

EVIDENCE ON THE CARCINOGENICITY OF
Fluoride and Its Salts

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**Reproductive and Cancer Hazard Assessment Branch
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PREFACE

Proposition 65¹ requires the publication of a list of chemicals “known to the state” to cause cancer or reproductive toxicity. It specifies that “a chemical is known to the state to cause cancer ... if in the opinion of the state’s qualified experts the chemical has been clearly shown through scientifically valid testing according to generally accepted principles to cause cancer ...” The “state’s qualified experts” regarding findings of carcinogenicity are the members of the Carcinogen Identification Committee (CIC) of the OEHHA Science Advisory Board².

The lead agency for implementing Proposition 65 is the Office of Environmental Health Hazard Assessment (OEHHA) of the California Environmental Protection Agency. After consultation with the CIC, OEHHA selected **fluoride and its salts** as a chemical for consideration for listing by the CIC. Upon selection, the public was given the opportunity to submit information relevant to the assessment of the evidence on the carcinogenicity of fluoride and its salts. OEHHA reviewed and considered those submissions in preparing this document.

OEHHA developed this document to provide the CIC with comprehensive information on fluoride carcinogenicity for use in its deliberations on whether or not the chemical should be listed under Proposition 65. The CIC is expected to meet to consider listing of fluoride and its salts in October 2011.

¹ The Safe Drinking Water and Toxic Enforcement Act of 1986 (California Health and Safety Code 25249.5 *et seq.*)

² Title 27 Cal. Code of Regs. §25302

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1. EXECUTIVE SUMMARY

Fluoride is the monovalent anion derived from the element fluorine. It is present in many salts, including sodium fluoride and stannous fluoride. When these salts are dissolved in water the free fluoride ion is released. Fluoride salts or other compounds that release fluoride ion may be added to drinking water to prevent dental caries. Fluoride releasing compounds are also added for this purpose to a wide variety of dental products including toothpastes and mouthwashes. The public is exposed to fluoride ion by drinking fluoridated water and by using fluoride-containing dental products and treatments. Exposure may also occur through naturally present fluoride in foods and beverages, and in some cases by inhalation of fluoride compounds in the air.

There are numerous studies in the published scientific literature investigating the potential carcinogenicity of fluoride. The human evidence consists primarily of epidemiological studies (ecological, cohort, and case-control) comparing cancer risks in exposed *versus* unexposed individuals. One ecologic study (Cohn, 1992) and one hospital-based case-control study (Bassin *et al.*, 2006) have reported increases in osteosarcomas in young males exposed to fluoride in drinking water. The possibility that chance, bias, inappropriate analyses or confounding played a role in these findings could not be ruled out, however.

The animal evidence consists of several drinking water and diet studies conducted in rats and mice. Statistically significant increases in rare malignant bone tumors (osteosarcomas) were observed in male rats in drinking water studies conducted by the National Toxicology Program (NTP, 1990), but not in female rats or male or female mice similarly exposed. A statistically significant dose-related increase in thyroid tumors (adenomas and carcinomas, combined) was also observed in this male rat study (NTP, 1990). These findings were not replicated in a subsequent NTP study in male rats of the same strain receiving a somewhat higher fluoride dose, also via drinking water (NTP, 1992). Increases in benign osteomas were observed in two-year diet studies in male and female CD-1 mice (Maurer *et al.*, 1993). However, the possible contribution of retroviral infection reported in the male and female mice to the development of osteomas could not be ruled out. No treatment-related tumor findings were observed in two-year diet studies conducted in male and female Sprague-Dawley rats (Maurer *et al.*, 1990).

Pharmacokinetic studies of fluoride show that fluoride ion is readily taken up and incorporated into bones and teeth of humans and laboratory animals. Comparison of fluoride pharmacokinetics between humans and rodents indicates that rodents must be exposed to much higher levels of fluoride in diet or water than humans to achieve the same levels of bone fluoride levels.

Osteosarcoma is the most common form of primary bone cancer in humans, occurring more frequently in males than females. It commonly arises in the metaphyses — a region that encompasses the epiphyseal (growth) plate — of long bones, near the joints. Osteosarcomas occur more frequently during periods of rapid bone growth. The age distribution of osteosarcomas is bimodal, with the first and largest peak in incidence in the second decade of life, and the second peak occurring in males over 50.

Fluoride is mitogenic to osteoblasts, directly stimulating cell division and bone growth *in vivo* and *in vitro*.

Fluoride has been tested in a variety of *in vitro* and *in vivo* test systems assessing mutagenicity and clastogenicity, including in a number of studies of exposed humans. A mix of positive and negative results have been reported across test systems, with positive findings more often associated with higher concentrations of fluoride. In humans, positive findings of mutagenicity and clastogenicity have been reported in some studies of occupationally exposed workers and in some populations exposed to elevated levels of fluoride in drinking water.

Fluoride induced malignant transformation in the Syrian hamster embryo cell transformation assay in experiments conducted in three different laboratories and in the BALB/c 3T3 (mouse) promotion assay, but not in the BALB/c 3T3 cell standard focus assay.

