Revisiting the Fluoride-Osteosarcoma connection in the context of Elise Bassin's findings: Part II

by

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Submitted to the NRC review panel on the Toxicology of Fluoride in Water

March 21, 2005
revised April 8, 2005

1. Introduction

In Part I of our submission we summarized Elise Bassin's findings in her 2001 PhD thesis obtained at Harvard University (Bassin, 2001). She suggests that:

> **... for males less than twenty years old, fluoride level in drinking water during growth is associated with an increased risk of osteosarcoma, demonstrating a peak in the odds ratios from ages six to eight years of age (OR=7.20, 95 percent CI 1.73-30.01 at age 7). All of our models are remarkably robust in showing this effect during the mid-childhood growth spurt,** which, for boys, occurs at ages seven and eight years....

Bassin concluded her thesis by suggesting that ongoing and future studies consider incorporating age-specific exposure analysis. She also explains why failure to look at age-specific exposure will tend to obscure evidence of an association between fluoride and osteosarcoma.

In this Part II of our submission, we discuss in more detail both previous and proposed studies on fluoride and osteosarcoma, as well as additional studies on radium and osteosarcoma.

2. A re-examination of other osteosarcoma studies in light of Bassin's findings

In the following tables we have collated relevant information on the studies on fluoride/osteosarcoma. Further discussion on some of these studies is provided below.

Table 2.1 Studies with positive findings

Researchers	Nat. Tox. Prog. (NTP)	Hoover et al.	Cohn	Freni & Gaylor*	Yiamouyiannis	Takahashi et al.
Date	1990	1991	1992	1992	1993	2001
Type of study	controlled animal lab experiments	"ecological", geographical correlation and time trend	"ecological", geographical correlation	"ecological", geographical correlation and time trend	"ecological", geographical correlation and time trend	"ecological", geographical correlation and time trend
Type(s) of cancer studied	osteosarcoma; all cancers	osteosarcoma; bone; most cancers	osteosarcoma	bone	osteosarcoma; bone; oral cancers	bone; most cancers
Methods summary	rats & mice given NaF in water up to max. tolerated dose	incidence rate comparison between counties	incidence rate comparison between towns	incidence rates comparison between nations or cancer registries	re-analysis of Hoover 1991 study using females as controls for males	incidence rate comparison between cancer registries
Sample size; Years of data; Population size	780 rats, 780 mice; 90 or 60 animals per study group: 3 doses, 2 controls, males, females; over 2 years	some analysis on all SEER data, others on just partial Iowa & Seattle with ~4 mil. pop for 15 years (1973-87)	71 cases ; 2.4 million population for 9 years (1979-87)	150 million population for ~20 years (~1958-87, varied)	re-examined Hoover 1991	22 million population for 15 years (1978-92)
Person-years	3,000 animal-years	~50 mil. pers-yrs IA/Seat.	20 million pers-yrs	3,000 million pers-yrs	see Hoover 1991	300 million pers-yrs
Geographic area	not applicable	US SEER cancer registries; IA & Seattle SEER areas	7 counties in New Jersey	US, Canada, Northern Europe, UK, Australia, New Zealand	SEER cancer registries see Hoover 1991	US, 9 SEER cancer registries
