

May 5, 2009

## Research on PFOA and fluoride carcinogenicity supports their high priority review for Proposition 65 listing

Re: Prioritization of Chemicals for Carcinogen Identification Committee Review

Environmental Working Group (EWG) is a non-profit public health and environmental research and advocacy organization with offices in Oakland, CA, Ames, IA, and Washington, DC. We focus much of our research on potential health risks from chemical contamination of food, water, consumer products, and the environment. With this letter, we urge the Carcinogen Identification Committee (CIC) to assign high priority ranking to 2 chemicals currently under its review, perfluorooctanoic acid (PFOA) and fluoride (OEHHA 2009a, b). This ranking will serve as the first, essential step for preparation of hazard identification materials and subsequent designation of PFOA and fluoride as listed carcinogens under California's Safe Drinking Water and Toxic Enforcement Act of 1986 (Proposition 65).

The health of millions of Californians may be at risk due to widespread and nearly unavoidable exposure to these 2 carcinogenic chemicals. Human and animal data provide ample evidence of their hazard, which is further supported by the growing scientific body of research on the mechanisms of toxicity and the cancer-promoting activity for each chemical. In this letter we provide to CIC a detailed analysis of the extensive corpus of science that signals the need for expedited Proposition 65 review of PFOA, its salts, and its transformation and degradation precursors, as well as fluoride and its salts used in water fluoridation.

**PFOA.** PFOA has been used for decades as a manufacturing aid for producing a wide range of everyday products, including non-stick cookware and stain-proof coatings on furniture, clothing, carpets and packaging. Nearly every American is exposed to PFOA due to contaminated consumer products, packaged food, or drinking water. Over a lifetime of incremental exposures, PFOA builds up in human body, leading to an increased risk of cancer in multiple organs and tissues. Strong evidence for PFOA carcinogenicity is provided by occupational studies where two long-running cohort mortality studies of workers in DuPont and 3M chemical plants detected a significant increase in prostate, bladder, and other types of cancers. Similarly, 2 PFOA feeding studies in rats found an increased incidence of liver, pancreatic, testicular, and mammary cancers. In 2006, the Science Advisory Board (SAB) of the Environmental Protection Agency (EPA) declared PFOA to be a likely human carcinogen. Overall, the weight of the evidence strongly supports PFOA priority review towards Proposition 65 listing which will serve to protect the health of all Californians from this toxic, carcinogenic chemical.

**Fluoride.** High quality, targeted epidemiological studies indicate that exposure to fluoride in tap water during the mid-childhood growth spurt between ages 5 and 10 increases the incidence of osteosarcoma in boys ages 10 through 19. The results of 3 focused epidemiological studies each show a strong association between fluoride in tap water and osteosarcoma in boys. Epidemiological studies that have failed to find this association do not examine the relationship between age of exposure to fluoride and the incidence of bone cancer in young males. The link between fluoride in tap water and bone cancer in boys is supported by significant biological evidence. Half of ingested fluoride is deposited in bones, where it acts as a mitogen that stimulates cellular proliferation in the growing

ends of the bones where osteosarcoma occurs. Fluoride's capacity to induce DNA damage, including sister chromatid exchange, suggests that it can cause genotoxic effects in bone cells where it is actively deposited. Animal studies add further credence to the potential link between fluoride and bone cancer in males; 2 animal cancer bioassays conducted with fluoride both show rare bone tumors, many of which were malignant, in male test animals. Finally, a study published just last month provides a missing link between fluoride and osteosarcoma, revealing higher levels of fluoride in serum from osteosarcoma patients relative to those suffering from other types of bone cancer, as well as controls.

## **PFOA, its salts, and its transformation and degradation precursors**

PFOA and related perfluorochemicals (PFCs) have been used for the past 5 decades in industrial and consumer product applications, including food packaging, non-stick cookware, stain-proof coatings on furniture, clothing, and carpets, and manufacture of various fluoropolymers. PFOA- and PFC-based products can be found in nearly every home, and PFOA pollution of surface and ground water has been detected in at least nine states. Nearly every American is exposed to PFOA and other PFCs from contact with PFOA-containing consumer products, or contaminated food and tap water. A nationwide biomonitoring study conducted by the Centers for Disease Control and Prevention (CDC) found that PFOA contaminates the bodies of 99.7% of the U.S. population (Calafat 2007). Most worrisome, the developing fetus is exposed to PFOA from the moment of conception, since PFOA can cross the placenta and is found in umbilical cord blood and amniotic fluid (Apelberg 2007; EWG 2005; Midasch 2007; Reiner 2009).

Widespread contamination of the population with PFOA raises great concern since extensive evidence indicates that PFOA is a carcinogen (Lau 2007; US EPA 2005). The scientific record on PFOA carcinogenicity includes dozens of studies that encompass epidemiological data, long-term animal studies, and mechanistic data on genotoxicity, hormonal effects, immune system effects, and cellular membrane stability, all of which may potentially contribute to PFOA's cancer-causing properties. In 2006, the Science Advisory Board (SAB) of the Environmental Protection Agency (EPA) declared PFOA "likely to be carcinogenic" to humans (SAB 2006). A number of additional studies supporting PFOA carcinogenicity have appeared in the 3 years since the SAB made that determination, setting the stage for strong government action on this toxic chemical.

### **Summary of the science**

As a result of PFOA contamination of consumer products and the environment, nearly all Americans have been polluted with this persistent chemical toxicant that resists degradation, accumulates in living tissues, and remains in the human body for many years (Calafat 2007; Emmett 2006; Lau 2007; Olsen 2007). This widespread contamination poses a significant human health concern since PFOA is a potent, multisite carcinogen. Researchers report elevated rates of cancer incidence and mortality for workers exposed to PFOA in every published record on the subject, including up to a 7-fold higher risk of dying from prostate cancer for workers with highest PFOA exposure (Alexander 2001; Leonard 2007; Lundin 2007). Two long-term animal studies detected an increased incidence of liver, pancreatic, and testicular cancers in PFOA-fed male rats and, additionally, increased incidence of mammary tumors in PFOA-fed female rats. Furthermore, PFOA affects multiple biological processes in the body and disrupts the endocrine and immune systems, whose integrity is essential to the body's ability to fight cancer successfully. Overall, the weight of the evidence strongly supports PFOA carcinogenicity to humans, warranting its priority review towards Proposition 65 listing.

## **Worker studies show increased rates of developing and dying of certain cancers**

Several occupational cohort mortality studies at 3M's and DuPont's chemical plants found an increased risk of developing prostate cancer (Gilliland 1993; Lundin 2007) as well as cancers of the kidney, bladder, rectum, liver, central nervous system, and lymphatic and hematopoietic tissues, all linked to PFOA exposure (Leonard 2007). At these facilities, PFOA was either directly produced or used for manufacture of fluoropolymer-based products, and PFOA exposure in both workplaces has been consistently linked to cancer mortality in workers.