Fluoride affects thyroid and parathyroid function in humans and animals, elevating thyroid stimulating hormone levels, altering levels of the thyroid hormones T3 and T4, and increasing levels of parathyroid hormone and calcitonin. These changes can affect the rate of formation of bone tissue and the overall rate of bone growth.

Fluoride can either stimulate or inhibit cellular immune responses in humans, rats, and mice. Decreases in cellular immune response may lead to a reduction in the ability of the immune system to identify and remove cancerous cells (*i.e.*, immune surveillance). Increases in cellular immune response may lead to inflammation, which may play a role in carcinogenesis.

Taken together, these multiple lines of evidence from mechanistic and other relevant data appear to support several plausible hypotheses: that fluoride is incorporated into bones (especially rapidly growing bones), where it can i) stimulate cell division of osteoblasts via direct mitogenicity and indirectly via effects on thyroid function and parathyroid function; ii) induce genetic changes; iii) induce other cellular changes leading to malignant transformation, and iv) alter cellular immune response, resulting in increased inflammation and/or reduced immune surveillance, thereby increasing the risk of development of osteosarcomas.

2. INTRODUCTION

2.1 Identity of Fluoride and Its Salts

Fluoride is the monovalent anion derived from the element fluorine. Fluorine is element number nine, with an atomic weight of 19. Fluorine is the most electronegative of the halogens. Fluorine exists as a diatomic gas with the molecular formula F_2 .

Fluoride ion (F^-) can form ionic bonds with cations, such as the sodium (Na^+), potassium (K^+) and stannous (Sn^{++}) ions, to form fluoride salts (e.g., sodium fluoride, potassium fluoride, stannous fluoride). Fluoride salts are highly soluble in water. Fluoride salts dissociate completely in aqueous solution, releasing the free fluoride ion and the free cation.

Fluoride ion can also be released from some fluorine-containing compounds as a result of hydrolysis of covalent bonds. Examples of fluoride compounds that release fluoride ion are fluorosilicic acid and sodium monofluorophosphate.

2.2 Occurrence and Use

Fluoride ion often occurs naturally in drinking water sources. Some geographical locations in California have relatively high concentrations of fluoride occurring naturally in ground or surface waters, e.g., at levels of a few parts per million (ATSDR, 2003). Fluoride ion also occurs in some foods and beverages such as tea or infant formulas³ (ATSDR, 2003, NRC, 2006, U.S. EPA, 2010).

The most important natural starting material for the production of fluorine-containing chemicals is fluorspar (calcium fluoride [CaF_2]), followed by fluorapatite ($Ca_5(PO_4)_3F$) and cryolite (Na_3AlF_6). Fluorspar has not been mined in the United States since 1996; it is imported, or purchased from the National Defense Stockpile. Some calcium fluoride is recovered from industrial waste streams, including uranium enrichment, stainless steel pickling, and petroleum alkylation. To supplement fluorspar supplies, fluorosilicic acid is recovered from phosphoric acid plants processing phosphate rock. Extraction of phosphates results in fluoride being a significant waste product (ATSDR, 2003).

Drinking water fluoridation is practiced in some municipalities in California, but not in others, for the purpose of preventing dental caries. Fluoride salts and other fluoride compounds, such as fluorosilicic acid, are used to fluoridate drinking water. Toothpastes and mouthwashes often contain fluoride salts or other fluoride-releasing compounds such as sodium monofluorophosphate, also for the prevention of dental

³ Although some fluoride in infant formula comes from the milk ingredient, the main source appears to be the processing water used to prepare the formula (ATSDR, 2003, p. 231).

caries. Children are commonly exposed to fluoride by in-office fluoride treatments. Fluoride can also be prescribed as a medication for treatment of osteoporosis.

3. DATA ON CARCINOGENICITY

3.1 Carcinogenicity Studies in Humans

A number of epidemiology studies have investigated the relationship between exposure to fluoride and the risk of cancer. These include more than 50 ecological studies investigating cancer risks associated with fluoride in drinking water, one cohort study of cryolite (a fluoride- and aluminum-rich mineral) production workers, and four case-control studies of osteosarcoma. The available studies were reviewed by the NRC in 1993 (Chapter 7, pp. 109-113, 121-123, Attachment 1) and again in 2006 (Chapter 10, pp. 320-339, Attachment 2). One case-control study (Bassin *et al.*, 2006, Attachment 3) and a related letter to the editor (Douglass and Joshipura, 2006, Attachment 4) have been published subsequent to the release of the 2006 NRC report and the 2003 ATSDR report.

