Fluoridation Report for the Secretary of Health

Findings from a Review of the Current Literature

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Executive Summary

VDH was approached in August 2009 by a concerned citizen regarding the fluoridation of drinking water and its health effects. After taking into consideration the time that has elapsed since VDH last reviewed the effects of fluoride in drinking water on humans and the magnitude of the debate a review of the current literature was undertaken by the public health toxicologist. Current peer reviewed literature was obtained and reviewed by the toxicologist after performing a search on PubMed. Keywords including: cancer, osteosarcoma, reproduction, development, neurotoxicity were used in combination with fluoride and or fluorine to generate a list of manuscripts to be reviewed. A number of review articles, which provided general and background information, as well as *in vivo*, *in vitro*, and epidemiological studies were collected. The NRC 2006 Fluoride in Drinking Water report was also reviewed.[1]

The toxicological review focused on the risk assessment of fluoride rather than the risk management of fluoridation or naturally occurring fluoride. Currently, the EPA has established a maximum-contaminant-level goal (MCLG) of 4 milligrams/Liter (mg/L) and a secondary maximum contaminant level (SMCL) of 2 mg/L for naturally occurring fluoride in drinking water. The MCLG is to prevent adverse health effects in the general population. The secondary level is to reduce the cosmetic tooth defect, enamel fluorosis, which may develop from consuming high levels of naturally occurring fluoride in drinking water at a young age. Guideline for fluoridation to protect the public from dental caries by the U.S. Public Health Service is 0.7-1.2 mg/L depending on mean

daily air temperature. Virginia's Fluoridation Program has adopted these guidelines and recommends 0.9 mg/L as the optimal level for fluoridated drinking water.

A consistent finding from epidemiological and toxicological papers reviewed as well as the NRC 2006 publication is that the adverse effects of fluoride occur at "high" concentrations. The use of "high" in this report is indicative of drinking water that contains more than 4 ppm fluoride or the highest dose administered to test animals. In the review by the NRC it is never stated that the practice of fluoridation should be halted. The NRC suggests lowering the EPA's MCLG to a level that is more protective of severe enamel fluorosis, clinical stage II skeletal fluorosis, and bone fractures. The review by the NRC in 2006 was not intended to address the benefits and risk from consuming drinking water with fluoride at concentrations recommended by Virginia's Fluoridation Program which is below the EPA's SMCL.

After reviewing the literature several findings stand out: (1) current epidemiological studies were conducted in places where the level of fluoride is higher than that recommended by Virginia's Fluoridation Program, (2) the high doses administered during animal studies produced the adverse effects in the majority of the studies, (3) and measured adverse effects of fluoride in one study are seldom replicated by others. Therefore, the current review of the literature does not warrant any change to Virginia's current fluoridation practice.

Introduction

Fluoride is the ionic form of fluorine, the 13th most abundant element in the earth's crust. Fluoride ion is negatively charged and found with positively charged ions such as calcium and sodium. Because of fluoride's high affinity for calcium it is associated with calcified tissues such as bone and teeth. Fluoride inhibits the demineralization of sound enamel and enhances the remineralization of teeth that have lost minerals such as calcium, phosphate, and carbonate. Fluoride is also thought to inhibit caries formation by inhibiting bacteria from metabolizing carbohydrates into acid.

The EPA sets a maximum allowable limit for naturally occurring fluoride in community drinking water at 4 mg/L and a secondary limit (i.e., non-enforceable guideline) at 2 mg/L. The MCLG (4 mg/L) is to protect the public against adverse health effects. The SMCL is to prevent cosmetic tooth defect that can occur at concentrations above 2 mg/L. EPA standards are for fluoride found in drinking water from natural contamination.

Since 1945, fluoride has been added to many drinking-water systems to control dental caries. The U.S. Public Health Service recommends fluoride concentration in fluoride treated drinking water range from 0.7 to 1.2 mg/L depending on the average maximum daily air temperature of the area. This range was selected to maximize caries prevention and limit enamel fluorosis. Decisions to fluoridate public drinking-water are made by state and local authorities. The Virginia Fluoridation Program recommends an optimal fluoride concentration equal to 0.9 mg/L.