Fluoride exposure: Classification system; Assessment method F = "fluoridated" nonF = "non-fluoridated"	direct measurement of fluoride in water and food	F/nonF; residence at diagnosis; duration of fluoridation in county	F/nonF; residence at diagnosis; F if >85% town pop. drank F water, nonF if <10% drank nonF water; excluded if >15% use private wells; info from NJ DEP	F/nonF/unknown; residence at diagnosis; F if >50% cancer registry pop. drank ~1ppm F water beginning by 1960s; many registries assigned Don't Know	see Hoover 1991	percent F/nonF for each cancer registry area input to linear regression models; >0.7 ppm F, <0.7 ppm nonF
Age range included and Age subsets	studied from 6 weeks old to 2 years	all ages; subsets: 0-20; 20-39, 40-79	all ages; subsets: 0-9, 10-19, 20-49, 50-69, 70+	0–74 and 10–29 years old	see Hoover 1991	all ages; no subsets
Age-specific exposure analysis?	no	no	no	no	see Hoover 1991	no
Male/Female analysis	yes	yes	yes	yes	see Hoover 1991	yes
Other covariates & confounders included	no, but controlled laboratory experiment	duration of fluoridation	race; source of water: ground, surface, mixed; air toxics	none	see Hoover 1991	solar radiation for skin cancers
Summary of findings	dose dependent increase in osteosarcoma in male rats, significant at 95% CI; dose-dependent increase in rare liver cancer in both sexes of fluoride-treated mice (identified by contractor but challenged by NTP); Increases in oral and thyroid cancers, but not statistically-significant	• RR of 1.4 for white males <20 in F/nonF counties osteosarcoma • RR of 1.7 for white males <20 in F/nonF counties bone & joint cancers • 79% increase in osteosarcoma over time in F counties compared to –4% decrease in nonF counties for <20 males • duration of fluoridation trend analysis insufficient data unlikely to reach statistical significance	• RR of 4.8 (95%CI 2.3-8.8) for white males 10-19 years old • RR of 8.0 (95%CI 3.9-15) for white males 10-19 in central 3 county study area • RRs not significantly elevated for females or older males	• "...significant increases in CR 10-29 [cumulative risk for 10-29 year olds] (P<0.1) were seen mainly in males and most frequently in the United States registry areas." • US and Canada (40-60% F) had much larger significant increases in male CR 10-29 than Northern Europe or UK which are <10% F.	• found increased relative risks for fluoridated areas when applying the technique of subtracting female rates from male rates for each area; reanalyzed Hoover 1991 results	• regression analysis shows statistically significant increase in bone cancer incidence rate with increasing percent fluoridation with p<0.01 • of 36 cancer sites examined 23 positively associated with percent fluoridation, 4 negatively associated, and 9 no statistically significant association (at p<0.1 or better)
Weaknesses		duration trend analysis based on insufficient data and compares different sets of counties; too broad exposure criteria; used small subset of population for most analyses	no age-specific exposures	F exposure misclassification; no confounders; no age-specific exposures	technique can potentially lead to false positive associations if there are confounders that influence sexes unequally	compares rates in different geographic areas instead of in similar areas which can lead to confounding
Strengths	controlled lab experiment; used doses within 10x of human doses rather than many times larger	for some analyses used entire SEER data which is large	F assessment by town rather than county; less misclassification on F exposure due to stricter criteria than other studies	large population size and period	can potentially control for many nonF confounders as long as they affect males and females equally	large population; use of % F in regression may be more accurate than simple F/nonF assignment, partly because it incorporates diffusion effect