In brief, some of the early concerns about the possibility that fluoride ingestion may cause cancer relate to a 1977 study by Yiamouyiannis and Burk, which was an ecologic study comparing overall cancer mortality rates over time between large U.S. cities with and without drinking water fluoridation. From 1940 to 1970, cancer death rates steadily increased in the fluoridated cities, while the rate of increase was less in the non-fluoridated cities. Other differences between the two groups of cities with regard to the major causes of cancers were not taken into consideration in this study. Importantly, the rate of cancer mortality increase in the fluoridated cities was similar before and after fluoridation began. In addition, although the rate of increase in the non-fluoridated cities was lower during 1950-1965, it was similar to the rate increase in the fluoridated cities both before and after that time. Both of these later findings suggest that any difference between the two groups of cities was not due to fluoride. In addition, subsequent analyses of the same data showed that differences in the cancer rates between the fluoridated and nonfluoridated cities were not seen when adjustments were made for age, sex, race, and the differences in cancer rates that existed before fluoridation began (Smith, 1980).

Other ecologic studies, including large studies by the National Cancer Institute, and several small case-control studies have failed to find clear associations between fluoride in water and either total cancer mortality, bone cancer or osteosarcoma (*e.g.*, Hoover *et al.*, 1991; Mahoney *et al.*, 1991; Hrudey *et al.*, 1990; Chilvers, 1983; Erickson, 1978; Rogot *et al.*, 1978; McGuire *et al.*, 1991; Operskalski *et al.*, 1987; Gelberg *et al.*, 1995; Moss *et al.*, 1995). However, not all these studies specifically examined young males.

Two studies have reported some evidence that fluoride may be associated with osteosarcoma in young males (Cohn, 1992; Bassin *et al.*, 2006), but several concerns limit the interpretability of these studies. In the ecological study by Cohn (1992), the relative risk for osteosarcoma, comparing fluoridated to non-fluoridated counties in New Jersey was 3.4 (95% CI, 1.8-6.0) in males under age 20. These findings were based on analysis of a limited number of cases of osteosarcoma in males under age 20 (12 exposed and eight unexposed cases). No increased risk was seen in females or other age groups. Fluoride exposure was based solely on residence at the time of cancer diagnosis; past drinking water exposures and other sources of fluoride were not considered. These issues would tend to decrease, not increase relative risks, although bias in either direction is possible. This study was published as a report, not in a scientific journal, and few details on the statistical analysis or other methods are provided.

In the hospital-based case-control study of osteosarcoma in people under age 20 in the U.S. by Bassin *et al.* (2006), elevated odds ratios (OR) were reported in males for fluoride drinking water levels above the U.S. Food and Drug Administration (FDA) target dose (*i.e.*, 1 ppm). ORs were calculated for each age of exposure, and the highest OR was reported for exposure to elevated levels of fluoride in drinking water at age seven (OR = 5.46, 95% CI, 1.50-19.90). Evidence of a dose-response relationship was seen and there was little change in ORs with adjustment for age, zip code, median income, county population, and use of fluoride supplements. By itself, this study seems to provide evidence of an association between fluoride in water and osteosarcoma in young males. However, in a Letter to the Editor accompanying this study (Douglass and Joshipura, 2006), researchers who were involved in the study (but are not listed as co-authors), warn that the Bassin *et al.* findings should be interpreted with caution. The reason given is that preliminary data from a second set of osteosarcoma cases and controls, which involves eight years of case accrual and a possibly more accurate method for assessing exposure (detailed interviews about water consumption and measurement of fluoride content in bone specimens), did not appear to confirm the elevated ORs observed in the first set of cases and controls (Bassin *et al.*, 2006). To date, no analysis of the association between fluoride exposure and osteosarcoma in this second set of cases and controls has been published.

The 2006 NRC report concluded that “The combined literature... does not clearly indicate that fluoride either is or is not carcinogenic in humans”. It appears that Bassin *et al.* (2006) is the only study published since that time which could potentially add insight into this issue. However, given the uncertainties discussed above regarding the findings of Bassin *et al.* (2006), the current body of epidemiologic research on the carcinogenicity of fluoride remains inconclusive.

See Attachments cited in this section:

1. NRC, 1993, Chapter 7, pp. 109-113, 121-123
2. NRC, 2006, Chapter 10, pp. 320-339
3. Bassin *et al.*, 2006
4. Douglass and Joshipura, 2006

3.2 Carcinogenicity Studies in Animals

The animal evidence for carcinogenicity of fluoride consists of nine rodent bioassays performed by two laboratories in the 1990s. These bioassays are well summarized by the NRC (2006, Chapter 10, pp. 316-320, Attachment 2).

In brief, these studies consist of: two-year drinking water studies conducted by the NTP in male and female F344/N rats and male and female B6C3F₁ mice (NTP, 1990), a two-year drinking water study in male F344/N rats (NTP, 1992), 99-week diet studies in male and female Sprague-Dawley rats (Maurer *et al.*, 1990), and 97-week diet studies in male and female CD-1 mice (Maurer *et al.*, 1993).

NTP 1990 studies

In the initial NTP (1990) studies, the animals were administered sodium fluoride in their drinking water at doses up to 175 mg/L. Significant dose-related increases in osteosarcomas were observed in male rats ($p < 0.05$, by Fisher pairwise comparison). Osteosarcomas are a rare malignant tumor in rats. A significant increasing trend with increasing dose ($p < 0.05$, Cochran-Armitage trend test) was also seen in thyroid tumors (adenomas and carcinomas combined) in these male rats. No significant increases in neoplasms were seen in the female rats or in the mice of either sex in the NTP studies. A non-significant increase in tumors of the oral cavity was observed in female rats.

