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
Sacramento, October 2011
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 B6C3F₁ mice
  - Female B6C3F₁ 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)**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>25 ppm</th>
<th>100 ppm</th>
<th>175 ppm</th>
<th>Trend</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Osteosarcomas</strong></td>
<td>0/80</td>
<td>0/51</td>
<td>1/50</td>
<td>4/80</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(3 skeletal)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>p=0.057</td>
</tr>
<tr>
<td><strong>Thyroid follicular adenomas and carcinomas</strong></td>
<td>1/80</td>
<td>1/51</td>
<td>1/50</td>
<td>4/80</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>p=0.171</td>
</tr>
</tbody>
</table>

**Office of Environmental Health Hazard Assessment**
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 \textit{in vivo} and \textit{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 B6C3F₁ 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