In a cohort mortality study of workers employed at the 3M PFOA production facility in Cottage Grove, Minnesota, employees who had been exposed to PFOA had an elevated risk of dying from prostate cancer (Lundin 2007). The study population included all employees of the Cottage Grove facility with a minimum of 1 year of cumulative employment, for a total of 3,993 workers. The cohort was followed between 1943 and 2002. Initial results from this cohort study were reported in 1993, observing that 10 years of employment in PFOA-related jobs was associated with a 3.3-fold increase in prostate cancer mortality, as compared to no employment in PFOA production (Gilliland 1993). The data have been confirmed in subsequent epidemiological analysis. The latest follow-up from the same cohort found that workers who had worked a job with probable exposure to PFOA, or worked a job with definite exposure for less than 6 months, were 3 times as likely to die from prostate cancer as workers without this job history (95% CI = 0.8-11.0). For employees who worked a job with definite PFOA exposure for 6 months or longer, the risk of dying from prostate cancer was 7 times greater than for unexposed workers (95% CI = 1.2-42.2) (Lundin 2007).

In a retrospective cohort mortality study of 6,027 employees at the Washington Works fluoropolymer production plant at Parkersburg, West Virginia (Leonard 2007), elevated mortality rates were observed from cancers of the kidney, bladder, liver, rectum, central nervous system, and lymphatic and hematopoietic tissues. The Parkersburg cohort was defined as all individuals who had worked at the Washington Works plant between 1948 and 2002. Mortality rates in these workers were compared to the DuPont regional worker reference file that included West Virginia (excluding Washington Works) and seven neighboring states, in order to decrease the influence of the healthy worker effect that frequently gives an appearance of minimal risk in occupational studies. Increased incidence of multiple, different cancers in this worker cohort is in agreement with animal carcinogenicity studies where PFOA exposure induces cancers in multiple organs.

### **Evidence of hazard: animal carcinogenicity data**

**Liver, pancreas, and testicular cancer.** Animal data strongly support the findings of cancer in chemical plant workers. In particular, 2 long-term diet studies in rats consistently found PFOA carcinogenicity to multiple organs and tissues, including:

- 2-year study in male and female Sprague-Dawley rats observed a significantly increased incidence of testicular (Leydig) cell tumors, development of hyperplastic liver nodules and increased incidence of hepatocellular carcinoma and pancreatic acinar cell tumors in PFOA-fed animals (Sibinski 1987). Female rats exposed to PFOA also had a twice higher incidence of mammary fibroadenomas compared to control animals and a statistically significant, dose-related increase in the incidence of ovarian tubular hyperplasia, a precursor to ovarian cancer (Sibinski 1987; U.S. EPA 2005a);
- 2-year study in male Sprague-Dawley rats found an increased incidence of liver adenomas, testicular (Leydig) cell tumors, and pancreatic acinar cell tumors in PFOA-fed animals compared to controls (Biegel 2001; Cook 1992).

**Mammary tumors.** Of special concern is the observed association of PFOA exposure with mammary fibroadenomas in female rats. In a 2-year bioassay sponsored by 3M and carried out by the Riker Laboratory, the incidence of mammary fibroadenomas in treated rats was significantly higher than that in study controls ( $p < 0.05$ , 42% in the low dose group versus 21% in the controls) (Sibinski 1987). In a 2002 review of this study, EPA determined that these findings were significant and relevant to humans, declaring the increased incidences of mammary fibroadenoma to be statistically significant ( $p < 0.05$ ) as compared to the concurrent controls (US EPA 2002).

EPA also judged the increases to be statistically significant as compared to the historical control indices for mammary fibroadenoma of 19% (US EPA 2002). EPA reversed this position in a subsequent review, but their Science Advisory Board did not agree with this reversal, finding it to be based on faulty assumptions. In justifying their reversal, EPA cited an elevated (37%) historical incidence rate of mammary fibroadenomas observed in female rats in the DuPont Haskell laboratory (US EPA 2005). As noted by the SAB, however, this comparison is not sound, since the historical reference data used in the 2005 EPA review were collected at the DuPont Haskell Laboratory in 1987, while the Sibinski study was carried out at the Riker Laboratory during 1981-1983. Since the DuPont data were gathered more than 3 years after the 3M study, their use for comparison with this study is inappropriate according to EPA's own guidelines on the appropriate use of data on relevant historical controls (U.S. EPA 2005b). The EPA's Science Advisory Board did not find merit in this reversal of position, and considered the exclusion of mammary tumors inappropriate "since the most appropriate control group is a concurrent control group" (SAB 2006). Thus, as indicated by the current body of data, the evidence for PFOA carcinogenicity to mammary tissue stands, and remains a concern.

The link between PFOA exposure and breast cancer is further supported by the fact that PFOA is an endocrine disruptor (as discussed in the following section) and a known mammary toxicant with transgenerational effects (Lau 2007). In animal studies, gestational PFOA exposure is associated with altered mammary gland development in dams and female offspring (White 2007; Yang 2009). Female mice orally dosed with PFOA during pregnancy had a significant reduction in mammary differentiation, with delays in normal epithelial involution and alterations in milk protein gene expression. Female pups exposed to PFOA in utero displayed stunted mammary epithelial branching and growth (White 2007). These findings are of great concern for human health, since toxic chemical exposures during early periods of development are particularly critical to later risk of developing breast cancer (Coyle 2004; Gray 2009; Hilakivi-Clarke 2006).

On the weight of the liver, pancreas, mammary, and testicular cancer evidence in both male and female animals, the Science Advisory Board to EPA declared PFOA to be a multi-site carcinogen and deemed these rat studies of PFOA carcinogenicity to be relevant to humans (SAB 2006).

**Tumor promotion.** The final piece of evidence for PFOA carcinogenicity in vivo comes from 3 independent tumor promotion studies, 2 of them carried out in rats (Abdellatif 1991; Nilsson 1991), and 1 in rainbow trout (Tilton 2008). In each study, PFOA was shown to promote liver carcinogenesis associated with toxic chemical exposures (Abdellatif 1991). There are likely to be different mechanisms of tumor promotion in different species, yet the outcome of increased tumor incidence is consistently observed. Interestingly, PFOA-promoted tumor development in rainbow trout may involve estrogenic signaling (Tilton 2008), while in rodents, tumor development may be driven by the peroxisomal proliferator effects of PFOA (Nilsson 1991). It is unknown what primary cancer-related process is triggered or disrupted by PFOA in humans, although disruption of membrane stability and intracellular junctions has been proposed as a possible mechanism of action (Trosko 2007).