There has been controversy since the practice of fluoridating water supplies began. The safety, motivation, and benefits are continuously challenged by opponents. There are also those who object because they view it as an infringement on their freedom of choice. Others ague how can individuals monitor the dose of fluoride they receive through large-scale fluoridation. Another dispute is between those that believe topically applied fluoride is more efficacious at preventing caries than fluoride that reaches the teeth systemically.

The practice of fluoridation has been hailed as one of the greatest health achievements and is endorsed by the Center for Disease Control and Prevention, the U.S. Surgeon General, American Dental Association, and the American Medical Association. A review by the Virginia Department of Health's Bureau of Toxic Substances 1980 concluded that further research was necessary to address the effects of fluoride on human health. Since then a number of organizations have reviewed the benefits and adverse effects of fluoride on human health including: The World Health Organization 1984, 1994 and 2006, NRC 1993 and 2006, and the Institute of Medicine 1997. As expected a growing body of literature surfaces each year regarding fluoride and its effects on health. Therefore, a review of the current peer reviewed literature was conducted by the Virginia Department of Health's public health toxicologist.

Method

A search on PubMed http://www.ncbi.nlm.nih.gov/PubMed using the following keywords: development, reproduction, cancer, osteosarcoma, mutagenesis, neurotoxicity, toxicology, toxicity, genotoxicity, thyroid in combination with fluorine and or fluoride was used in the initial search. To keep the review current only manuscripts published after 1999 were initially considered. After the initial review several other papers were gathered that were referenced in the initial papers. A few review papers on fluoridation were also added to the initial selection. A 1996 manuscript studying developmental effects of fluoride was included because a more recent article was not available. This report was also limited to peer reviewed journal articles and the NRC 2006 Fluoride in Drinking Water report.

Due to time constraints and to limit any bias the author of this paper did not review any manuscripts that were submitted by the Virginia Fluoridation Program or concerned citizens. However, the Virginia Fluoridation Program did provide a copy of the NRC 2006 Fluoride in Drinking Water report.

There were a few papers that could not be retrieved from the Virginia Commonwealth University Library. Two were requested from the authors and supplied by the authors. A few papers were not available when the review period began (e.g., abstract ahead of print) and were still not available at the end of the review period.

Review articles were read for general information and are listed in the reference section of this manuscript. The author is aware that there is debate whether severe enamel fluorosis is an adverse effect or not. This is not discussed in detail in this manuscript and neither is systemic vs. topical application of fluoride in preventing caries.

Results

The results presented are from current peer reviewed journals available using the methodology described above and are representative of current fluoride research. The findings from the journal articles are presented below with complete references given at the end of this manuscript. The results are presented in four sections: Human Studies, Animal Studies, *In Vitro*, and the NRC 2006 Report.

Human Studies

Bassin *et al.* performed a matched case-control study to examine the relationship between age-specific fluoride exposure in drinking water and osteosarcoma.[2] An odds ratio of 5.46 (95% CI 1.5, 19.90) for males aged 7 years was produced when confounders were eliminated. Several limitations to this study as cited by the author include: estimating fluoride in drinking water at each resident may not reflect actual consumption, the estimation of fluoride concentration at each residence is subject to measurement error, lack of data that may implicate another factor in drinking water that is correlated with fluoride in drinking water, and possibility of selection bias.

Douglass and Joshipura describe Bassin *et al*. paper[2] as containing "…subset of participants in our ongoing study of fluoride and osteosarcoma."[3] The complete study is made up of two sets of cases. The first set of cases between 1989 and 1992 were used by Bassin *et al*., and the second set of cases is from 1993 and 2002. Douglass and Joshipura found an association between fluoride and osteosarcoma in the overall first set of cases (not age specific), but could not replicate the findings in the second set of cases. In the second set of cases bone specimens were provided from many of the cases and controls, and no association between fluoride level and excess risk of osteosarcoma was identified. The authors caution the reader to wait for the publications from the full study before making any conclusions from the Bassin *et al*. study.

A fluoride study by Wang *et al*. was part of a larger evaluation of the health effects of arsenic in rural China.[4]   The mean concentration of fluoride in drinking water for the "high-fluoride" group studied was 8.3 ± 1.9 mg/L, and the range was $3.8 - 11.5$ mg/L. In the study the number of IQ tests administered was higher than the number of analysis performed on water and urinary fluoride. The average IQ of Chinese children in 2005 was 103.5 ± 17.7. The average IQ of the control and the high-fluoride group was 104.8 ± 14.7 and 100.5 ± 15.8, respectively, and considered significantly different ($p<0.05$).