* Freni & Gaylor 1992: See text for why we have included this study amongst those which show positive findings. F = "fluoridated", nonF = "non-fluoridated"

Table 2.2 Recent case-control studies with larger sample sizes

Researchers	Gelberg et al.	Bassin
Date	1994, 1995, 1997	2001
Type of study	case-control	case-control with age-specific exposure analysis
Type(s) of cancer studied	osteosarcoma	osteosarcoma
Methods summary	interview subjects for residential history and exposure to other F sources	interview with subjects for residential history
Sample size; Years of data; Population size	130 cases, 130 controls matched by age, sex; from New York State except NYC with 10 million pop. for 10 years (1978-88);	91 cases, 188 controls matched by age, sex, residence distance from hospital for 11 hospitals throughout US for 3 years (1989-92); pop. roughly 15 million
Pop. size in person-years	100 million person-years	not specified but roughly 50 million person-years
Geographic area	NYS	US, in service areas of 11 hospitals
Fluoride exposure: Classification system; Assessment method F = "fluoridated" nonF = "non-fluoridated"	cumulative lifetime fluoride doses from drinking water, toothpaste, fluoride supplements and treatments; CDC F Census; F water assumed 1 ppm F, nonF water 0 ppm	age-specific drinking water F levels; CDC Fluoridation Census supplemented with careful cross-checks; water samples taken of well water; bottled water fluoride exposure assessed
Age range included and Age subsets	<25 years old	<20 years old
Age-specific exposure analysis?	no	**yes**, by exposure at each year of age
Male/Female analysis	yes	yes
Other covariates & confounders included	race, sociodemographic, parent risk factors such as occupation and maternal age at birth, subject and maternal exposure to dental x-rays or medical x-rays	distance from hospital; socioeconomic status; population density; use of bottled or well water; use of fluoride supplements
Summary of findings	• statistically significant increased risk for some exposed groups with drinking water: OR 10.6 (95%CI 1.2-91) for females in 2nd "quartile"; OR 2.8 (95%CI 1.0-8.1) for males in 2nd "quartile"; did not present analysis other than by "quartile" of exposure • no statistically significant results (95%CI) for F exposure through F toothpaste, F supplements, or F treatments , although a weak trend was found for decreasing risk with increasing exposure • of ~10 other nonF variables examined, only exposure to dental x-rays showed statistically significant risk OR 4.0 (95%CI 1.3-12) for both sexes combined	• OR 7.2 (95%CI 1.7-30) statistically significant increased risk for exposure at age 7 for males when covariates included; consistently increased risk for exposure at other ages with peak risk for exposure at ages 6, 7, and 8 • OR 5.1 (95%CI 1.3-20) for males exposed at age 7 to even the "medium" level of F in drinking water (~0.3-1.0 ppm F) • "window of vulnerability" for males was exposure from age 5-10; for exposures outside of this time window risk dropped off • increased risks for females consistently greater than OR 2.0 for exposure at most ages when covariates included in models, but did not reach statistical significance
Weaknesses	no analysis by age-specific exposure although data was collected which would allow such analysis; failed to report ORs for simple analysis using two categories of exposure: F and nonF; limited control for socioeconomic status, pop. density, or other factors besides age and sex; faulty assumptions in F assessment; gross errors (reversing sexes and reversing cases with controls) raise questions about work; excluded all cases from NYC even though NYC makes up almost half the pop. of NYS; also, NYC has high non-white pop. which Gelberg found had significantly higher risk of osteosarcoma, and Freni & Gaylor (1992) found very rapid increase of osteosarcoma in young males during study time period	less comprehensive control for non-drinking water F exposures than Gelberg (1994, 1995)
Strengths	assessed F exposure from more sources than other studies, but used questionable unverified assumptions for these non-drinking water exposures; relatively large sample size case-control study	most detailed drinking water F exposure assessment methods; only study to analyze for age-specific exposures; controlled for a variety of potential confounders