## Mechanism of PFOA carcinogenicity

PFOA simultaneously targets multiple biological pathways and systems, including the immune system, endocrine system, lipid balance, membrane stability, and cellular metabolism and oxidative status. Disruption of any of these pathways by itself can be sufficient for cancer promotion, and when multiple systems are affected jointly, risk of cancer and other chronic diseases may prove even greater.

The human health consequences of PFOA exposure can be seen in the most extensive study of PFOA's impact on people, the 69,000-person C8 Health Project, carried out by an independent panel of academic scientists and paid for by DuPont (C8 Science Panel 2009b; West Virginia University School of Medicine 2008). This ongoing study encompasses residents of West Virginia and Ohio counties in the vicinity of the DuPont Washington Works fluorochemical production facility, whose drinking water has been contaminated with PFOA emitted from the DuPont plant. The C8 Health Project has already revealed increased risk for damage to the immune and endocrine systems related to PFOA exposures in this large study group.

**Immune system damage.** Healthy, intact and alert immune response is essential for the body's ability to fight cancer, while weakened or suppressed immune function, viral infections, and autoimmune inflammation are frequently associated with accelerated tumor development (Haynes 2008; Schuster 2006; Tesniere 2008). Weakening the immune system may be the one of the mechanisms of PFOA carcinogenicity in people. Higher PFOA levels in the C8 study participants were correlated with lower levels of serum immunoglobulins IgG, IgA and IgE, key proteins that help the body fight pathogenic microorganisms and suppress tumor development (Frisbee 2008). Similarly, EPA researchers reported PFOA-exposed mice had low immunoglobulin levels (DeWitt 2008). The C8 study also found higher PFOA levels associated with decreased serum concentration of C-reactive protein, a marker of the body's innate immune response. Finally, higher PFOA correlated with elevated levels of total antinuclear antibodies, which indicates an increase in the risk of autoimmune diseases (C8 Science Panel 2009a). Together, these findings of suppressed and disrupted immune systems in PFOA-exposed people agree with animal studies where PFOA has been linked with death of immune cells and weakening of the body's ability to protect itself (DeWitt 2008; Dewitt 2009; Son 2008; Yang, Abedi-Valugerdi 2002; Yang, Xie 2002; Yang, Xie 2000; Yang 2001). Thus, the adverse effect of PFOA exposure on the immune system may contribute to tumor development and progression, especially in vulnerable populations such as children and the elderly.

**Endocrine disruption.** Endocrine disruption is likely another pathway of PFOA carcinogenicity. Estrogenic properties of PFOA and related chemicals have been established in various types of experimental assays. Studies demonstrate elevated estradiol and estrogen-related increases in testicular cancer incidence in male rats following dietary exposure to PFOA (Cook 1992; Liu 1996); confirmed estrogenic activity from in vitro studies using human estrogen receptors in yeast cells and human breast cancer cells (Ishibashi 2007; Maras 2006); as well as estrogenic effects in both in vitro and in vivo studies with fish (Liu 2007; Tilton 2008). An increased level of serum estradiol was observed in 3M workers occupationally exposed to PFOA (Olsen 1998). PFOA has been also linked to disruption of the hypothalamic-pituitary-gonad axis in exposed animals (Cook 1992), as well as altered levels of thyroid hormones in PFOA-exposed participants of the C8 Health Project (Frisbee 2008). Endocrine disruption has been strongly associated with increased cancer risk, especially for breast and prostate cancer (Prins 2008; Soto 2008). PFOA association with these cancers may be linked to its disregulating effect on normal endocrine function.

**PFOA-related cellular damage.** Finally, multiple studies have examined PFOA's impact on essential functions of a living cell. PFOA is not directly genotoxic and does not induce mutations. However, in vitro treatment of human cells with PFOA leads to intracellular generation of reactive oxygen species (Panaretakis 2001), which produce oxidative DNA damage and DNA strand breaks in a dose-dependent manner (Yao 2005). In gene array studies, PFOA exposure of developing mouse fetuses altered expression of multiple genes, including genes associated with lipid transport, ketogenesis, glucose metabolism, lipoprotein metabolism, cholesterol biosynthesis, steroid metabolism, bile acid biosynthesis, phospholipid metabolism, retinol metabolism, proteasome activation, and inflammation (Rosen 2007).

The key effect of PFOA on cellular metabolism is decreased membrane stability and induction of membrane permeability (Liu 2008; Matyszewska 2007; Trosko 2007). In cellular in vitro experiments, PFOA induced mitochondrial dysfunction and leaking of the inner mitochondrial membrane (Starkov 2002); PFOA also inhibits gap junctional intercellular communication, an adverse cellular effect that has been linked to the tumor-promoting properties of many carcinogens (Upham 1998).

### **Recommendations**

Widespread exposure of Californians to PFOA and related perfluorochemicals raises serious health concerns. Expedited review of PFOA, its salts, and its transformation and degradation products for listing as carcinogens under Proposition 65 is clearly warranted by the data. Furthermore, the fact that PFOA does not degrade in the environment makes such a review an even higher priority. Because every molecule of this chemical will be circulating in the environment essentially in perpetuity, its carcinogenicity is of particular concern.

## **Fluoride and its salts used for tap water fluoridation**

EWG urges CIC to nominate fluoride for high priority review as a potential carcinogen, based on its ability to cause osteosarcoma in males less than 20 years of age through exposures to fluoridated tap water. The science supporting the link between fluoride and bone cancer in boys is compelling, and includes 3 focused epidemiological studies, 2 long-term animal studies, a wealth of mechanistic information on the effect of fluoride on the developing bone, and a new study published this year that detected higher fluoride levels in osteosarcoma patients compared to patients with bone-forming tumors other than osteosarcoma and controls reporting musculo-skeletal pain but having no tumors.

The question of fluoride's carcinogenicity is an extremely high priority public health concern, given the widespread exposure to fluoride in tap water. While the value to dentistry of topical application of fluoride to teeth is clearly established, a substantial and growing body of peer reviewed science strongly suggests that adding fluoride to tap water may not be an effective way to achieve the dental health benefits of fluoridation, and may have potentially serious side effects in at least some of those exposed. Based on a number of serious health concerns with fluoride, in 2006 the National Research Council (NRC) unanimously concluded that EPA's current maximum contaminant level goal (MCLG) of 4 parts per million (ppm) is not health-protective and should be lowered. The widespread exposure to fluoride in California's tap water causes millions of boys to be exposed during critical periods of development and growth, when their bones are especially vulnerable to fluoride-induced osteosarcoma. Expedited review of fluoride carcinogenicity by CIC may be essential for protecting the health of boys across the entire state of California.