A Spearman's correlation coefficient of -0.107 ($p<0.05$) was found between the IQ scores and urinary fluoride concentration in the control group and high-fluoride group. The author suggests that this negative correlation is indicative of fluoride's affect on children's intelligence. The study also examined the affect of fluoride on development

and did not find anything statistically significant except the affect of high-fluoride on height ($p < 0.05$). The authors conclude that there is a need for more careful evaluation of the effects of fluoride on intelligence.

Amador *et al.* reported that when school children exposed to fluoride and arsenic in drinking water, a statistically significant reduction in IQ scores is observed.[5] The children were exposed to 5.3 ± 0.9 mg/L fluoride and 169 ± 0.9 µg/L arsenic in one rural community and 9.4 ± 0.9 mg/L fluoride and 194 ± 1.3 µg/L arsenic in a second community. The authors conclude that these levels of fluoride and arsenic in drinking water produce a neurotoxic effect in children. Arsenic has been associated in other epidemiological studies with a decrease in IQ and may confound the findings in this study when present in drinking water at these concentrations with fluoride.

A study by Tang *et al.* reviewed the literature from 1988 to 2008 to determine whether fluoride exposure in China was associated with a low intelligence quotient.[6] The literature search was done on MEDLINE, SCI, and CNKI using fluorosis, fluoride, intelligence, and IQ as keywords. The authors also searched on the website www.fluorideresearch.org for studies. The study performed a meta-analysis on 16 case-control studies that met the author's eligibility criteria for inclusion. Using a weighted mean difference, meta-analysis found on average that children living in fluorosis areas in China where 5 times more likely to develop low IQ than those living in non-fluorosis or slight fluorosis areas. A funnel plot of the meta-analysis generated a bias that the authors attributed to publication and language biases.

Animal Studies

Riberiro *et al.* administered fluoride to male Wistar rats as sodium fluoride in drinking water for 6 weeks at 0, 7, and 100 ppm.[7] Peripheral blood, oral mucosa and brain cells in vivo were analyzed for DNA damage using the single cell gel (comet) assay. As depicted by the mean tail moment and tail intensity there was no statistically significant difference between the control group and the rats treated with sodium fluoride.

Guadarrama *et al.* evaluated the *in vivo* effect of sodium fluoride on increasing the rate of sister chromatid exchanges (SCE) in mouse bone marrow cells.[8] The author dosed (2.0, 4.0, 8.0, 16.0, 24.0 mg/kg) male mice up to 75% of the author's previously determined LD_{50} with sodium fluoride intraperitoneally. A significant SCE increase was found with the three highest sodium fluoride doses. The study found no significant difference in cellular proliferation kinetics between control animals and dosed animal. The authors could not draw a conclusion from the mitotic based index comprised of one thousand cells because of the variability. The authors conclude that their *in vivo* research is in agreement with others reporting low doses of fluoride found in the environment does not pose a danger to genetic material.

Leite *et al.* examined fluoride's acute affect on DNA strand breaks in blood and various organs in rats using the comet assay.[9] Six doses of sodium fluoride (10, 20, 40, 60, 80, 100 mg/kg body weight) were administered by gastrogavage to rats including potentially lethal doses. The author found no significant DNA damage at any dose as

expressed by tail moment in blood, liver, kidney, urinary bladder, and thyroid gland cells in rats when compared to controls. The author concludes that acute lethal doses of fluoride were unable to induce genotoxicity in rats as depicted by the single cell comet assay in the cell types tested.

Chouhan and Flora exposed male rats to one of three doses of sodium fluoride (10, 50, or 100 ppm in drinking water) for a period of 10 weeks.[10]   The effects of fluoride on blood and tissue oxidative stress and apoptosis were investigated. Glutathione (GSH) blood level decreased in a dose-dependent manner; however, the dose-dependent decline between the low and middle dosed animals was not evident. A decrease in GSH indicates an overproduction of reactive oxygen species (ROS) and a decreased antioxidant state. The ROS concentration was increased significantly in the middle and high dosed animals. There was no significant difference between the controls and mid and high dosed animal's δ-aminolevulinic acid dehydratase (ALAD) activity. ALAD activity was decreased significantly in animals receiving the low fluoride dose. This trend was also observed in WBC counts.