Table 2.3 Studies that found no association or inconclusive results

Researchers	Procter & Gamble (Maurer/FDA)	Hrudey et al.	Mahoney et al.	McGuire et al.	Freni & Gaylor	Moss et al.	McGuire & Douglass et al.
Date	1990, 1993	1990	1991	1991	1992	1995	1995 (abstract only)
Type of study	Controlled animal lab experiment	"ecological", geo. corr. & time trend	"ecological", geo. corr. & time trend	case-control	"ecological", geo. corr. & time trend	case-control	case-control **but no matching in analysis**
Type(s) of cancer studied		osteosarcoma	osteosarcoma, bone	osteosarcoma	bone	osteosarcoma	osteosarcoma
Methods summary		incidence rate comparison between cities	incidence rate comparison between counties	cases and matched controls obtained from hospital patients; telephone interviews for drinking water histories	incidence rates comparison between nations or cancer registries	cases and matched controls obtained from cancer registry, controls had other forms of cancer	cases and matched controls obtained from hospital patients; interviews for drinking water histories; **no matching analysis**
Sample size; Years of data; Population size		55 cases over 19 years (1970-88); population total 1 million: 0.5 million nonF (Calgary), 0.5 million F (Edmonton); cancer registry incidence data	pop. 10 million (1955-87 for some analyses 1976-87 for others)	22 cases, 22 controls matched by age, sex, and county of residence; 10 years, 3 million?	150 million population for ~20 years (~1958-87, varied)	167 cases, 989 controls matched by age (±7 or 5 yrs), sex, and race; from WI cancer registry pop. 5 million for 11 yrs (1979-1989)	147 cases, 248 controls matched by age, sex and residence distance from hospital for 10 hospitals throughout US for 3 years (1989-92); pop. roughly 15 million
Person-years of data		20 million pers-yrs	100-300 mil. pers-yrs	30 million? pers-yrs	3,000 million pers-yrs	50 million pers-yrs	~50 million pers-yrs
Geographic area		Calgary and Edmonton, Alberta Canada	New York State excluding NYC	parts of Iowa and Nebraska	US, Canada, Northern Europe, UK, Australia	Wisconsin	US, in service areas of 10 hospitals
F exposure: Classification system; Assessment method F = "fluoridated" nonF = "non-fluoridated"		F/nonF; residence at diagnosis in single fluoridated city or single non-fluoridated city	F/nonF based on county of residence; 3 counties nonF, but all others assumed fluoridated even though only 56% F	F/nonF; residential history; CDC F census or DNR query; well water samples analyzed; 3 methods of assignment between 2 categories.	F/nonF/unknown; residence at diagnosis; F if >50% cancer registry pop. drank ~1ppm F water beginning by 1960s; many registries assigned Don't Know	F/nonF; residence at diagnosis, no histories taken;	
Age range included and age sub-sets		all ages; 2 year subsets up to age 15, =>15, **but no sex grouping for age group analysis**	all ages; <30, 30+	0-40 years old; no subsets	0–74 and 10–29 years old	all ages; two subsets: <45, 45+ years old	
Age-specific exposure analysis?	no	no	no	no	no	no	no
Male/Female analysis	yes	yes, **but not with age**	yes	yes	yes	yes, **but not with age**	no
Other covariates & confounders included	No, but controlled laboratory experiment		urban/non-urban		none	alpha radiation	
Summary of findings	Occurrence of malignant bone tumors in fluoride-treated rats, but not statistically significant and without dose-dependent trend; Dose-dependent & statistically significant increase in benign bone tumors in both sexes of fluoride treated mice.	no statistically significant results	time trend showed statistically significant increasing trend of incidence rate over 35 years during which F increased in NYS; but did not find higher rates in F areas compared to nonF areas	due to small sample sizes very wide CIs and no statistically significant results for any models employed	**Note positive findings:** • "...significant increases in CR 10-29 [cumulative risk for 10-29 year olds] (*P*<0.1) were seen mainly in males and most frequently in the United States registry areas." • US and Canada (40-60% F) had much larger significant increases in male CR 10-29 than North Europe or UK which are <10% F • F areas of US tended to have greater increases than nonF	no statistically significant results for F or alpha radiation	no association, but see text for fuller discussion

F = "fluoridated", nonF = "non-fluoridated"

Table 2.3 Studies that found no association or inconclusive results (cont.)

Researchers	Procter & Gamble (Maurer/FDA)	Hrudey et al.	Mahoney et al.	McGuire et al.	Freni & Gaylor	Moss et al.	McGuire & Douglass et al.
Date	1990, 1993	1990	1991	1991	1992	1995	1995 (abstract only)
Weaknesses	Many, See FDA 1990	very small sample size; no analysis by both sex and age groupings; no age-specific exposure analysis	no age-specific exposure analysis; high degree of misclassification of F exposure; excluded all cases from NYC even though NYC makes up almost half the pop. of NYS; also, NYC has high non-white pop. which Gelberg found had significantly higher risk of osteosarcoma, and Freni & Gaylor (1992) found very rapid increase of osteosarcoma in young males during study time period	very small sample size; no age-specific exposure assessment; overmatching: matched by county of residence, many small pop. counties in IA and NE	F exposure misclassification; no confounders; no age-specific exposures; only examined bone cancers, did not examine osteosarcoma	controls were cancer patients with cancers other than osteosarcoma but some studies show F increases rates of several cancers; age matching only within ± 7 or 5 years which is too broad for childhood osteosarcoma; control for Ra was crude; too small a sample; no analysis by sex and age group; age groups were too broad: <45 and 45+ years old; no age-specific exposure analysis	no analysis using matching to control for age or sex; no age-specific exposure assessment
Strengths				case-control study where detailed drinking water source histories were obtained	large population size and period	only study to consider both fluoride and radionuclide exposure	

2.1 Studies showing positive findings

Hoover 1991

Prompted by NTP's finding of increased osteosarcoma in fluoride-treated male rats, Hoover 1991 surveyed the incidence of osteosarcoma and other cancers using the data collected in the Surveillance, Epidemiology and End Results (SEER) program. This program, begun in 1973, consists of nine cancer registries and provides continuous incidence coverage for about 10% of the US population. The Hoover study of fluoridation and cancer was included as Appendices E and F of the 1991 DHHS report, but never formally published in a peer-reviewed journal.