## Summary of the science

The overall weight of the evidence strongly supports the conclusion that exposure to fluoride in tap water during the mid-childhood growth spurt between ages 5 and 10 increases the incidence of osteosarcoma in boys ages 10 through 19. The results of 3 focused epidemiological studies each show a strong association between fluoride in tap water and osteosarcoma in boys (Bassin 2006; Cohn 1992; DHHS 1991). While several epidemiological studies have failed to find an association between fluoride and cancer, these studies typically did not look for a relationship between age of exposure to fluoride and the incidence of bone cancer in young males. Negative findings from studies probing a variety of different questions must not distract from positive findings with respect to fluoride's link to bone cancer in young boys.

Biologically, the link between fluoride in tap water and bone cancer in boys is highly plausible. Half of ingested fluoride is deposited in bones where it reaches tissue concentrations thousands of times higher than in blood or soft tissues, and fluoride is a mitogen that stimulates cellular proliferation in the growing ends of the bones where the osteosarcoma occurs (NRC 2006). Multiple studies have demonstrated fluoride to be genotoxic to mammalian cells, eliciting chromosomal aberration (Hayashi 1993; Kishi 1993; Zeiger 1993). Fluoride is not a typical mutagenic chemical, yet its capacity to induce DNA breaks and translocations suggests that it can cause genetic damage in bone cells where it is actively deposited, in this case precisely where the osteosarcoma arises. Animal studies add further credence to the potential link between fluoride and bone cancer in males. 2 animal cancer bioassays conducted with fluoride both show rare bone tumors, many of which were malignant, in male as opposed to female test animals (Maurer 1990; Maurer 1993; NTP 1990). Finally, new findings reported last month provide a missing link between fluoride and osteosarcoma, revealing higher levels of fluoride in serum from osteosarcoma patients relative to those suffering from other types of bone cancer, as well as controls (Sandhu 2009).

## Fluoride's link to osteosarcoma in epidemiological studies

Osteosarcoma accounts for about 3% of all childhood cancers, and occurs with an incidence of 0.3 cases per 100,000 (NRC 2006). While rare, this cancer is deadly – the 5-year mortality rate is around 50%, and nearly all survivors have limbs amputated, usually legs.

As mentioned above, a recent publication documents measurements of fluoride levels in serum samples collected from 25 people diagnosed with osteosarcoma, age- and sex- matched with 25 people suffering from other forms of bone cancer, and a control group of 25 suffering from musculo-skeletal pain (Sandhu 2009). These measurements indicate that osteosarcoma patients have significantly higher levels of fluoride compared to the other groups examined, "suggesting a role of fluoride in the disease" (Sandhu 2009).

Concern about the ability of fluoride to cause bone cancer arose first in a 1977 NAS review of fluoride safety, where the academy committee expressed concerns about a near doubling of the incidence of bone structure defects in the population of one of the nation's first fluoridated communities (Newburgh, New York, incidence rate 13.5%), as compared to that of a nearby non-fluoridated community (Kingston, New York, incidence rate 7%). At that time, NAS recommended a full study of fluoride's potential to cause osteosarcoma in young boys. The resulting Department of Health and Human Services (DHHS) study was completed in 1991, and found a significant association between fluoride exposure and bone cancer in boys.

The 1991 DHHS study was based on data collected by the National Cancer Institute from 1973 through 1987. The first phase compared osteosarcoma rates in males under 20 years of age in fluoridated and non-fluoridated communities in Iowa and around Seattle. The researchers found a 79% increase in osteosarcoma from 1973 through 1987 in fluoridated communities, compared to a 4% decrease over the same time period in non-fluoridated communities. A second phase of the study expanded the analysis nationwide, and found that the rates of osteosarcoma were 57% higher in fluoridated communities than in communities with non-fluoridated water supplies (DHHS 1991).

As a follow-up to the DHHS study, the New Jersey Department of Health (NJDH) commissioned a similar study at the municipal level based on an individual's residence at the time of osteosarcoma diagnosis. The NJDH found that young males living in fluoridated communities had significantly higher rates of osteosarcoma than young males living in non-fluoridated areas; males 10-19 years old in fluoridated areas were 6.9 times more likely to develop osteosarcoma than those in non-fluoridated areas. According to the study authors, the findings "support the importance of investigating the possible link between osteosarcoma and overall ingestion of fluoride" (Cohn 1992).

Some experts questioned the significance of the DHHS study findings when it was published, citing the lack of an association between osteosarcoma and the length of time that individuals were exposed to fluoride in tap water. The overall weight of the scientific evidence, however, including recent research from Harvard that closely examined timing of exposure in relationship to osteosarcoma incidence (Bassin 2006), provides compelling evidence that fluoride exposure during distinct mid-childhood periods of rapid bone growth is a much better indicator of osteosarcoma risk than total duration of exposure or average lifetime exposure to fluoride.

The Harvard study mentioned above measured the risk of osteosarcoma before age 20 based on exposures to fluoride in drinking water during each year of age in childhood (Bassin 2006). The methodology employed was rigorous, and fluoride levels in tap water for each study participant were confirmed for each year of exposure during childhood. The analysis shows significantly elevated risks of bone cancer in boys exposed to fluoridated water during a window of vulnerability, from ages 5 through 10, with a peak risk associated with exposures at 7 years of age.

Elevated bone cancer risks were identified by Bassin (2006) at fluoride levels that are commonly found in American water supplies. For drinking water systems with fluoride levels from 30-99% of the amount recommended by CDC (ranging from 0.7 to 1.2 ppm as a function of climate), Bassin reports elevated risks for osteosarcoma with fluoride exposures from ages 5 through 10, with a 5-fold risk of osteosarcoma for those exposed at age 7 (4.94 (1.23-19.8) at 95% CI). At 100% or more of the recommended water fluoridation level (and still far below legal maximum levels), the risk for exposure at 7 years old rises to 5.46-fold (1.50-19.90) at the 95% confidence interval (Bassin 2006).

Notably, Bassin's doctoral dissertation was based on a reanalysis of data from another study that found no association between drinking water fluoride levels and bone cancer (McGuire 1991). In her reanalysis, Bassin examined the same cases and controls used by the earlier study. Dr. Bassin, however, refined the analysis by limiting cases to individuals exposed at less than 20 years old, and conducted a more detailed analysis of fluoride exposure and age-specific effects. The result was a very strong correlation between fluoride exposure and bone cancer, particularly for boys exposed at ages 6 through 8.