Significant ROS elevation in liver, kidney, and brain was not observed. There was an exceptionally high concentration of ROS in the kidney of animals receiving the high dose. Tissue oxidative stress was evaluated by determining the GSH:GSSG ratio in the liver, kidney, and brain. The liver ratio decreased more than any other tissue. The greatest effect on the liver ratio was observed in the low dosed animals. There was no significant

change in the ratio between the control and all three doses in the brain. In the kidney there was no significant difference between the control and high dosed animal's ratio.

Fluoride induced cell death was studied by measuring caspase 3 and ATPase activity in cells. There was a decreased caspase activity in the middle and high dose animals. Also, DNA damage studies did not confirm fluoride induced cell death at all doses administered. The authors conclude that fluoride may exert it toxic effect through enhanced oxidative stress. The study also finds it "interesting" that fluoride's effects are more deleterious at low concentrations. The author ascribes this to the ionic mobility of fluoride. Lastly, short term fluoride exposure did not produce apoptosis.

Adult male mice were exposed to a single high dose (5 mg/kg) of sodium fluoride in a study by Mittal *et al.* orally each day for 8 weeks and then sacrificed.[11] A number of assays were performed on the liver, kidneys, and whole blood to examine fluoride's affect on tissue oxidative stress and cell injury. There was a significant decrease from the control in ALAD activity and GSH level in animals exposed to sodium fluoride. The study also found a significant increase from control in ROS in animals exposed to sodium fluoride. Fluoride was found to have no significant effect on hematological variables in mice.

The study found that in the liver fluoride did not produce a significant change in catalase or glutathione peroxidase (GPx) activity. However, fluoride dosed animals did have a significant decrease in superoxide dismutase (SOD) activity. Fluoride exposure

had no effect on GSH and GSSG levels. Biochemical variables indicative of liver damage, ACP, ALP and ALT, all remained unchanged in fluoride dosed mice with AST activity showing a marginal increase. G-6-P activity was not affected in fluoride dosed animals when compared to control animals.

In the kidneys fluoride dosed animals showed an increase in thiobarbituric acid reactive substance (TBARS) level compared to control animals. Fluoride dosed animals did not show a significant change in antioxidant enzyme activity except significant decrease in SOD activity. The authors conclude that the effects are based on using doses of fluoride which may not reflect human fluoride exposure.

Wang *et al.* examined fluoride's effect on membrane lipid changes in rat liver following long term exposure to drinking water containing 30 or 100 ppm of sodium fluoride.[12] Wistar rats were given treated water for seven months at which time they were sacrificed and their livers analyzed. The study produced a dose dependent decrease in total liver phospholipid with respect to increasing fluoride. Cholesterol, dolichol, and ubiquinone, three end products of the mevalonate pathway were analyzed in the study to examine fluoride's mechanism of toxicity. Cholesterol and dolichol content in rat liver were not significantly influenced by fluoride. Ubiquinone in rat liver was significantly decreased by fluoride. The authors suggest that the decreased phospholipid, unsaturated fatty acids, and loss of ubiquinone, are related to lipid peroxidation resulting from increase in free radicals due to long term fluoride exposure.

Chlubek *et al.* exposed Male Wistar Fl rats to distilled water containing sodium fluoride (50 or 100 mg F⁻/L) or vehicle for 4 months before being sacrificed and examined for fluoride's effect on the pancreas.[13] Serum concentrations of glucose and fluoride increased significantly in treated rats compared to control rats. Treated rats also had a significant decrease in pancreatic CuZn-SOD activity but there was no significant decrease in pancreatic Mn-SOD. Lipid peroxidation as measured by a decrease in MDA levels was not statistically significant. The authors conclude that the cytoplasmic CuZn-SOD is more susceptible than the mitochondrial Mn-SOD and that explains why the later failed to reach a statistical significance. The study also failed to observe any pancreatic lipid peroxidation, but rather a weak inhibition. The authors found it improbable that elevated fluoride serum levels were the major factor for pancreatic dysfunction resulting in hyperglycemia. Rather the authors suggest that the hyperglycemia was a result of increasing levels of cAMP leading to an increased hepatic glycogenolysis.