There was also a non-significant increase in hepatoblastomas in both male and female mice. Hepatoblastomas are rare tumors in B6C3F₁ mice⁴. The NTP committee did not consider the slight numerical increase in hepatoblastomas to be biologically significant. The incidence data for hepatocellular neoplasms including hepatoblastomas are given for male and female mice in Table 21 from the NTP Technical Report (NTP, 1990, Attachment 5).

NTP 1992 study

No treatment-related increases in tumors were observed in male F344/N rats administered 250 mg/L sodium fluoride in drinking water for two years (compared with

⁴ Historical incidence (prior to 1990): 1/2397 in males, 1/2439 in females, Turusov *et al.*, 2002

175 mg/L as the highest dose in the NTP, 1990 study). This study did not replicate the findings of the earlier male rat study.

Maurer et al. 1990 and 1993 studies

In the studies conducted by Maurer *et al.* sodium fluoride was administered to male and female Sprague-Dawley rats (1990, Attachment 6) and male and female CD-1 mice (1993, Attachment 7) in the diet at doses up to 25 mg/kg/day. No treatment-related increases in tumors were observed in male or female Sprague-Dawley rats. Significant increases in non-malignant osteomas⁵ were observed in the high dose groups of both male and female CD-1 mice. In these studies the mice became infected with a retrovirus which may have caused the osteomas.

See Attachments cited in this section:

2. NRC, 2006, Chapter 10, pp. 320-339
5. NTP, 1990, Table 21
6. Maurer *et al.*, 1990
7. Maurer *et al.*, 1993

3.3 Mechanistic Evidence and Other Relevant Data

3.3.1 Pharmacokinetics

Information regarding uptake, distribution and excretion of fluoride in humans and animals is well summarized in Chapter 3, pp. 89-102 of the NRC report (2006, Attachment 8).

Ingested fluoride is readily absorbed in the gastrointestinal tract, distributed into tissues (primarily teeth and bones) and excreted in the urine. Fluoride not absorbed in the gastrointestinal tract is excreted in the feces. Fluoride exists in body fluids as fluoride ion and/or as hydrofluoric acid. Fluoride is taken up into the bones at a higher rate in younger animals, especially during active bone growth. Fluoride readily incorporates into calcified tissues by substituting for hydroxyl groups in the hydroxyapatite crystals of bones and teeth. Fluoride accumulates in the skeleton over the course of a lifetime, depending on the amount of fluoride in the diet (food and water).

Comparison of bone accumulation of fluoride in rats and humans leads to the conclusion that rats must be exposed to at least an order of magnitude higher fluoride

⁵ Osteomas are benign tumors, however there are a few clinical reports of human patients with osteomas that progress to malignant osteoblastomas (distinct from osteosarcomas)(Pieterse *et al.*, 1983).

concentration to achieve the same bone concentrations as humans. This should be kept in mind when considering the relevance of rodent experiments to humans.

See Attachment cited in this section:

8. NRC, 2006, Chapter 3, pp. 89-102

3.3.2 Tumor types

Male F344/N rats given fluoride in drinking water showed a significant increase in osteosarcomas and a dose-related increase in thyroid follicular cell adenoma and carcinoma (combined) (NTP, 1990). Osteosarcomas are rare in F344 rats (Leininger and Riley, 1990). Thyroid follicular cell adenomas and carcinomas are not uncommon in male and female F344 rats (Hardisty and Boorman, 1990). Thyroid adenomas may progress to carcinomas, so it is legitimate to combine them for risk assessment purposes. These findings are uncertain since they were not replicated in a second NTP study using similar dose levels. Nonetheless, a statistically significant increase in a rare tumor cannot be completely discounted.

Male CD-1 mice given fluoride in the diet showed a significant increase in osteomas (Maurer *et al.*, 1993). Osteomas are benign bone tumors, usually occurring on the surface of the bone (Nilsson and Stanton, 1994). Osteomas do not progress to osteosarcomas or other types of malignant tumors, although there are rare reports in humans of progression to malignant osteoblastomas (Pieterse *et al.*, 1983). Osteomas may occur spontaneously or they may be caused by retrovirus infection (Nilsson and Stanton, 1994). The type of retrovirus that has been shown to cause osteomas in mice is murine leukemia virus (Murray *et al.*, 1986). The C-type retrovirus particles identified by electron microscopy in the mice in the Maurer *et al.* 1993 studies are consistent with this type of virus.

Osteosarcoma is the most common primary bone cancer in humans, affecting approximately 1000 new patients each year in the United States (Withrow *et al.*, 1991). The mean age of new cases is 14. It occurs more frequently in males than in females by a ratio of 1.5:1. It occurs more often in heavier individuals. Ninety percent of the osteosarcomas in humans occur in the long bones. There is a peak in osteosarcoma occurrence in adolescence (age 10 to 20 years), and a second peak in individuals over 50 (Mirabello *et al.*, 2009). The etiology is generally unknown, but ionizing radiation has been demonstrated to induce osteosarcomas in humans and in dogs (Withrow *et al.*, 1991).

