 female residents of 3 small communities in which the water fluoride concentrations were 52.6 µmol/l (1 mg/l) or 210.4 µmol/L (4 mg/l). The mean serum fluoride concentration in the high-fluoride community, 2.11 ± 0.05 µmol/l, was significantly higher than serum fluoride concentrations in the control (1.6 ± 0.04 µmol/l) and high-calcium (1.22 ± 0.05 µmol/L) communities with water fluoridation to 52.6 µmol/l. Serum fluoride was not significantly related to BMD after adjusting for covariates including age and body size. The mean distal radius BMD was significantly higher in the high-fluoride community. Serum fluoride concentrations were not related to incident osteoporotic fractures with 4 years of observation nor it was associated with BMD or osteoporotic fractures among female residents of communities with water fluoride concentrations of 52.6 or 210.4 µmol/l.

**Retrospective cohort**

In a retrospective cohort study (Finland) based on record linkage, Kurttio et al (1999) studied a cohort of persons born in 1900-1930 (n = 144,627) and who had lived in the same rural location at least from 1967 to 1980. Natural fluoride concentrations (median, 0.1 mg/liter; maximum, 2.4 mg/liter) in well water in each member participant were estimated. Information on hip fractures was obtained from the Hospital Discharge Registry for 1981-1994. No significant association was observed between hip fractures and estimated fluoride concentration in the well water in either men or women when all age groups were analyzed together. Higher fluoride levels increased the risk of hip fractures in women aged 50-64 years; age- and area-RR was 2.09 (95% CI: 1.16 - 3.76) for women from this group who were the most exposed (>1.5 mg/liter) when compared with those who were the least exposed (≤ 0.1 mg/liter). The results suggest that fluoride (>1.5 mg/liter) increases the risk of hip fractures only among women.
Li et al (2001) investigated the prevalence of bone fracture, including hip fracture, in six Chinese populations with water fluoride concentrations (natural) ranging from 0.25 to 7.97 ppm in men and women (n= 8,266; ≥ 50 years). A U-shaped pattern was detected for the relationship between the prevalence of bone fracture and water fluoride level. The prevalence of overall bone fracture was lowest in the population of 1.00-1.06 ppm fluoride in drinking water, which was significantly lower (p < 0.05) than that of the groups exposed to water fluoride levels ≥ 4.32 and ≤ 0.34 ppm. The prevalence of hip fractures was highest in the group with the highest water fluoride (4.32 - 7.97 ppm). The value is significantly higher than the population with 1.00-1.06 ppm water fluoride, which had the lowest prevalence rate. Whereas, long-term fluoride exposure from drinking water containing ≥ 4.32 ppm increases the risk of overall fractures as well as hip fractures, lower levels (1.00-1.06 ppm) decrease the risk of overall fractures relative to negligible fluoride in water.

Case-control study

In a case-control study, Hillier et al (2000) compared men and women (≥ 50 years: 514 cases with hip fractures and 527 controls) from the English county of Cleveland. Exposures to fluoride in water were estimated from the residential histories and from information provided by water suppliers. Hip fracture was strongly associated with low body-mass index (p for trend <0.001) and physical inactivity (p for trend <0.001). Estimated average lifetime exposure to fluoride in drinking water ranged from 0.15 to 1.79 ppm. After adjustment for potential confounders, the odds ratio associated with an average lifetime exposure to fluoride ≥0.9 ppm was 1.0 [95% CI 0.7-1.5]. Authors concluded that fluoridation of water at concentrations of about 1 ppm is not likely to have important effect on the risk of hip fracture and the low risk should not be a reason for withholding fluoridation of water supplies.

Since 1995, several additional human studies of various types (e.g., ecological, case-control, prospective and retrospective cohorts) have looked at the impact of water fluoride on bone mineral density and fractures. Overall, these studies showed that, whereas exposure to fluoride concentrations at 1.0 -1.5 ppm was occasionally associated with a positive effect on BMD, it did not increase significantly the risk of fractures. The study by Kurttio et al (1999) is the only one that provides some evidence of increase rate of hip fracture at fluoride level exceeding 1.5 ppm. However, these studies indicate an increase risk of bone fracture for exposure to high fluoride levels (e.g., 4 ppm).