For the main part of their analysis a subset of SEER data was chosen: the Seattle and Iowa registries. This decision was made because:

> "Seven of the nine SEER areas had inadequate variation in fluoridation exposure levels to allow for meaningful analysis. Two SEER areas, Iowa and the Seattle metropolitan area, included both fluoridated and control counties: 11 exposed and 14 non-exposed counties in Iowa, and one exposed and seven non-exposed counties in Seattle" (Hoover 1991 p. E-3).

Note that Iowa has 99 counties so Hoover was using only about a quarter of the Iowa data in this analysis. This small study population represented less than a tenth of total SEER data.

They compared two time periods:1973-80 and 1981-87. A dramatic difference was found between fluoridated counties and non-fluoridated counties in the incidence rates of osteosarcoma for young males over time:

**Table 2.4 Changes in rates of osteosarcoma and bone & joint cancers
in fluoridated and non-fluoridated counties for males <20 years old**

	osteosarcoma 33 Iowa & Seattle counties	osteosarcoma 205 US counties	bone & joint 205 US counties
fluoridated counties	**+79%**	**+69%**	**+39%**
non-fluoridated counties	**-4%**	**+40%**	**-5%**

Percent change in rates between period 1973-80 and period 1981-87 (Hoover 1991 p. F-3, F-5, F-6, F-7)

Hoover's data also show that amongst the 205 US SEER counties the Relative Risk (RR) for osteosarcoma in fluoridated counties compared to non-fluoridated was 1.4. The RR for bone & joint cancers was 1.7.

However, after finding this association between osteosarcoma and fluoridation, Hoover examined the data to see if he could find a relationship between duration of fluoridation and osteosarcoma. First, he narrowed the comparison to counties that had fluoridated before 1955 versus those that had fluoridated after 1965. The very small set of counties fluoridated before 1955 showed faster rates of increase in osteosarcoma than in the set of counties fluoridated after 1965. Hoover argues this is evidence fluoride could not have been causing the overall increases in rates because:

> "For this group, you would expect to see no influence of fluoridation, since essentially all persons under age 20 in *both* time periods [1973-80 and 1981-87] from these early-fluoridated counties would have been exposed for their entire life-times." (Hoover 1991 p. F-3)

But when one sees the very small numbers on which these rates are based (3 osteosarcoma cases of both males and females in the 1973-80 period and 7 in 1981-87 period) it is clear the rates would be very

unstable. Hoover does not provide confidence intervals or any statistical tests of significance for these rates. No reliable conclusions can be derived from comparison of such unstable rates.

Hoover then went on to investigate at a finer level the relationship between duration of fluoridation and osteosarcoma. Using the Iowa/Seattle subset Hoover found no relationship between the duration of fluoridation and the onset of osteosarcoma when cases were divided into four duration categories.

A similar no effect finding was obtained from the larger subset of the 205 SEER counties. But this analysis used areas Hoover himself had initially rejected as being unsuitable due to insufficient variation in fluoridation exposure levels.

Hoover used the absence of a dose-response trend in Iowa/Seattle and to a lesser extent in the subset of the 205 SEER counties to argue that the overall increases of osteosarcoma in fluoridated compared to non-fluoridated areas could not have been caused by fluoridation. But these trends are based on a subdivision of his data which would lead to wider confidence intervals. He never presents confidence intervals for these subdivisions nor any statistical tests of significance for the trends. From the small numbers of cases in each duration category (1, 9, 8, and 5 for males) it is probable the rates would be unstable and unsuitable for determining a trend:

Table 2.5 Osteosarcoma RRs for fluoridated compared to non-fluoridated counties by "duration of fluoridation" for county of residence (Iowa/Seattle data 1972-1987) Males <20 years old

duration of fluoridation	<5 years	5-9 years	10-14 years	15-19 years
observed number of cases in fluoridated counties	1	9	8	5
Risk Ratios	1.0	2.9	0.7	0.4

(Hoover 1991 p. F-7 Table 5)

Note how small the sample size is on which the RRs are based. For the first duration category a single osteosarcoma case determines the RR.

