Evidence on the Carcinogenicity of Fluoride and its Salts

David W. Morry, Ph.D.

Craig Steinmaus, M.D., M.P.H

Office of Environmental Health Hazard Assessment

Carcinogen Identification Committee

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Identity of Fluoride and Its Salts

- Fluoride is monovalent anion derived from the element fluorine.
- Fluorine is the most electronegative of the halogens.
- Fluoride can form salts with positive ions such as Na^{+}, Sn^{++}.
- Fluoride salts are highly soluble in water; most dissociate completely, releasing fluoride ion.
- Fluoride is also released from some fluoride-containing compounds.

Occurrence of Fluoride

- Fluoride often occurs naturally in drinking water sources.
- Fluoride naturally occurs in some foods and beverages.
- Fluoride is obtained from a number of naturally occurring minerals – calcium fluoride, fluoroapatite, cryolite.

Fluoride Exposure

- Human exposure comes from a variety of sources.
- Drinking water fluoridation results in widespread exposures.
- Fluoride is also added to dental products.
- Fluoride occurs in some foods and beverages.
- Exposures vary geographically.

Carcinogenicity Studies in Humans

- **Epidemiological studies were reviewed by the NRC in 2006.**
- **NRC judged the studies to be inconclusive.**
- **We will discuss**
  - 2 studies that found increased osteosarcoma risk in young males
  - New studies available since NRC review

Cohn (1992)

- **Ecological study compared areas with and without fluoridation in New Jersey.**
- **Found increased risk (OR=3.4, 95% CI=1.8-6.0) for young (<20 yrs) males living in fluoridated areas.**
- **Limitations of study:**
  - Ecological design
  - Fluoridation status based on residence at time of diagnosis
  - Small numbers of osteosarcomas observed (12 in exposed areas, 8 in unexposed areas)
  - Not published in peer-reviewed journal

Bassin *et al*. (2006)

- US hospital-based case-control study of osteosarcoma in young people < age 20.
- 103 cases and 215 hospital-based controls (other orthopedic patients)
- Highest OR was in males at age 7 with fluoride exposure above recommended level, OR=5.46 (95% CI, 1.5-19.9).
- Unknown if assessment of exposure was blinded.
- Details of statistical analysis not provided.
- Conclusion: Provides some evidence of an association in young males for exposures around age 7

Kim *et al.* (2011)

- **US case-control study of bone fluoride & osteosarcoma.**
  - Cases: 137 incident primary osteosarcoma, all ages
  - Controls: 51 subjects with other malignant bone tumors
- **Exposure: fluoride levels in tumor-adjacent bone**
- **Found no association (Overall OR = 1.23, p = 0.65)**
- **Limitations of study:**
  - Small sample size
  - Validity of bone-sampling approach uncertain
  - Age difference between cases and controls.
  - Poor participation rates among controls
- **Not conclusive.**

Sandhu *et al*. (2009)

- Case-control study in India on osteosarcoma and fluoride levels in serum
- Measured fluoride in serum at time of treatment in 25 osteosarcoma cases and 50 controls (25 "other bone forming tumors" and 25 musculoskeletal pain patients)
- Found higher levels of fluoride in patients with osteosarcoma than controls (0.14 mg/L *cases* vs. 0.072 *control II* vs. 0.042 *control I*, $p < 0.001$)
- Cross-sectional exposure assessment: so which came first, osteosarcoma or high serum fluoride?

Comber *et al*. (2011)

- Ecological study of osteosarcoma and fluoridation in Ireland, 1994-2006 (183 cases)
- Exposure based on whether a person lived in Northern Ireland (unexposed) or Republic of Ireland (where exposure was based on population density)
- Overall, no difference in osteosarcoma rates between fluoridated and non-fluoridated areas (RR = 1.07, 95% CI = 0.75-1.54).
- Very broad exposure categorization, no data on past exposure

Summary of Epidemiological Studies

- **NRC (2006) said, "The combined literature…does not clearly indicate that fluoride either is or is not carcinogenic to humans."**
- **Studies published since then include Bassin *et al.* (2006), Sandhu *et al*. (2009), Kim *et al*. (2011) and Comber *et al.* (2011).**
- **Taking these more recent studies into account, the current body of epidemiologic research remains inconclusive.**

Nine Rodent Bioassays

- **NTP (1990) drinking water studies**
  - Male F344/N rats
  - Female F344/N rats
  - Male B6C3F_1 mice
  - Female B6C3F_1 mice
- **NTP (1992) drinking water study (higher dose)**
  - Male F344/N rats
- **Maurer *et al.* (1990) diet studies**
  - Male S-D rats
  - Female S-D rats
- **Maurer *et al*. (1993) diet studies**
  - Male CD-1 mice
  - Female CD-1 mice