2.2 CANCER
Case-control

Gelberg et al (1995) conducted a population-based case-control (retrospective) study was conducted among residents of New York State, excluding New York City. Cases (n = 130) were diagnosed with pediatric osteosarcoma between 1978 and 1988, at age 24 years or younger. The lifetime exposure to each source of fluoride was assessed (Total life time exposure index). The fluoride level was assumed to be 1.0 mg/liter for fluoridated areas and 0 mg/liter for non-fluoridated areas. Based on the parents' responses, total lifetime fluoride exposure was not significantly associated with osteosarcoma among all subjects combined or among females. However, a significant protective trend was observed among males. Protective trend was associated with fluoridated toothpaste (estimates of quantities are
considered high according to NRC 2006), fluoride tablets, and dental fluoride treatments among all subjects and among males. Based on the subjects' responses, no significant associations between fluoride exposure and osteosarcoma were observed. Fluoride exposure does not increase the risk of osteosarcoma and may be protective in males.

Using proxy exposure measures and readily available data from the Wisconsin Cancer Reporting System, Moss et al (1995) compared 167 osteosarcoma cases with 989 frequency-matched cancer referents reported during 1979-1989. Differences in potential exposure to water-borne radiation and fluoridated drinking water, population size for the listed place of residence, and seasonality were assessed. No association was found between potential exposure to fluoridated drinking water and osteosarcoma (OR 1.0, 95% CI: 0.6-1.5).

Recently, Bassin et al (2006) explored age-specific and gender-specific effects of fluoride level in drinking water and the incidence of osteosarcoma based on data from a matched case-control study conducted through 11 hospitals in the United States that included a complete residential history for each patient and type of drinking water (public, private well, bottled) used at each address. Analysis was limited to cases less than 20 years old and standardized fluoride exposure estimates were based on CDC-recommended target levels that take climate into account. Exposure was categorized into three groups (<30%, 30-99%, >99% of target) and used conditional logistic regression to estimate odds ratios. Analysis, based on 103 cases under the age of 20 and 215 matched controls discloses that for males, the unadjusted odds ratios for higher exposures were greater than 1.0 at each exposure age, reaching a peak of 4.07 (95% CI 1.43, 11.56) at age 7 years for the highest exposure. Adjusting for potential confounders produced similar results with an adjusted odds ratio for males of 5.46 (95% CI 1.50, 19.90) at age 7 years. However, this association was not apparent among females. This exploratory analysis found an association between fluoride exposure in drinking water during childhood and the incidence of osteosarcoma among males but not consistently among females.

**Ecological**

The relationship between fluoride concentration (0.02 mg/l - 0.37 mg/l) in drinking water and uterine cancer mortality rate, before and after termination of fluoridation, was studied in 20 municipalities of Okinawa. Significant positive correlation was found between fluoride concentration in drinking water and uterine cancer mortality ($r = 0.626, p < 0.005$). Even after adjusting for the potential confounding variables, such as tap water diffusion rate, primary industry population ratio, income gap, stillbirth rate, divorce rate, this association was considerably significant. Furthermore, the time trends in the uterine cancer mortality rate appear to be related to changes in water fluoridation practices. (Tohyama et al, 1996).

Yang et al (2000) compared the 1982-1991 age-adjusted mortality rates (SMRs) for cancer for 10 municipalities whose water supplies contained the highest naturally occurring fluoride concentrations [mean (median) : 0.24 (0.25 mg/l)] in Taiwan with those rates for 10 matched municipalities with unfluoridated water (< 0.01 mg/liter). According to the results fluoridation of water (at low levels) supplies is not associated with an increase in cancer mortality in Taiwan (including Cervix uterus). However, this study showed a statistically significant RR of 2.79 (CI 1.41 – 5.55) in females for bladder cancer. Since the measure of effect in this study was mortality rather that the incidence,
migration during the interval between cancer diagnosis and death must have been considered since cancer diagnosis may have influenced a decision to migrate and possibly introduced bias.