Osteosarcoma commonly arises in the metaphyses of long bones, especially near the major growth centers (epiphyseal plate) of the distal femur, proximal tibia, and proximal

humerus (Unni and Dahlin, 1989; Jones, 2011). Osteosarcoma in humans does not have any known benign precursor tumor type (Jones, 2011).

3.3.3 Hypothetical mechanisms

A number of hypothetical mechanisms by which fluoride could be carcinogenic may be postulated, including: genotoxicity, stimulation of cell division (mitogenesis), effects on thyroid function, and effects on immune function.

Genotoxicity and cell transformation

With regard to the possible genotoxic action of fluoride, the extensive body of data generated in a variety of *in vitro* and *in vivo* mutagenic and clastogenic test systems includes some positive and some negative results for most of the endpoints tested. In addition, fluoride caused the morphological transformation of Syrian hamster embryo cells in culture in experiments conducted in three separate laboratories. These data have been reviewed by the U.S. EPA (2007) and the NRC (1993; 2006) (see more detailed discussion of the NRC reviews below). The U.S. EPA stated that “positive mutagenicity results have been reported in mouse lymphoma assays, in chromosome aberration assays, in unscheduled DNA synthesis assays, and *in vitro* sister chromatid exchange assays.” The NRC (2006) report described the *in vitro* evidence for genotoxicity of fluoride as inconsistent and inconclusive, and the *in vivo* human studies as of questionable relevance to the “practical genotoxic potential in humans,” because these studies involved populations exposed to very large amounts of fluoride. It should be noted, however, that the human populations studied were not exposed to fluoride intentionally, but rather as a result of occupational or environmental conditions (e.g., naturally occurring levels in drinking water).

Massive genomic rearrangement is frequently observed in human osteosarcomas (Stephens *et al.*, 2011; Martin *et al.*, 2011). These rearrangements may be involved in the etiology of the disease, perhaps by changing the expression of genes involved in differentiation of osteoblasts (Martin *et al.*, 2011). The finding that fluoride has often been positive in assays for chromosomal aberrations and sister chromatid exchanges (NRC, 1993) is consistent with the hypothesis that fluoride may act on the genome to bring about DNA rearrangements. Such rearrangements could lead to changes of expression of genes such as those coding for RUNX2 proteins or the canonical Wnt pathway which appear to play key roles in osteosarcomagenesis (Martin *et al.*, 2011).

The NRC has twice reviewed the genotoxicity data for fluoride (1993, Chapter 6, pp. 91-108, Attachment 9; 2006, Chapter 10, pp. 304-316, pp. 334-339, Attachment 2). The first review (NRC, 1993) covered genotoxicity data that were available up to that time. It included data on *in vitro* mutagenicity tests (Attachment 9: Table 6-1), cytogenetic effects in cultured mammalian cells (Table 6-2), tests of DNA repair and unscheduled

DNA synthesis (Table 6-3), *in vitro* cell transformation assays⁶ (Table 6-4), *in vivo* mutation effects in *Drosophila* (Table 6-5), and *in vivo* chromosomal effects in rodents (Table 6-6). In all of these tables of data there are some positive and some negative results. In the narrative text and discussion sections that accompany these tables, the report frequently discounted the reported positive results based on technical considerations, such as doses too high to be relevant, lack of adequate controls, or inconsistency of results from different investigators at similar doses, and suggested that few if any of the positive results can be relied upon. With regard to the relevance of high doses, one should keep in mind that fluoride concentrates in the bone, and that it is the concentration of fluoride to which osteoblasts are exposed that would be relevant to a genotoxic mechanism of carcinogenesis (see Section 3.3.1, Pharmacokinetics). The high doses should not be used as a rationale for dismissing the positive genotoxicity findings.

Near the end of the genotoxicity chapter (NRC, 1993) there is a discussion of proposed mechanisms of genotoxicity. This discussion suggested that there may be some genotoxicity of fluoride, but it must be of an unusual kind, as “fluoride is not a typical mutagen.” For example, fluoride cannot intercalate between DNA bases or form DNA adducts. The report speculated that fluoride may be mutagenic by a mechanism involving inhibition of protein synthesis, possibly by inhibiting synthesis of DNA polymerases or other enzymes involved in DNA replication.

The 1993 NRC report concluded:

“The *in vitro* data indicate that (1) the genotoxicity of fluoride is limited primarily to doses much higher than those to which humans are exposed, (2) even at high doses, genotoxic effects are not always observed, and (3) the preponderance of the genotoxic effects that have been reported are of the types that probably are of no or negligible genetic significance.

In vivo tests in rodents for genotoxicity of fluoride provide mixed results that cannot be resolved readily because of differences in protocols and insufficient detail in some reports to allow a thorough analysis.” (NRC, 1993, Chapter 6, p.108, Attachment 9)

In the 2006 NRC report, studies produced since the 1993 NRC review are summarized, and the available genotoxicity data are evaluated in their entirety. Genotoxicity studies available since the 1993 review included:

⁶ Induction of morphological cell transformation may occur through either genotoxic or non-genotoxic mechanisms, or a combination of genotoxic and non-genotoxic mechanisms.