Heindel *et al.* administered sodium fluoride at various concentrations in drinking water to Sprague-Dawley rats (0, 50, 150, or 300 ppm) and New Zealand White rabbits (0, 100, 200, or 400 ppm) during gestational days 6-15 and 6-19, respectively.[14] The rats and rabbits were killed on day 20 and 30, respectively, and examined for maternal and embryo-fetal effects. No maternal effects were detected in either species and no animals died during the course of the study. The rats exposed to high doses of sodium fluoride consumed less water during the exposure period. Water consumption returned to normal after dosing was stopped. The same pattern was seen in the rabbits. This decrease in water consumption in the high dose animals was probably due to the palatability of the

water. In the rabbit there was no significant reduction in body weight except for the high dosed group during gestation days 6-8 probably due to decrease water and food intake. Likewise, the high dosed rat group had decrease maternal weight during gestational days 6-16. Maternal liver and kidney weights were not significantly different from the control in either the rat or rabbit model.

The study reported no significant differences between the treated animals and controls in the average number of implantations, live fetuses, percentage of early deaths (resorptions), late fetal deaths/litter, or corpora lutea. Morphological abnormalities of rabbit and rat fetuses in the study showed no significant effects for pairwise comparison of treated animals with the control group. The percentage of externally malformed fetuses and the percentage of skeletally malformed fetuses per rat liter showed an increase with increasing dose. Also the percentage of litters with malformed rat fetuses showed an increase with dose. These trends occurred in the absence of a significant groupwise difference among the control and treated group. The study concluded that rats drinking 27 mg/kg/day and rabbits drinking 29 mg/kg/day sodium fluoride throughout organogenesis did not produce any definitive developmental toxicity.

In vitro

Anuradha *et al.* examined fluoride induced apoptosis in HL-60 cells.[15] Cells exposed to sodium fluoride (2 mM) for 24 h induced apoptosis in approximately 50% of the cells. The fluoride induced cell death was inhibited by pretreatment with either antioxidants N-acetyl cysteine (NAC) or GSH, or by the caspase inhibitor, z-VADFMK.

Sodium fluoride increased lipid peroxides as measured by estimating the end products of lipid peroxidation, malondialdehyde and 4-hydroxy-2(E)-nonenal in HL-60 cells. Fluoride induced apoptosis was also examined by measuring membrane potential in cells treated with sodium fluoride. There was a decrease in membrane potential in cells treated with sodium fluoride and this decrease in potential could be lessened with antioxidants (NAC and GSH). The accumulation of cytochrome c in the cytoplasm as measured by the Western Blot analysis in sodium fluoride treated cells compared to controls was significant. The role of fluoride in cell death was also examined by measuring the decrease in Bcl-2 protein in treated and non-treated cells. Western Blot analysis showed a significant decrease in Bcl-2 protein in cells treated with sodium fluoride compared to controls. Treating cells with antioxidants and z-VADFMK during sodium fluoride treatment yielded no change in Bcl-2 protein level.

The authors propose that the mechanism by which sodium fluoride induces apoptosis is mediated by oxidative stress-induced lipid peroxidation. Lipid peroxidation affects the membrane potential of the mitochondria causing the release of cytochrome c into the cytosol increasing the caspase cascade leading to apoptosis in HL-60 cells.

NRC 2006 Report

The report by the NRC was in response to the EPA's request to have its MCLG (4 mg/L) and SMCL (2 mg/L) independently evaluated for adequacy to protect children and others from adverse health effects of fluoride at these levels in drinking water. The

review of the NRC report is summarized below in two main sections (Findings and Research Needs).

Findings

Severe enamel fluorosis occurs among children in U.S. communities with fluoride concentrations near 4 mg/L. Severe enamel fluorosis compromises the health-protective function of the tooth by damaging the structure of the tooth. Not all committee members judged this to be consistent with the prevailing definition of an adverse health effect.

Skeletal fluorosis is a bone and joint condition that occurs with chronic exposure to fluoride and results in joint stiffness and pain. Based on the current literature and existing epidemiologic literature the committee could not determine if stage II skeletal fluorosis is occurring in the U.S. where individuals consume water with fluoride at 4 mg/L. The committee suggests more research is needed in this area.

Fluoride can weaken bone and increase risk of fractures under certain conditions. The majority of the committee agreed that there was an increase risk of bone fractures from consuming water with 4 mg/L fluoride over a lifetime.

Reproductive and developmental studies report adverse outcomes at very high concentrations that would not be encountered by the U.S. population.