Takanashi et al (2001) used the percentage of people supplied with "optimally" fluoridated drinking water (≥ 0.7 mg/l) obtained from the Fluoridation Census 1985, U.S.A. for regression analysis of incidence rates of cancers at thirty six sites (ICD-WHO, 1957). About two-thirds of sites of the body were associated positively with fluoridated water, but negative associations were noted for lip cancer, melanoma of the skin, and cancers of the prostate and thyroid gland. In digestive organs the stomach showed only limited and small intestine no significant link. However, cancers of the oral cavity and pharynx, colon and rectum, hepato-biliary and urinary organs were positively associated with fluoridated water as well as bone cancers in male. Brain tumors and T-cell system Hodgkin's disease, Non-Hodgkin lymphoma, multiple myeloma, melanoma of the skin and monocytic leukaemia were also correlated with FD. Of the 36 sites, 23 were positively significant (63.9%), 9 not significant (25.0%) and 4 negatively significant (11.1%). This may indicate a complexity of mechanisms of action of fluoride in the body, especially in view of the coexisting positive and negative correlations with the fluoridation index. The likelihood of fluoride acting as a genetic cause of cancer requires consideration. According to NRC (2006) the statistical methods used in this study are flawed suggesting that care should be exercised in considering those results.

Two case-control studies on cancer were published in 1995. On study concluded that there was no association between lifetime fluoride exposure and pediatric osteosarcoma when concentration was assumed to be 1.0 mg/l (fluoridated areas; another one confirmed this observation. The last study published in 2006 suggested the existence of an age-specific association between exposure to fluoride in drinking water (approx. 1 mg/l) and osteosarcoma during childhood. However, this association was apparent only in male subjects at age between 4 and 12 with a peak between 6 and 8 years. Regarding the latter study, Douglass and Joshipura (2006) made the following comments: «In the course of a study of fluoride and osteosarcoma that stared in 1992, the Harvard School of Dental Medicine in collaboration with NIEHS, NCI, NIDCR, and Harvard has been collected 2 sets of cases each with their own control groups. The first set of cases, which was studied by Bassin et al, was recruited from existing cases between 1989 and 1992. A second set of cases was recruited between 1993 and 2000. Douglass’s group also found some positive associations between fluoride and osteosarcoma in the overall (not age specific) analysis of the first set of cases. However, their preliminary findings from the overall analysis of the second set of cases (1993–2000) do not appear to replicate the overall findings from the first part of the study. Their findings do not suggest an overall association between fluoride and osteosarcoma. This seems particularly important since the cases had been accrued essentially from the same hospitals within the same orthopedic departments with the same providers, and the same pathology departments making the diagnosis of the osteosarcoma and also using similar methods of fluoride exposure.»

Accordingly, the authors suggested that care should be exercised in order to avoid any generalization and over-interpretation of the results of the Bassin et al. paper and to await the publications from the full study, before making conclusions, and especially before influencing any related policy decisions.

Two ecological studies reported an association between relatively low levels of fluoride and uterine cancers. Finally, an ecological study using regression analysis reported several positive associations
between fluoride exposure and some cancer sites. However, the statistical methods used in this later study were considered to be flawed in a recent review (NRC, 2006).

2.3 GENOTOXICITY

Li et al (1995) investigated the genotoxic risks of long-term ingestion of drinking water containing fluoride (0.2, 1.0, or 4.8 ppm) in humans from Chinese population with normal or inadequate nutrition. Blood lymphocytes were examined to determine the frequency of sister chromatid exchange (SCE). The average daily fluoride intake as well as urine and plasma fluoride levels increased with increase in the fluoride content of the drinking water. They observed that while the numerical differences were small, the subjects with low fluoride in the water (0.11 and 0.23 ppm) had significantly higher SCE frequencies than those with higher fluoride exposures.

An ecological study (199 subjects) by Jackson et al (1997) disclosed an increase in SCEs in residents (≥ 30 years residence) receiving water containing 4 mg/l fluoride compared to residents of 0.2 mg/l and 1.0 mg/l. However, a follow-up second study (58 subjects) aimed at determining whether the increased frequency was potentially related to the fluoride level of the communal water supply showed that the increased sister chromatid exchange frequency was not related to the fluoride level of the communal water. The investigation provided evidence that the long-term ingestion of water containing 4.0 mg/l fluoride did not have any clinically important physiologic or genotoxic effects in healthy adults.