**Table:** Positive findings provided by epidemiological studies of fluoride carcinogenicity in young boys

Type of study	Findings	Reference
Ecological study of general populations that examined the relationship between the incidence of osteosarcomas in adult males and water fluoridation, using Surveillance, Epidemiology and End Results (SEER) Program data from 1973 through 1987.	The first phase (Iowa and Seattle): 79% increase in osteosarcoma rates in males under 20 years of age in fluoridated communities, compared to a 4% decrease in non-fluoridated communities. The second phase (nationwide): osteosarcoma rates 57% higher in fluoridated communities.	(DHHS 1991)
Ecological study limited to younger age groups in municipalities of New Jersey, and examining the link between osteosarcoma and residence at the time of diagnosis.	Young males in fluoridated communities had significantly higher rates of osteosarcoma than young males living in non-fluoridated areas; males 10-19 years old in fluoridated areas were 6.9 times more likely to develop osteosarcoma than those in non-fluoridated areas.	(Cohn 1992)
U.S. hospital-based case-control study of fluoride exposure and osteosarcoma in persons less than 20 years of age, specifically probing links between age of exposure to fluoride and cancer.	Elevated osteosarcoma risks for the top 2 terciles of fluoride exposure for boys ages 5-10, with a 5 fold risk of osteosarcoma for those exposed at age 7.	(Bassin 2006)

A recent study that found a correlation between fluoride exposures through tap water and osteosarcoma (Takahashi 2001), along with a number of other cancers, has received more legitimate criticism due to its comparison of large metropolitan areas without accounting for urbanization, industry, and demographic differences, and due to its use of the percentage of individuals receiving fluoridated water, rather than actual fluoride concentrations in the water, in establishing links to cancer.

Of the studies that did not report an association between fluoride in tap water and bone cancer (Kinlen 1975; Hoover 1976; Goodall 1980; Chilvers 1983, 1985; Operskalski 1987; Hrudey 1990; Mahoney 1991; McGuire 1991; Freni 1992; Gelberg 1995; Moss 1995; Yang, Cheng 2000; Steiner 2002), most have basic methodological issues that readily explain the negative findings. For instance, 13 of the 14 studies referenced below failed to analyze for osteosarcoma in young boys, and/or effects of age-specific fluoride exposures, making it impossible for them to find such an association.

For example, frequently-cited study by Operskalski (1987) claims to find no link between age-specific fluoride exposures and osteosarcoma in young men and boys under age 25. A careful reading of this study reveals a few crucial flaws that call into question this conclusion. First, the study was conducted in Los Angeles County, at a time when water in the area was not fluoridated. In fact, fewer than 5% of people in the city of Los Angeles consumed fluoridated water during the study period (Takahashi 2001), making conclusions about the effect of fluoridation difficult to probe using this study population. Furthermore, researchers never examined fluoride exposure through drinking water, assessing instead use of fluoride supplements during childhood.

In addition, Operskalski (1987) used friends and neighbors as controls, which, according to Dr. Bassin, produced a phenomenon called overmatching, where “detecting a benefit or risk for fluoride would be unlikely” (Bassin 2006). In fact, this study was better designed to probe relative osteosarcoma risk related to height at time of diagnosis, periods of rapid skeletal growth over the subject’s lifetime,

birth measurements, bone trauma, a family history of bone disease, and a number of other factors, so it is unsurprising that no link between fluoride exposure and osteosarcoma was observed. Overall, as summarized by Bassin, “Prior studies have primarily evaluated fluoride exposure at the time of diagnosis or as an average lifetime exposure, and have not evaluated exposures at specific ages during growth and development when cell division is occurring rapidly” (Bassin 2006).

**Table:** Studies finding no link between fluoride and cancer are not asking the right question

<b>Type of study</b>	<b>Methodological constraints preventing assessment of age-specific fluoride exposure and osteosarcoma in young boys</b>	<b>Reference</b>
Ecological study of cancer incidence in relation to fluoride level in water supplies.	General bone cancer incidence in areas of England with high natural fluoride levels in the water were compared with other regions of England and the globe with low fluoride levels. Fluoridated and non-fluoridated districts were also compared. No attempt was made to examine more specific forms of bone cancer (osteosarcoma), specific age groups (boys younger than 20), or age-specific fluoride exposures.	(Kinlen 1975)
Ecological study of the relationship between fluoridated drinking water and the occurrence of cancer in the U.S.	Crude analysis of cancer mortality rates and trends. Very small number of bone cancer cases. Mortality data are less reliable than incidence data, given that many cancers metastasize to bone.	(Hoover 1976)
Ecological study of fluoridation and cancer mortality in individuals greater than 45 years of age in New Zealand.	This study examines osteosarcoma in adults of at least middle age, rather than young boys.	(Goodall 1980)
Ecological cancer mortality study of 35 U.S. cities.	Examined trends in overall cancer mortality in U.S. cities. Incidence of cancer rose in 7 of 9 fluoridated cities and 6 of 9 non-fluoridated locations, making a fluoride-related effect difficult to determine. No specific focus on bone cancers or on the age and gender most at risk.	(Chilvers 1983)
Ecological cancer mortality study in England.	No analysis of bone cancer incidence or mortality.	(Chilvers 1985)
Case-control study of osteosarcoma in young persons.	Study population received limited exposure to fluoridated tap water overall. Use of friends and neighbors as controls results in statistical overmatching, preventing detection of the influence of fluoride on the cancer. This study design is appropriate for determining relative osteosarcoma risk related to height at time of diagnosis, periods of rapid skeletal growth over the subject’s lifetime, birth measurements, bone trauma, a family history of bone disease, and a number of other factors.	(Operskalski 1987)

Ecological study of the relationship between drinking water fluoridation and osteosarcoma in Alberta, Canada.	Evaluated osteosarcoma rates in a city with fluoridation (1 ppm) and without (0.3 ppm). Due to time of fluoridation, the author's expected that differences between rates in the 2 cities wouldn't be fully reported "before 1993." However they only present cancer incident cases through 1988. Other limitations include very small sample size and no examination by both age and sex.	(Hrudey 1990)
Ecological time-trend study of bone cancer incidence rates in New York State related to fluoridation of drinking water.	Examined bone cancer, specifically osteosarcoma by age and gender in New York State. As an ecological study did not account for specific fluoride levels and population mobility. Also, slightly higher bone cancer and osteosarcoma rates were observed in young men in non- metropolitan fluoridated areas.	(Mahoney 1991)
Case-control study of fluoridated water and osteosarcoma in general population of Iowa and Nebraska.	Examined 22 patients and age-, gender-and county-matched, hospital-based controls. Surveyed drinking water at current and previous residences. No data were presented about the variety of fluoride levels in the region. Fluoride was assessed as a categorical variable (time with water greater than 0.7 ppm fluoride). Most cases and controls had similar fluoride levels in water, which limits study power. Statistical overmatching prevents focused examination of fluoride in drinking water, because cases and matched controls both came from the same county. Other weaknesses in the study were the very small sample size and lack of a young male grouping; the only age group was 0-40 years old.	(McGuire 1991)
Ecological study of international time-trends in the incidence of bone cancer.	This study made no effort to determine age-specific (or overall) fluoride exposure of bone cancer patients reported in the multiple cancer registry databases analyzed. Instead, cancer cases were linked to fluoride if they were reported in areas – in some cases, areas as large as the state of Iowa, upstate New York, or entire countries – if water fluoridation was introduced in or before the 1960s for at least 50% of the population.	(Freni 1992)
Population-based case-control study of the relationship between fluoride exposure and childhood osteosarcoma.	Extensive data was collected for each individual on fluoride exposure from water, as well as from toothpaste, mouth rinses, and other sources. These data were summed to produce a total lifetime exposure or average annual exposure for purposes of analysis, preventing age-specific analysis. The study was not controlled for age, or confounders like socio-economic status or urban versus rural location.	(Gelberg 1995)
Ecological study of osteosarcoma, seasonality and environmental factors in Wisconsin.	No interviews were conducted with study participants to ascertain residence or drinking water history. Instead, fluoridation status for listed place of residence at time of diagnosis was used to link cancer cases to fluoride, preventing analysis of the effects of age-specific exposures to fluoridated tap water.	(Moss 1995)