NTP Bioassays in Male Rats

- **NTP (1990) drinking water bioassay (175 ppm)**
  - Significant increase in rare osteosarcomas ($p<0.05$) in male F344/N rats
  - Osteosarcomas are rare malignant bone tumors
  - NTP judged osteosarcoma data as “equivocal evidence” of carcinogenic activity.
  - Significant increase in thyroid adenomas and carcinomas in male F344/N rats ($p<0.05$)

- **NTP (1992) drinking water bioassay (250 ppm)**
  - No increase in osteosarcomas or other malignant tumors

Tumors in Male Rats
NTP (1990)

	Control	25 ppm	100 ppm	175 ppm	Trend
Osteo-sarcomas	0/80	0/51	1/50	4/80 (3 skeletal) p=0.057	p<0.05
Thyroid follicular adenomas and carcinomas	1/80	1/51	1/50	4/80 p=0.171	p<0.05

NTP Bioassays – Negative Findings

- **No significant increases in tumors in:**
  - NTP (1990)
    - Female F344/N rats
    - Male CD-1 mice
    - Female CD-1 mice
  - NTP (1992)
    - Male F344/N rats

Maurer *et al*. (1993) Bioassays in CD-1 Mice

- 97-week diet studies in male and female mice (25 mg/kg/day).
- Significant increase in osteomas in males.
- Significant increase in osteomas in females.
- Osteomas are benign bone tumors, not related to malignant osteosarcomas*.
- All osteomas (control and treated) showed retrovirus (by EM) – may have caused tumors.

***There have been reports of human osteomas progressing to malignant osteoblastomas.**

Maurer *et al.* (1990) Bioassays in S-D Rats

- **No significant increase in malignant tumors in:**
  - 99-week diet studies (25 mg/kg/day)
    - Male S-D rats
    - Female S-D rats

Mechanistic and Other Evidence

- **Pharmacokinetic studies show fluoride is taken up and incorporated into bones and teeth.**
  - Rodents must be exposed to much higher levels of fluoride to achieve same bone levels as humans.
- **Fluoride stimulates cell division in osteoblasts *in vivo* and *in vitro*.**
  - Could be an indication of early transformation.
  - Could facilitate progression.

In Vitro Genotoxicity Data

- **Positive**
  - L5178Y Mouse Lymphoma assay (+/- S9)
  - Sister chromatid exchange in Chinese hamster ovary (CHO) cells (+/-S9)
  - Chromosome aberrations (CA) in CHO cells (-S9)
  - UDS in human oral keratinocytes
- **Negative**
  - *S. typhimurium* (gene mutation) (+/- S9)
  - Chromosome aberrations in CHO cells (+S9)

In Vivo Genotoxicity Data

- **Positive**
  - Some studies of humans in India and China showed increased chromosomal effects.
  - Some studies of chromosomal effects in rats and mice were positive.
- **Negative**
  - Some studies of humans in India and China showed no chromosomal effects.
  - Studies of chromosomal effects in rats and mice were generally negative.

Recent Genotoxicity Studies

- **Positive**
  - *Drosophila* somatic mutation and recombination test
  - *In vitro* SCE and comet assay in cultured human lymphocytes
  - *In vitro* CA and comet assay in human peripheral blood lymphocytes
  - *In vivo* CA in mouse bone marrow

In Vitro Cell Transformation Assays

- **Syrian hamster embryo transformation assay:**
  - Positive in three laboratories
- **BALB/c 3T3 mouse cell focus assay**
  - Standard focus assay: negative
  - Promotion assay: positive

Thyroid and Parathyroid (PTH) Function

- **Fluoride elevates TSH, PTH and calcitonin levels, and alters T3 and T4.**
  - These changes can affect the rate of growth of bone tissue.
  - Increased rate of bone growth could increase the risk of osteosarcoma.
  - Osteosarcomas arise in the metaphyses of long bones, near the joints, and occur more frequently during periods of rapid bone growth.

Effects on Immune Response

- Fluoride can either stimulate or inhibit cellular immune response in humans, rats and mice.
- Decreases in cellular immune response may reduce immune surveillance of nascent cancer cells.
- Increases in cellular immune response may lead to inflammation, known to be involved in carcinogenesis.
- Osteosarcomas are often found near the joints of long bones, where inflammation is also common.

Summary of Evidence

- **Human evidence**
  - Mostly negative findings (many studies).
  - Some findings of increased osteosarcoma.
  - Overall the evidence is inconclusive.

Summary of Animal Evidence

- Increased osteosarcomas in male rats in one study; trend for thyroid tumors
- No tumor findings in follow-up study of male rats exposed to a higher dose
- Increases in benign osteomas in male and female mice; possible confounding by retroviral infection
- No tumor findings in female F344/N rats, male and female S-D rats or male and female $B6C3F_1$ mice

Summary of Mechanistic Evidence

- **Some findings of genotoxicity, including**
  - In exposed humans
  - Findings of rearrangement of genetic material
- **Stimulation of bone growth**
- **Effects on immune system**
- **Effects on thyroid and parathyroid function**