Frequency of sister chromatid exchanges (SCE) and chromosome aberrations in peripheral blood lymphocytes were assessed in residents of three villages (14 samples/village; 7 males and 7 females) with same fluoride levels and one nearby township (control) (14 samples; mean age: 28 years) in South Gujarat. Overall, fluoride concentrations in drinking water ranged from 1.56 - 3.46 in the three villages, and 0.6 - 0.8 ppm (control), respectively. The rates of SCEs and chromosome aberrations in persons living in only one of the villages were significantly higher than in the others, and their lymphocytes were more susceptible to the clastogen Mitomycin-C. However, it should be mentioned that the mean age of subjects from this village is 35 years compared to 29 for the others (Joseph et al, 2000).

Ramesh et al (2001) analyzed p53 mutations in various exons in tissue of osteosarcoma, and correlated the findings with the bone fluoride levels in Indian patients. Tissue samples from 20 osteosarcoma patients were examined for possible genetic alterations including mutations for the extent of fluoride accumulation in bone. Mutation which was observed in samples of two cases (10% incidence) was associated with very high levels of fluoride in bone (64,000 and 89,000 ppm) compared subjects without mutation exceeding the maximum theoretical level of 37 000 ppm predicted in the NRC review (2006) based on a pharmacokinetic model. According to authors, the high levels of bone fluoride levels and the similarity of the mechanisms of action between fluoride-induced DNA damage and chemically-induced p53 mutations suggested that high fluoride bone content might have been a major factors causing osteosarcoma in subjects with p53 mutations.
Overall, the results of the human studies on genotoxicity described above do not provide evidence of any link between exposure to fluoride in drinking water and any potential genotoxic effects in humans at concentration below 1.5 mg/l.

2.4 SYSTEMIC EFFECTS

Acute effects

A group of students were exposed during an incident that resulted in a high concentration of fluoride (92 ppm) in drinking water at an elementary school (Portage, Michigan). No dose estimation is reported. Seven students who drank water from the school fountain reportedly suffered nausea and vomiting. Toxicological evaluations were made by conducting a risk assessment. Fluoride had irritated the stomach causing nausea and vomiting. This mild oral exposure to fluoride was, however, deemed too low to cause any long-term appreciable adverse health effects (Sidhu and Kimmer, 2002).

Intelligence (Cognitive effects)

The impact of fluoride exposure on intelligence (IQ) was measured in 2 studies in China. The first one involved 907 children aged 8-13 years living in areas which differed in the amount of fluoride present in the environment. The IQ of children living in areas with a medium or severe prevalence of fluorosis was lower than that of children living in areas with only slight dental fluorosis or no fluorosis. The development of intelligence appeared to be adversely affected by fluoride in the areas with a medium or severe prevalence of fluorosis but to a minor extent only in areas with only a slight prevalence of fluorosis. A high fluoride intake was associated with a lower intelligence (Li et al, 1995).

In a subsequent study with a stronger design according to NRC (2006), IQ was measured in 512 children, aged 8-13 years, living in two villages: in the high-fluoride village (water fluoride: 2.47±0.79 mg/L; range: 0.57-4.50 mg/L), the mean IQ of 222 children was significantly lower (92.02 ± 13.00; range: 54-126) than in the low-fluoride (water fluoride: 0.36 ± 0.15 mg/L; range: 0.18-0.76 mg/L), where the mean IQ of 290 children was higher (100.41±13.21; range: 60-128). Higher drinking water fluoride levels were significantly associated with higher rates of mental retardation (IQ <70) and borderline intelligence (IQ 70-79). The Benchmark Concentration (BMC) for the concentration-response relationship between IQ <80 and the drinking water fluoride level was 2.32 mg/L, and the lower-bound confidence limit (BMCL) of the BMC was 1.85 mg/L. Taking dental fluorosis and other sources of dietary fluoride into account, the reference value concentration (RfC) for fluoride was calculated to be 0.925 mg/L, which is very close to the current national Chinese standard of <1.0 mg/L. In endemic fluorosis areas, drinking water fluoride levels greater than 1.0 mg/L may adversely affect the development of children's intelligence (Xiang et al, 2003).