Ecological study of fluoridated drinking water and cancer mortality in Taiwan.	Fluoride-exposed regions only had 0.24 ppm in water, which is a quarter of the target level for American communities. The study only measured cancer mortality and used a crude 8-category "urbanization index" to control for "a large number of explanatory variables, such as socioeconomic status and differential exposures to environmental conditions, which are related to the etiology of mortality."	(Yang, Cheng 2000)
Ecological global study of cancer incidence rates and environmental factors including fluoridated drinking water.	This study examined trends in regional cancer incidence rates, with no specific analysis of osteosarcoma. Drinking water fluoride concentrations were obtained from the literature, and estimated for regions as large as states or countries. No age-specific effects were assessed.	(Steiner 2002)

In summary, a critical review of the epidemiological data reveals the consistent finding of increased risk of osteosarcoma for young boys exposed to fluoride in drinking water, every time this key age and sex relationship is examined. The studies noting this relationship (DHHS 1991; Cohn 1992; Bassin 2006) are reputable, high quality, recent, and consistent with supporting data discussed below. All studies indicating no association between fluoride and cancer neglect this key relationship between the timing of exposures to fluoridated drinking water and osteosarcoma in young males. Furthermore, the quality of these apparently negative studies must be considered variable at best. OEHHA is best advised to use high quality studies probing the most relevant questions of carcinogenicity in its review of fluoride.

### **Carcinogenicity of fluoride is strongly supported by data from animal studies.**

2 long-term animal cancer bioassays with fluoride found bone tumors in male test animals; one by the National Toxicology Program (NTP 1990), and another by Procter and Gamble, each of which involved both rats and mice (Maurer 1990; Maurer 1993). Both found an increase in rare bone tumors among male animals exposed to fluoride.

In the NTP study, a dose-dependent increase of osteosarcoma was seen in the bones of fluoride-treated male rats (NTP 1990). These findings are highly significant for a number of reasons:

- Osteosarcoma is extremely difficult to induce in rats; the only other environmental agent known to provoke osteosarcoma in rats is high doses of radiation;
- The levels of fluoride in the treated rats' bones were in the same range as fluoride found in human bones;
- The levels of fluoride in the tested drinking water were only somewhat higher than that used in fluoridation, in contrast to most animal cancer bioassays where the concentrations are typically thousands of times higher than common human exposures;
- Bones are the site of fluoride accumulation; and,
- The osteosarcomas were evident before the end of the study, indicating an age dependent vulnerability similar to that seen in human males.

The NTP study authors were unequivocal about their findings: "The neoplasms were clearly malignant (one metastasized to the lung) and there was complete agreement concerning the diagnoses at both the quality assessment and Pathology Working Group stages of the histopathology review" (NTP 1990).

An additional animal study was conducted by Procter & Gamble, using both mice and rats. The first part of the study found a large, dose-dependent increase in rare bone tumors (osteomas) in fluoride-treated mice (Maurer 1993). In this study, male and female mice exposed to 10 mg/kg/day fluoride in diet had a 5% incidence rate of osteomas, while animals exposed to 25 mg/kg/day fluoride had 26% incidence of osteomas – a statistically significant increase relative to 2-3% incidence of osteomas in control animals. The second part of the study, in rats, again found bone tumors and a rare tooth tumor in the treated rats, but not the controls (Maurer 1990). In this case, an increase in osteosarcomas was discovered in both male and female rats, though not at statistically significant rates when compared to controls (NRC 2006). Osteosarcoma is rare in the human population and virtually non-existent in laboratory animals. Its occurrence at elevated rates in a small population of animals exposed to fluoride cannot be dismissed.

### **Fluoride can cause genetic damage in humans**

A compound's ability to cause genetic damage is considered an important indicator of cancer-causing potential (NRC 2006; Parodi 1991; Tice 1996). Many studies have investigated and found positive evidence of fluoride's genotoxicity, often through measurements of increased sister chromatid exchange (SCE), or how often the ends of DNA strands break off and the pieces switch positions when they reattach themselves (Sheth 1994; Wu 1995; Joseph 2000). Notable among studies of fluoride genotoxicity is a 1996 study that reported that sodium fluoride was genotoxic to rat cortical bone, the same tissue in which osteosarcoma forms (Mihashi 1996). Importantly, ape and human cells have shown greater susceptibility to fluoride's mutagenic effects than rodent cells (Kishi 1993). These findings suggest that humans may be more susceptible to fluoride's genotoxic properties, and consequently, more susceptible to a potential carcinogenic effect.

Since 1994, 4 of 5 published genotoxicity studies report increased incidence of genotoxic damage in humans exposed to fluoride in drinking water. 2 studies reported clear findings at exposure levels that were well within legal limits for fluoride in tap water in the United States (1.9 - 2.2 ppm and 1.6 - 3.5 ppm respectively) (Sheth 1994; Joseph 2000). A third study reported findings for exposure levels of 4 to 15 ppm (Wu 1995).

The fourth study presented both positive and negative findings regarding the genotoxic effects of fluoride in drinking water, though only the negative findings are highlighted in the title of the manuscript: "Lack of effect of long-term fluoride ingestion on blood chemistry and frequency of sister chromatid exchange in human lymphocytes" (Jackson 1997). An initial study involving 199 volunteers identified a statistically significant increase in sister chromatid exchange (SCE) frequency in the 4.0 ppm fluoride community, as compared to the other communities examined. The authors extended this work to compare the SCE frequency of 30 individuals using city water fluoridated to 4.0 ppm with 28 individuals using well water with fluoride levels of less than or equal to 0.3 ppm, and found both groups to exhibit similar, higher levels of genotoxic damage. Overall, this study performed in 3 communities in Indiana (Connersville (0.2 ppm fluoride), Brownsburg (1.0 ppm fluoride), and Lowell (4.0 ppm fluoride)) significantly increases the weight of evidence for the potential genotoxicity of fluoride in people.