Epidemiologic studies addressing fluoride's effect on neurotoxicity and neurobehavioral effects from foreign countries lack sufficient detail and the committee questions the relevance to the U.S. population. More compelling studies were from molecular, cellular, and anatomical changes in the nervous system research. The committee recognizes that functional changes could occur but more research is needed.

The committee list several endocrine effects from consuming fluoride in drinking water at 4 mg/L or less. The chief effects are decreased thyroid function, increased calcitonin activity, impaired glucose tolerance, increased parathyroid hormone activity, secondary hyperparathyroidism, and possible effects on sexual maturity timing. Many of the effects are considered subclinical and are not considered adverse health effects. More research is needed to explore mild imbalances or perturbations in hormone concentrations.

No well conducted studies examining the effects on gastrointestinal system, liver, kidneys, and immune system from humans drinking water containing 4 mg/L were available. There are case reports and animal studies showing 4 mg/L fluoride in drinking water irritates the gastrointestinal system, and can affect the kidneys, liver, and immune system. The committee says that such effects are unlikely to be a risk for the average individual drinking water containing fluoride at 4 mg/L.

Genotoxicity studies of fluoride include the more important *in vivo* assays in humans, and to a lesser extent, *in vitro* studies including human cell lines and *in vivo*

rodent studies. The committee finds the *in vivo* human studies to be mixed and the *in vitro* tests conflicting and not contributing to the interpretation of the existing data. Also cytogenetic effects of fluoride at environmental concentrations are considered contradictory. Because fluoride deposits in bone it is considered a possible site for cancer. The committee acknowledges a 1990 study showing a dose-response trend for fluoride in drinking water and osteosarcoma in male rats. Many epidemiological investigations have limited methodology to draw a conclusion between linking fluoride and cancer. Some studies report a positive association and others report a negative association. The committee collectively considers data from all studies examining fluoride's ability to initiate or promote cancer as tentative and mixed. The committee is awaiting a study from Harvard School of Dental Medicine to add insight to fluoride causing osteosarcoma.

Research Needs

The NRC committee identified several research areas of opportunity that will fill in gaps that prevented the committee from making some judgments about the safety of fluoride between 2 and 4 mg/L in drinking water.

Future exposure assessments should be characterized by individuals instead of communities and epidemiologic studies should be grouped by exposure level rather than exposure source, location, or drinking water fluoride concentration. Fluoride should be included in nationwide biomonitoring surveys and nutritional studies.

Concentration of fluoride in human bone as a function of exposure, duration of exposure, age, sex, and health should be evaluated. Individuals with renal function changes would benefit from plasma and bone fluoride concentration studies in these types of patients. Pharmacokinetic models should be improved.

Enamel fluorosis longitudinal studies should be done in U.S. communities with fluoride concentrations greater than 1 mg/L.

Methods should be developed to objectively assess enamel fluorosis and aesthetic consequences should also be better addressed. Increase research to study fluoride exposure and its relation to dental fluorosis and delayed tooth eruption patterns.

More research to clarify the relationship between fluoride ingestion, fluoride bone concentration, and clinical symptoms. Increase study in communities where fluoride in drinking water is above 2 mg/L to assess fluoride increasing risk of bone fracture. Quantitative measures other than self-reported fractures or hospital records should be used in these studies.

Carefully conducted studies in the U.S. population exposed to various concentrations of fluoride should be done with appropriately documented exposure to determine emerging health effects (e.g., endocrine effects and brain function).

Discussion and Conclusion

Human Studies

The review of the literature and the NRC 2006 report suggests a number of associations between fluoride and adverse effects. However, the effects of fluoride in many of these studies reviewed are only significantly different when comparing the control animal and the high dosed animal.

Review of recent epidemiologic studies from China and Mexico indicate that high levels of fluoride are associated with lower IQ scores. These studies include areas where fluoride concentrations in drinking water are greater than those found in the U.S. In the study from Mexico the drinking water also contained high levels of arsenic. The meta-analysis study of the literature by Tang *et al.* contains biases as indicated by a funnel plot presented by the author. The negative correlation between IQ and urinary fluoride concentrations reported by Wang *et al.* is remarkable with a $p<0.05$. However, a strongly negative correlation would be closer to -1 rather than the reported -0.107 which can be considered weak.