Spina bifida

Gupta et al (1995) compared children (5 to 12 years, weighing 15 to 30 kg) consuming fluoride rich drinking water 4.5 and 8.5 ppm fluoride (Group A) and manifesting either clinical, dental and/or skeletal fluorosis to age and weight-matched children, consuming less than or equal to 1.5 ppm (Group B). A total of 22 (44%) of the 50 children in group A and 6 (12%) of the 50 children in group B revealed spina bifida occulta in the lumbosacral region. Since this defect occurs during the antenatal
period, the observations indicate that an association may exist between spina bifida occulta and high fluoride intake during the antennal period.

**Testosterone level/Reproductive effect**

Serum testosterone concentrations were measured in patients with skeletal fluorosis (fluoride in drinking water: 1.5 – 14.5 ppm). Serum testosterones were compared for patients afflicted with skeletal fluorosis (n = 30) and healthy males consuming water containing less than 1 ppm fluoride (Control 1, n = 26) and a second category of controls (Control 2, n = 16): individuals living in the same house as the patients and consuming same water as patients but not exhibiting clinical manifestations of skeletal fluorosis. Serum testosterones in skeletal fluorosis patients were significantly lower than those of Control 1 (p < 0.01). Testosterone concentrations of Control 2 were also lower than those of Control 1 at p < 0.05 but were higher than those of the patient group (Susheela and Jethanandani, 1996).

**Gastroduodenal effects**

Dasarathy et al (1996) compared ten patients with documented osteofluorosis (High endemic level of $F^- = 4.9 \pm 4.7$ mg/l) with ten age- and sex-matched healthy volunteers ($F^- = 0.34 \pm 0.1$ mg/l). All patients with osteofluorosis had gastrointestinal symptoms, the most common being abdominal pain. None of the control subjects had any clinical symptoms or mucosal abnormalities. It was concluded that gastrointestinal symptoms as well as mucosal abnormalities are common in patients with osteofluorosis.

**Otosclerosis**

Using a retrospective chart review and a residential history questionnaire Vartainen et al (1997) investigated the effect of drinking water fluoridation on the prevalence of clinical otosclerosis in an area where the natural waters have very low fluoride content, in subjects born between 1948 and 1962. In this age group, the prevalence of clinical otosclerosis was found to be 0.35 per cent of persons exposed to fluoridated tap water and 0.32 per cent of those consuming fluoride-poor water. It seems that a sodium fluoride intake of 1 to 3 mg daily cannot prevent the development of clinical otosclerosis in a low-fluoride area.

**Immune system**

Careful examination of various studies on fluoride and immune function do not support the suggestion that fluoridation might affect immunity. While fluoride at high concentrations can have inhibitory effects on lymphocyte and polymorphonuclear leucocyte function, these concentrations are many times higher than levels which would be expected from fluoridation. Fluoride can act as an immunological adjuvant. There is no evidence of any deleterious effect on specific immunity following fluoridation nor any confirmed reports of allergic reactions. Fluoride at high concentration can be an adjuvant for specific immunity but there is no evidence of any deleterious effect of fluoride on specific immunity and no conformed reports of allergic reactions (Challacombe, 1996).
Sudden Infant Death Syndrome (SIDS)

Dick et al (1999) reported that infants exposed to fluoridated water supplies during pregnancy were not at increased risk for SIDS, adjusted odds ratio (OR) 1.19 (95% confidence interval (CI) 0.82, 1.74). For breast-fed infants at the time of death/nominated sleep, fluoridated water exposure was not associated with an increased risk for SIDS, adjusted OR 1.09 (95% CI 0.66, 1.79). Similarly, fluoridated formula feeding, when compared with unfluoridated formula feeding, showed no increased risk of SIDS, adjusted OR 1.25 (95% CI 0.73, 2.13). There was no evidence of an interaction between fluoridation and infant feeding for the last two days (chi2 = 0.171, df = 1, p = 0.68). Exposure to fluoridated water supplies prenatally or postnatally at the time of death did not affect the relative risk for SIDS.