Finally, a fifth study instead reported decreased genotoxic effects in individuals of normal or inadequate nutritional intake exposed to higher concentrations of fluoride in drinking water (1.0 and 4.8 ppm) as compared to lower concentrations (0.11 and 0.23 ppm) (Li 1995). This study has been praised for its large size (700 participants) and attempts to control for variables such as nutritional status. However, the results are difficult to interpret, since no other study has reported fluoride to be able to protect cells from DNA breaks and chromosomal aberrations.

The most commonly observed genetic damage due to fluoride exposure has been increased sister chromatid exchange (Sheth 1994; Wu 1995; Joseph 2000). Wu (1995), who found an increase of SCE among humans drinking water with 4 - 15 ppm fluoride, described the significance of SCE as follows:

“In recent years, SCE analysis has been considered to be a sensitive method for detecting DNA damage. There is a clear relationship between a substance's ability to induce DNA damage, mutate chromosomes, and cause cancers. The SCE frequency in the human body in peripheral blood lymphocytes is very steady, and does not vary with age or sex. Any increase of the SCE frequency is primarily due to chromosome damage. Thus using a method to detect SCE for exploring the toxicity and harm caused by fluoride is of great importance” (Wu 1995).

Similarly, the National Research Council report noted: “SCE is considered a generic indication of exposure to substances that can affect chromosomal structure, many of which are also carcinogens. The SCE assay is a helpful and widely used assay because of its greater sensitivity at lower concentrations than chromosomal aberrations” (NRC 2006).

The finding of increased SCE in fluoride-exposed humans has reinforced the possibility — as suggested by numerous in vitro studies — that fluoride is a genotoxic agent that causes chromosomal aberration during the DNA duplication part of cellular cycle (Hayashi 1993). In its comprehensive analysis, the National Research Council suggested that the underlying mechanism of the chromosomal aberrations might be interference by fluoride with DNA synthesis and repair (NRC 2006).

Additionally, 2 studies of worker exposures to fluoride via inhalation at a fertilizer plant noted SCE effects (Meng 1995, 1997); while this is a different route of exposure, the findings of these studies may be treated as further indication of potential genotoxicity concerns

### **Overall weight of evidence supports fluoride carcinogenicity**

When the results of the DHHS, New Jersey, and Harvard (Bassin) studies (DHHS 1991; Cohn 1992; Bassin 2006) are combined with the results of animal tests, human genotoxicity studies, and the known biochemistry and metabolism of fluoride, the overall weight of the evidence strongly supports a conclusion that fluoride causes the rare and often fatal bone cancer osteosarcoma in boys. Beyond human epidemiologic studies, the core supporting evidence includes the following:

- The 2 animal cancer bioassays conducted to date each found an increase in extremely rare bone tumors among male test animals in 2 species, rats and mice, exposed to fluoride (Maurer 1990; Maurer 1993; NTP 1990). 1 of these studies found an increase in osteosarcoma, a rare tumor in the tissue most likely to be affected by fluoride, adding considerable weight to this evidence. The guidelines that OEHHA uses to judge evidence specifically note that a single strong animal study, where the tumor detected is rare and occurs in a tissue with a priori suspicion of biological effect, is enough to classify the agent as a likely carcinogen.
- 4 separate studies have reported that fluoride in water may be linked to genomic instability in humans (Sheth 1994; Wu 1995; Jackson 1997; Joseph 2000). Additional studies show that human cells appear to be more sensitive to the genotoxicity of fluoride than rodent cells (Kishi 1993).
- The link between fluoride and osteosarcoma during periods of rapid growth is biologically highly plausible. Fluoride is a proven mitogen, meaning that it increases the proliferation of

osteoblasts (bone formation) during periods of rapid skeletal growth (Bassin 2006; Gruber 1991; Kleerekoper 1996; Whitford 1996).

- Over 90% of fluoride in the human body is stored in the bones, leading to fluoride concentrations that elicit genotoxicity and chromosomal aberrations. A novel study published this year reports higher fluoride levels in osteosarcoma patients compared to patients with bone-forming tumors other than osteosarcoma (Sandhu 2009).

## Recommendations

The safety of fluoride in California's tap water is a pressing health concern. More than 9 million residents of California live in cities and towns with fluoridated water (Bailey 2008), and the weight of the evidence strongly supports the conclusion that millions of boys in these communities are at significantly increased risk of developing bone cancer as a result of fluoride exposure. EWG urges the Carcinogen Identification Committee to place fluoride into an expedited review for inclusion in the Proposition 65 list of chemicals known to the state of California to cause cancer.

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## Serum Fluoride and Sialic Acid Levels in Osteosarcoma

R. Sandhu · H. Lal · Z. S. Kundu · S. Kharb

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**Abstract** Osteosarcoma is a rare malignant bone tumor most commonly occurring in children and young adults presenting with painful swelling. Various etiological factors for osteosarcoma are ionizing radiation, family history of bone disorders and cancer, chemicals (fluoride, beryllium, and vinyl chloride), and viruses. Status of fluoride levels in serum of osteosarcoma is still not clear. Recent reports have indicated that there is a link between fluoride exposure and osteosarcoma. Glycoproteins and glycosaminoglycans are an integral part of bone and prolonged exposure to fluoride for long duration has been shown to cause degradation of collagen and ground substance in bones. The present study was planned to analyze serum fluoride, sialic acid, calcium, phosphorus, and alkaline phosphatase levels in 25 patients of osteosarcoma and age- and sex-matched subjects with bone-forming tumours other than osteosarcoma and musculo-skeletal pain (controls, 25 each). Fluoride levels were analyzed by ISE and sialic acid was analyzed by Warren's method. Mean serum fluoride concentration was found to be significantly higher in patients with osteosarcoma as compared to the other two groups. The mean value of fluoride in patients with other bone-forming tumors was approximately 50% of the group of osteosarcoma; however, it was significantly higher when compared with patients of group I. Serum sialic acid concentration was found to be significantly raised in patients with osteosarcoma as well as in the group with other bone-forming tumors as compared to the group of controls. There was, however, no significant difference in the group of patients of osteosarcoma when compared with group of patients with other bone-forming tumors. These results showing higher level of fluoride with osteosarcoma compared to others suggesting a role of fluoride in the disease.