- *in vitro* cytogenetic, DNA damage, and cell transformation assays in mammalian cells
- *in vitro* cytogenetic and DNA damage studies in human cells
- *in vivo* cytogenetic and DNA damage studies in rodents
- *in vivo* cytogenetic and p53 mutation studies in humans.

These more recent studies reported a mix of positive and negative results, with the positive results occurring generally at higher doses. The 2006 NRC report summarized the *in vivo* rodent genotoxicity studies as indicating “a very low probability of a mutagenic risk to humans.” The *in vivo* human genotoxicity studies were summarized as follows:

“The positive *in vivo* genotoxicity studies described in the chapter were conducted in India and China, where fluoride concentrations in drinking water are often higher than those in the United States. Further, each had a dearth of information on the selection of subjects and was based on small numbers of participants. Therefore, *in vivo* human genotoxicity studies in U.S. populations or other populations with nutritional and socio-demographic variables similar to those in the United States should be conducted.” (NRC, 2006, pp. 338-339, Attachment 2)

The overall conclusions of the 2006 NRC report regarding the genotoxicity of fluoride, based on data from model systems (*in vivo* and *in vitro*) and on human occupational and ecological studies, is that the results are inconsistent and do not provide a basis for any firm conclusions about the potential of fluoride to be genotoxic in humans.

A few additional genotoxicity studies of fluoride have been published since the 2006 NRC review. Fluoride demonstrated positive genotoxic activity in *Drosophila* in the Somatic Mutation and Recombination Test (SMART) at doses that were toxic to the flies (Erciyas and Sarikaya, 2009). Fluoride increased the frequency of structural and numerical chromosomal aberrations, and was positive in the comet assay in human peripheral blood lymphocytes (Tiwari and Rao, 2010). Fluoride increased the frequency of sister chromatid exchanges and was positive in the comet assay in cultured human lymphocytes (Pant and Rao, 2010). Fluoride increased the frequency of chromosomal aberrations (mainly chromatid breaks) in mouse bone marrow cells (Podder *et al.*, 2010, 2011).

While the 1993 NRC report questioned the positive *in vitro* cell transformation results observed in multiple studies with Syrian hamster cells, the 2006 NRC report discounted them, saying that this assay is not a reliable predictor of effects in other animals or humans. However, no data were provided to support such a conclusion regarding the lack of utility of the Syrian hamster cell transformation assay. A recognized expert on the Syrian hamster embryo cell transformation assay confirmed in March 2011 (Dr. J. C.

Barrett personal communication), that the Syrian hamster cell transformation assay continues to be considered a valid test for use in carcinogen testing. A 1999 International Agency for Research on Cancer (IARC) review of cell transformation assays (LeBoeuf *et al.*, 1999, Attachment 10) concluded that “the results [of Syrian hamster embryo and BALB/c 3T3 cell transformation assays] can have high predictive value for the outcome of bioassays in rodents and ... for predicting human carcinogens.” The 1999 IARC Consensus Report from this review of the utility of short- and medium-term tests for carcinogens (IARC, 1999, pp. 1-18, Attachment 11) found that “the existing data strongly support the biological relevance of cell transformation to *in vivo* carcinogenesis and, in that respect, make it a logical approach for predicting the carcinogenic potential of chemicals and mechanisms of action.” The report goes on to say, “Of the transformation assays used at present, the largest databases exist for those involving BALB/c-3T3 and Syrian hamster embryo cells. Analyses of these databases indicate that these assays are highly sensitive and specific for detecting the carcinogenic activity of chemicals.” (IARC, 1999, p. 17, Attachment 11).

Stimulation of cell division (mitogenesis)

Fluoride directly stimulates proliferation of osteoblasts (Farley *et al.*, 1983). Based on fluoride’s stimulation of bone growth, it has been used to treat osteoporosis (Farley *et al.*, 1987). Stimulation of cell proliferation can lead to carcinogenesis by increasing the probability of mutations in genes related to cell cycle control, or by expanding clones of initiated cells.

Thyroid function effects

With regard to a possible mechanism involving effects on thyroid function, fluoride exposure has been reported to affect thyroid and parathyroid function in animals (rats, cattle and other livestock animals) and humans (NRC, 2006). Fluoride elevates thyroid stimulating hormone (TSH), alters concentrations of thyroid hormones T3 and T4, and increases parathyroid hormone (PTH) and calcitonin activity in humans and animals (NRC, 2006). Thyroid effects in humans and animals are complex, depending on such factors as the sufficiency of iodine in the diet. These effects on thyroid function in turn may affect rate of formation of bone tissue and overall growth rate. Osteosarcomas are known to be related to the rate of bone growth, as they occur more frequently during periods of rapid bone growth. These effects of fluoride on thyroid function also may be related to the observed dose-related increase in thyroid tumors observed in male rats in the NTP 1990 drinking water study.