Nephrolithiasis (Kidney stones)

The prevalence of uroliathiasis was 4.6 times higher in fluoride endemic area (EA) than in non-endemic area. The prevalence was almost double in subjects with fluorosis than without fluorosis in the endemic area (NEA). No relationship was observed between urolithiasis and the duration of fluorosis. The fluoride levels in drinking water ranged from 3.5 to 4.9 ppm in EA. A comparison of normal subjects (NS) from EA and NEA revealed that endemic subjects tend to have slightly higher mean serum thiobarbituric acid reactive substance (TBAR) levels and excrete more oxalate and fluoride than their non-endemic counterparts. The urinary stone formers (SF) from the two areas showed a similar tendency, though again the difference was not significant. Citrate excretion in SF was almost normal in the EA, but NEA SF had significantly lower excretion levels. Urinary stones from endemic patients had higher fluoride, oxalate and calcium levels than those from non-endemic patients. The data suggest that fluoride in vivo may behave as a mild promoter of urinary stone formation by (a) excretion of insoluble calcium fluoride, (b) increasing oxalate excretion and (c) mildly increasing the oxidative burden (Singh et al, 2001).

Biochemical markers (enzymes)

The prevalence of dental and skeletal fluorosis was determined among children of Kheru Nayak Thanda of Gulbarga district, where the fluoride concentration in drinking water ranges from 0.6 to 13.4 ppm and the water has low levels of copper and zinc. These children were investigated clinically, radiologically and biochemically. The study revealed that 96% of the children had dental fluorosis and 39% exhibited skeletal fluorosis. Serum samples of these children showed elevated levels of alkaline phosphatase (ALP), alanine transaminase (ALT), aspartate transaminase (AST), and decreased levels of total protein, albumin, and potassium. Radiographic changes suggestive of osteoporosis, osteosclerosis, and genu valgum were observed (Shivashankara et al, 2000).

Down’s syndrome

Whiting et al 2001 (50) made systematic review of research studies in all languages which investigated the incidence of Down's syndrome in areas with different levels of fluoride in their water supplies was made. All were ecological in design and scored poorly on the validity assessment. The estimates of the crude relative risk ranged from 0.84 to 3.0. Four studies showed no significant associations between the incidence of Down's syndrome and water fluoride level and two studies by the same
author found a significant (p < 0.05) positive association (increased Down's syndrome incidence with increased water fluoride level). Only two of the studies controlled for confounding factors and only one of these presented summary outcome measures. The evidence of an association between water fluoride level and Down's syndrome incidence is inconclusive.

**Hyperparathyroidism**

In a cross sectional clinical study, Gupta et al 2001 (19) evaluated the effect of ingestion of drinking water containing high fluorides and its effect on serum parathyroid hormone. 200 children (6 to 12 years) were selected from four areas (50 from each area) consuming water containing 2.4, 4.6, 5.6 and 13.5 mg/l of fluoride. Serum calcium levels were well within normal range in the patients of all areas but an increase in serum parathyroid levels (S. PTH) was noted. The increased S. PTH was well correlated with increase in fluoride ingestion. The severity of clinical and skeletal fluorosis was observed to increase with increase in S. PTH concentration. According to authors high Fluoride ingestion has a definite relationship with increased parathyroid hormone secretion, which may be responsible for maintaining serum calcium levels and may have a role in toxic manifestations of fluorosis.

Since 1995, several studies have investigated and disclosed a spectrum of effects associated with exposure to fluoride in drinking water. Overall, the results from these studies showed that the effects are usually associated with high levels of fluoride except for the impact of endemic fluoride on intelligence (cognitive effects) that seems to occur at lower levels (less than 1 ppm). The findings regarding the impact of fluoride exposure on intelligence (cognitive effects) possibly occurring at lower levels need further investigation in order to confirm 1) whether or not this effect is observable in other areas of the world and, 2) to describe and better characterize the dose-response relationship if any.

2.5 REFERENCES


Douglass, C.W. and Joshipura, K., Caution needed in fluoride and osteosarcoma study (Letter to the editor). Cancer Causes Control, 2006; 17: 481-482.