The matched case-control study examining a correlation between osteosarcoma and fluoride in drinking water by Basin *et al.* from Harvard is followed by a manuscript in the same journal from Douglass and Joshipura from Harvard. Douglass and Joshipura caution readers in interpreting the study from Basin *et al.* The NRC 2006 report reviewed Basin's unpublished dissertation and describes the use of hospital-based

controls in the study a deficit because it introduces serious selection bias. The NRC concludes that a follow-up study confirming Basin's findings would give more weight to fluoride causing cancer. Douglass and Joshipura's preliminary findings suggest a lack of association between fluoride and osteosarcoma in cases from essentially the same hospitals (1993-2000) used in the study by Bassin *et al.* The NRC writes that there has been partial but incomplete fulfillment of its recommendations on cancer studies on humans since 1993.

Animal Studies

Several recent studies suggest that one of the biological pathways that fluoride induces its toxic effect is through oxidative stress. Authors in these studies also conclude that more studies are needed to fully understand fluoride's toxic mechanism of action. The research is complicated by findings that fluoride produces oxidative stress at low levels in some studies and at high levels in other studies.

Genotoxicity

Several recent studies looked at fluorides affect on the genetic code. These studies measured DNA damage or chromosome aberrations. Leite *et al.* did not find any fluoride toxicity in multiple organs examined by comet assay in rats dosed up to 100 mgF/kg body weight. Likewise, Velazquez-Guadarrama *et al.* reported genotoxic effects in mice began with animals dosed intraperitoneally with 8 mg/kg sodium fluoride and a duplication of basal level began at 24 mg/kg. Ribeiro *et al.* did not find any DNA damage

in rats exposed to drinking water containing 100 ppm sodium fluoride as depicted by the comet assay.

Developmental Toxicity

Because a current developmental study was not available the study by Heindel *et al.* (1996) was reviewed. This study determined a no-observed-adverse-affect-level (NOAEL) for maternal toxicity for the rat and rabbit to be 150 and 200 ppm sodium fluoride in drinking water, respectively. The NOAEL for developmental toxicity was greater than 300 ppm sodium fluoride in drinking water for the rat and greater than 400 ppm for rabbits. The author concludes that the mid- and high-dosed animals were exposed to fluoride that was 100 fold higher than what humans are exposed to daily drinking water containing 1 ppm fluoride.

Conclusion

The three toxicity end points used by the EPA to determine the MCLG for which there was sufficient relevant data are severe enamel fluorosis, skeletal fluorosis, and bone fractures. In addressing the MCLG the NRC 2006 report concludes:

> *"In light of the collective evidence on various health end points and total exposure to fluoride, the committee concludes that EPA's MCLG of 4 mg/L should be lowered."*

Lowering the MCLG will prevent children from developing severe enamel fluorosis and decrease a lifetime accumulation of fluoride into bone that may put individuals at increased risk of bone fracture and possible skeletal fluorosis.

More important to this report is that the NRC 2006 report also concludes that the SMCL (2 mg/L) should be reevaluated because it does not completely prevent the occurrence of moderate enamel fluorosis. In addressing the SMCL the NRC 2006 report concludes:

> "…Additional studies, including longitudinal studies, of the prevalence and severity of enamel fluorosis should be done in the U.S. communities with fluoride concentrations greater than 1 mg/L. These studies should focus on moderate and severe enamel fluorosis in relation to caries and in relation to psychological, behavioral, and social effects among affected children, among their parents, and affected children after they become adults."

In the above conclusion there is no mention of any other adverse effect at this level which is above Virginia's Fluoridation Programs optimal fluoridation goal, 0.9 mg/L. Also, the NRC also does not suggest a halt in fluoridation while further studies are conducted.

In summary, the present review of the literature, while not complete, is representative of current peer reviewed articles published on fluoride and its adverse health effects. Supplementing the articles found on PubMed with the NRC 2006 report does not generate any substantial findings indicating that the current practice of fluoridation in Virginia should be halted.

The author of this manuscript would recommend that the Virginia Health Department: (1) continue to review the literature, especially studies fulfilling the NRC's research recommendation; (2) continue to educate the public regarding fluoridation and

naturally occurring elevated fluoride; (3) encourage reducing the level of fluoride in

naturally fluoridated drinking water to the level recommended by U.S. Public Health

Service, 0.7-1.2 mg/L.

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