Immune function effects

Fluoride has been shown to have a number of immunological effects, either stimulating or inhibiting cellular immune responses in humans, rats and mice, depending on the

conditions (NRC, 2006). Since fluoride is concentrated in the bone tissue, the bone marrow where immune cells develop may be exposed to higher concentrations of fluoride than other tissues. Fluoride stimulates hematopoietic activity along the granulocytic pathway and away from the monocytic pathway. This may affect cellular immunity, which is a main line of defense against foreign antigens, including antigens that identify cancer cells. Fluoride may also increase the risk of inflammatory response, which in turn may influence the risk of cancer. In humans, osteosarcomas are most common around the knee joint (distal end of the femur, proximal end of the tibia). Inflammation of the knee joints may play a role in osteosarcomagenesis, especially in heavy and/or fast growing humans.

See Attachments cited in this section:

2. NRC, 2006, Chapter 10, pp. 304-316, pp. 334-339
9. NRC, 1993, Chapter 6, pp. 91-108
10. LeBoeuf et al., 1999
11. IARC, 1999, pp. 1-18

4. REVIEWS BY OTHER AGENCIES

Fluoride has not been classified as to its potential carcinogenicity by the U.S. Food and Drug Administration, NTP, the National Institute for Occupational Safety and Health, or IARC.

Fluoride was reviewed by the U.S. EPA (2007) and classified in Group D (inadequate evidence of carcinogenicity). In explaining this classification, U.S. EPA cited the statement by the National Academy of Sciences (NRC, 2006) that “the evidence on the potential of fluoride to initiate or promote cancers, particularly of the bone, is tentative and mixed.”

The NRC (2006) reviewed the health effects of fluoride in drinking water, and concluded:

“On the basis of the committee’s collective consideration of data from humans, genotoxicity assays, and studies of mechanisms of action in cell systems (e.g., bone cells *in vitro*), the evidence on the potential of fluoride to initiate or promote cancers, particularly of the bone, is tentative and mixed.”

Fluoride was reviewed by the Agency for Toxic Substances and Disease Registry (ATSDR, 2003). ATSDR concluded that “the weight of evidence indicates that fluoridation of water does not increase the risk of developing cancer.”

5. SUMMARY AND CONCLUSIONS

5.1 Summary of Evidence

The evidence on the carcinogenicity of fluoride and its salts comes from:

- Numerous epidemiological studies (ecological, cohort, and case-control) in human populations exposed to fluoride, primarily via drinking water
 - Most studies are negative or inconclusive.
 - An ecological study by Cohn (1992) found an increased relative risk of osteosarcoma in young males (under age 20) living in fluoridated areas compared to areas without fluoridation. Limitations of this study include the ecological design, drinking water fluoridation status based on residence at the time of diagnosis, small numbers of osteosarcomas observed (twelve in the exposed and eight in the unexposed populations), and limited reporting.
 - A hospital-based case-control study of osteosarcoma in people under age 20 by Bassin *et al.* (2006) found an increased risk in young males exposed to fluoride in drinking water at levels above 1 ppm. However, uncertainties regarding these findings stem from a (still) preliminary report published as a Letter to the Editor, that a related series of osteosarcoma cases and controls did not appear to confirm the association between fluoride exposure and osteosarcoma risk.
- Nine rodent bioassays performed by two laboratories in the 1990s.
 - Positive findings were reported in a two-year drinking water study in male F344/N rats (NTP, 1990)
 - Statistically significant increases in rare osteosarcomas ($p < 0.05$ by Fisher pairwise comparison) [These results were not replicated in a subsequent two-year drinking water study (*i.e.*, NTP, 1992) in male F344/N rats receiving a somewhat higher dose of fluoride.]
 - Statistically significant dose-related increase in thyroid tumors (adenomas and carcinomas, combined) in male rats ($p < 0.05$, Cochran-Armitage trend test) [These results were not replicated in the 1992 NTP male rat drinking water study.]
 - Positive findings were reported in two-year diet studies in male CD-1 mice and female CD-1 mice, although these animals were infected with a retrovirus that may have caused the tumors observed (Maurer *et al.*, 1993)
 - Statistically significant increases in osteomas in males