**Keywords** Fluoride · Sialic acid · Alkaline phosphatase

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Z. S. Kundu  
Department of Orthopaedics, Pt B D Sharma University of Health Sciences, Rohtak 124001 Haryana,  
India

R. Sandhu · H. Lal · S. Kharb (✉)  
Department of Biochemistry, Pt B D Sharma University of Health Sciences, H No 1447, sector-1,  
Urban Estate, Rohtak 124001 Haryana, India  
e-mail: simmikh@rediffmail.com

## Introduction

Bone cancer is the third most common malignancy in the age group 10–24 years and osteosarcoma is by far the most frequent pathologic type [1]. Various etiological factors for osteosarcoma include family history of bone disorders and cancers, chemicals (fluoride, beryllium, and vinyl chloride), exposure to ionizing radiations, and viruses [2].

Fluoride prevents dental caries at drinking water levels of 1 ppm. Fluoride is also recognized as stimulator of bone formation via osteoblast proliferation. Although an excess intake of fluoride has been reported to cause skeletal fluorosis [3], very little is known about the mechanism of adverse effects of fluoride in bone.

Recent reports have indicated that there is a direct link between fluoride exposure and osteosarcoma [4]. Exposure to high fluoride for prolonged duration affects all the three components of bone and teeth, namely collagen, proteoglycan, and ground substance, hence resulting in increased in serum sialic acid and calcium levels in patients of osteosarcoma [5]. Also, increased sialylation helps to disguise their immunogenic sites. Also, sialic acid levels have been reported as a sensitive index to detect fluoride toxicity at early stages in human and animal models.

To the best of our knowledge, we could not come across any study where fluoride levels have been estimated in osteosarcoma and other bone-forming tumors. Hence, the present study was planned to estimate serum sialic acid, calcium, phosphorus, and alkaline phosphatase levels along with fluoride in patients with osteosarcoma and other bone-forming tumors.

## Materials and Methods

The present study was planned to carry out in 75 patients in department of biochemistry in collaboration with department of orthopaedics Pt BDS PGIMS, Rohtak (India).

They were subdivided into three groups:

Group I (control,  $n=25$ ) with musculo-skeletal pain; group II (control,  $n=25$ ) with bone-forming tumors other than osteosarcoma; and group III (study,  $n=25$ ) with localized osteosarcoma without metastasis.

Five milliliters of venous blood was collected aseptically from antecubital vein and serum was separated by centrifugation. Serum fluoride, sialic acid, alkaline phosphatase, calcium, and phosphorus levels were estimated [6–10]. The data so obtained was analyzed using one-way ANOVA.

## Results

Serum fluoride levels were significantly higher in patients with osteosarcoma (group III) as compared to controls (Table 1,  $p<0.05$  at all levels). The values were double in group III as compared to group II. Serum calcium levels were raised in osteosarcoma cases as compared to two groups ( $p<0.05$ ) and the phosphorus levels were comparable in the three groups. Alkaline phosphatase levels were significantly raised in osteosarcoma patients as compared to group I and non-significant difference as compared to group II. Serum sialic acid levels were raised in both osteosarcoma patients and those with other bone-forming tumors as compared to group I ( $p<0.05$ ). The levels were higher in osteosarcoma as compared to group II, but the difference was not significant (Table 1).

**Table 1** Various Lab Parameters in Various Groups (mean±SD)

	Group I Controls	Group II Other bone-forming tumors	Group III Osteosarcoma
S. fluoride (mg/l)	0.0421±0.03480	0.072±0.07792*	0.1430±0.1056*****
S. sialic acid (mg%)	36.79±3.65	52.94±13.709*	60.40±18.66*
S. ALP (IU/l)	221.20±57.51	469.64±180.21*	461.76±210.67*
S. calcium (mg%)	9.26±0.35	9.312±0.54568	9.62±0.66*
S. phosphorus (mg%)	5.00±0.34	4.94±0.41028	4.86±0.85

\* $p < 0.05$  as compared to group I, \*\* $p < 0.001$  as compared to group I, \*\*\* $p < 0.05$  as compared to group II

## Discussion

Fluoride is an effective anabolic agent to increase bone density in osteoporotic patients and this action is mediated by osteoblastic stimulation. Fluoride can be mutagenic when present at sufficiently high concentrations above normal range [11]. There are studies indicating relationship of high fluoride levels in drinking water with incidence of osteosarcoma [12, 13]. Fluoride gets incorporated into lattice of bone by forming fluorapatite which is more stable than hydroxyapatite in humans. Bassin et al. showed mitogenic property of fluoride by increasing osteoblast proliferation [4]. Fluoride uptake in bone increases during periods of rapid skeleton growth since hydroxyapatite crystal structure of bone mineral in young age exist as extremely small crystals with ion-rich hydration shell to provide greater surface area facilitating greater fluoride exchange.

In the present study, serum fluoride levels were significantly higher in osteosarcoma patients as compared to controls. The values were double in osteosarcoma patients as compared to other bone-forming tumors (group II). There are no reports available in literature where serum fluoride levels are estimated in osteosarcoma patients, although reports are available where drinking water fluoride levels and incidence of osteosarcoma have been studied [12, 13]. On the other hand, few reports could not find a link between drinking water fluoride and incidence of bone cancers [14, 15]. In vitro studies have demonstrated mitogenic effect of fluoride on bones [3].

Also, fluoride has been reported to increase intestinal absorption of calcium [16] and in osteoporosis, fluoride has been reported to increase intestinal absorption of calcium and phosphorus to improve calcium balance.

Serum alkaline phosphatase levels were significantly raised (nearly doubled) in osteosarcoma patients as compared to group I and the difference was not significant between osteosarcoma patients and group II. Similar reports are available in literature where 40–80% rise in alkaline phosphatase levels in osteosarcoma have been reported [17, 18]. Fluoride exposure has been reported to increase the number of osteoblasts and rate of bone formation [19]. Also, in vitro studies have shown elevation of alkaline phosphatase activity in the presence of NaF [20].

Ingestion of high quantity of fluoride for prolonged duration affects proteoglycans and calcium [21]. Glycosaminoglycans have been considered as an index to assess fluoride toxicity and fluorosis at very early stages [21]. Also, sialic acid acts as an antigenic determinant and aberrant sialylation occurs in malignant cells to disguise their immunogenic sites and influences cell shape, anchorage that results in shedding of malignant cells. Various studies have reported rise in sialic acid levels in various

malignancies, bone cancers, and osteosarcoma [22, 23]. In the present study, serum sialic acid levels were significantly raised in osteosarcoma and group II patients as compared to control group I. These findings are in agreement with those reported in literature [24, 25]. A positive correlation was observed between rise in sialic acid levels and fluoride levels, although the difference was not statistically significant ( $r=0.00017$ ,  $p<0.05$ ).

These findings of raised fluoride sialic acid and alkaline phosphatase levels in osteosarcoma patients indicate that raised fluoride levels stimulate osteoblastic activity. Also, this report proves a link between raised fluoride levels in serum and osteosarcoma.

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