- Statistically significant increases in osteomas in females
 - No treatment-related tumor findings were observed in two-year drinking water studies in
 - Male F344/N rats (NTP, 1992)
 - Female F344/N rats (NTP, 1990)
 - Male B6CF₁ mice (NTP, 1990),
 - Female B6C3F₁ mice (NTP, 1990)
 - No treatment related tumor findings were observed in two-year diet studies in
 - Male Sprague-Dawley rats (Maurer *et al.*, 1990)
 - Female Sprague-Dawley rats (Maurer *et al.*, 1990)
- Mechanistic evidence and other relevant data considerations.
 - Pharmacokinetic studies indicate that fluoride is taken up and incorporated mainly into bones and teeth.
 - Rodents must be exposed to much higher levels of fluoride in diet or water than humans, in order to achieve the same bone fluoride levels.
 - Fluoride stimulates cell division in osteoblasts *in vivo* and *in vitro*.
 - Mitogenicity (stimulation of cell division) may lead to carcinogenesis by facilitation of mutational or epigenetic changes or by expansion of initiated clones.
 - An increase in the rate of cell division may itself be an indication of a cellular change that is consistent with progression toward a neoplastic state.
 - Fluoride has been tested in a variety of *in vitro* and *in vivo* test systems assessing mutagenicity and clastogenicity, generating a mix of positive and negative results (NRC, 1993, Attachment 9; NRC, 2006, Attachment 2; additional studies reviewed in Section 3.3.3).
 - *In vitro* mutagenicity studies in bacteria and animal cells yielded some positive and some negative results. Generally, positive findings were associated with studies testing higher concentrations of fluoride.
 - *In vitro* clastogenicity studies in animal and human cells yielded some positive and some negative results.
 - *In vivo* mutagenicity and clastogenicity studies in humans and animals yielded some positive and some negative results.
 - Positive findings in humans were seen in some studies of occupationally exposed workers and in some populations with elevated levels of fluoride in drinking water.

- Positive findings in animals and humans of cytogenetic and DNA damage suggest the possibility that fluoride is capable of producing massive genomic rearrangement.
 - Some human osteosarcomas exhibit massive genomic rearrangements.
- Fluoride induced malignant transformation of mammalian cells in culture.
 - Fluoride induced malignant transformation in the Syrian hamster embryo cell transformation assay in tests conducted in three different laboratories.
 - Fluoride induced malignant transformation in the BALB/c 3T3 (mouse) promotion assay, but not in the BALB/c 3T3 cell standard focus assay.
- Fluoride affects thyroid and parathyroid function in humans and animals.
 - Fluoride elevates TSH, alters T3 and T4 levels, and increases PTH and calcitonin activity.
 - These changes can affect the rate of formation of bone tissue and the overall rate of bone growth.
 - An increased rate of bone growth could increase the risk of osteosarcoma.
 - Osteosarcomas commonly arise in the metaphyses of long bones, near the joints, and occur more frequently during periods of rapid bone growth.
- Fluoride affects cellular immune response in humans and animals.
 - Fluoride has been shown to either stimulate or inhibit cellular immune responses in humans, rats, and mice.
 - Decreases in cellular immune response may lead to a reduction in the ability of the immune system to identify and remove cancerous cells (*i.e.*, immune surveillance).
 - Increases in cellular immune response may lead to inflammation, which is known to play a role in carcinogenesis.
 - Osteosarcomas are often found near the joints of long bones, where inflammation is also common.

Taken together, these multiple lines of evidence from mechanistic and other relevant data appear to support several plausible hypotheses: that fluoride is incorporated into bones (especially rapidly growing bones), where it can i) stimulate cell division of osteoblasts via direct mitogenicity and indirectly via effects on thyroid function and parathyroid function; ii) induce genetic changes; iii) induce other cellular changes leading to malignant transformation, and iv) alter cellular immune response, resulting in

increased inflammation and/or reduced immune surveillance, thereby increasing the risk of development of osteosarcomas.

5.2 Conclusions

In summary, the evidence for carcinogenicity of fluoride and its salts consists of:

- Some positive findings in epidemiology studies, including reported increases in osteosarcomas in young males in an ecological study and in a hospital-based case-control study. However, the contribution of chance, bias, inappropriate analyses or confounding to these findings could not be ruled out. Overall, the current body of epidemiologic evidence on the carcinogenicity of fluoride is considered inconclusive.
- Some positive findings in animal carcinogenicity studies.
 - Increased incidences of thyroid tumors and rare osteosarcomas in a two-year drinking water study in male F344/N rats, which were not replicated in a follow-up drinking water study.
 - Increased incidences of benign osteomas in two-year diet studies in male and female CD-1 mice. The possible contribution of retroviral infection reported in the male and female mice to the development of osteomas could not be ruled out.
- Mechanistic and other relevant data considerations.
 - Fluoride is taken up and incorporated into bones.
 - Fluoride stimulates cell division in osteoblasts *in vivo* and *in vitro*.
 - Some positive findings of genotoxicity *in vitro* and *in vivo*, including studies in exposed humans.
 - *In vitro* mutagenicity studies in bacteria and animal cells yielded some positive and some negative results.
 - *In vitro* clastogenicity studies in animal and human cells yielded some positive and some negative results.
 - *In vivo* mutagenicity and clastogenicity studies in humans and animals yielded some positive and some negative results.
 - Fluoride induced malignant transformation of mammalian cells in culture.
 - Fluoride affects thyroid and parathyroid function in humans and animals. Such functional changes may increase rates of bone growth.
 - Fluoride affects cellular immune response in humans and animals. Decreases in cellular immune response may reduce the effectiveness of immune surveillance; increases in cellular immune response may lead to inflammation.

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ATTACHMENTS

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5. NTP 1990, Table 21 (p. 65)
6. Maurer *et al.*, 1990
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