While Hoover does not make clear how he defines the categories of "duration of fluoridation" he does confirm that there will be "... a different mix of counties in each grouping [duration category]...." (Hoover 1991 p. E-7). This means the particular set of counties on which the Risk Ratios are based differs for each duration category. Deriving a trend by comparisons between duration categories is therefore suspect. The analysis is no longer controlled by geographic area and large variations would be expected simply because different counties are included in each category.

A further limitation of the statistical power of Hoover's analyses was the imprecise fluoride exposure classification system chosen. A county was classified "fluoridated" if more than 60% of residents had fluoridated water. It was classified "non-fluoridated" if less than 10% had fluoridated water. Substantial misclassification will result from this exposure assignment. Even with these criteria, many counties in the nine SEER areas were still eliminated resulting in a much reduced study population.

Especially for a very rare cancer like osteosarcoma, we believe it was premature for Hoover to have discounted his positive findings simply on the duration of exposure issue. His duration analyses trends do not appear to have had enough data to be considered reliable and are based on rates from differing mixes of counties. Furthermore, in light of Bassin's findings of a narrow developmental window of susceptibility Hoover's duration-dependency analysis may well have lacked the sensitivity to detect such an age-specific effect.

Buried amidst the attention Hoover gives to bone cancers are many pages of data in Appendix E which give results on other types of cancer. All of this data has been analyzed by duration of fluoridation which breaks the data into five duration subgroups. If sufficient data is available it is further broken down into male and female.

Information on single cancer sites is not always available; the data is often by groupings of cancers that affect related organs. Thus, there is the likelihood that an effect on just one of the cancers in a grouping will not be observable because it is diluted by the other cancer sites. Also, analyzing the data by five subgroups of duration instead of simply dividing all cases into two categories (exposed/unexposed) leads to even smaller sample sizes in each subgroup. This reduces the power of the analysis to detect effects. No analysis is presented for a division into just two categories. This is a weakness of the study because small increases in cancer rates might not be observable when the data is divided into more than two categories.

A further limitation for Hoover's other cancer type analyses is a result of the very small population size he has chosen to work with: certain counties in Iowa and the Seattle area. Hoover never presents any analysis for all the SEER areas combined or even for the selected 205 counties that he used in Appendix F to supplement the Iowa/Seattle data.

Nevertheless, despite these limitations to his approach's statistical power, several cancer site groups did show statistically significant increasing risk ratios for "fluoridated" counties with duration of fluoridation. They are colon & rectum cancers (p. E-21), prostate cancers (p. E-21), and non-Hodgkin's lymphoma (p. E-22). For colon & rectum cancers in both sexes in the Iowa counties, the trend with increasing fluoridation duration was positive and significant with a p-value of <0.001. For prostate cancer, both Iowa and Seattle counties showed p-values for a positive trend of <0.02. For non-Hodgkin's lymphoma the p-value for both sexes combined in Seattle counties was 0.01. Hoover dismisses these findings because some are not present in both Iowa and Seattle and because cancers of other organs did not exhibit similar positive associations. But considering the small population size these results are derived from, any such positive findings should be taken seriously and followed-up with studies on larger populations.

Takahashi 2001

Following Hoover's analysis, Takahashi (2001) analyzed the SEER data, using a method for assigning fluoridation status that allowed him to retain all cases from each cancer registry. For each SEER region the percent of the total population drinking fluoridated water was used as a continuous variable in a regression analysis against incidence rate of cancer for that SEER area. Nine SEER areas were included with a total population of 22 million over 15 years for 300 million person-years of data. There was a wide range of percent fluoridation from 2% in Utah to 84% for San Francisco. Regression analysis found 23 of 36 cancer sites to be significantly positively associated with degree of fluoridation, 4 sites to be negatively associated, and 9 to have no significant association (at $p < 0.1$ or less). Bone cancer in males was positively associated with fluoridation at $p < 0.001$. This was the highest significance level achieved by any cancer site. The study is weaker than some others because it compares rates between differing geographical areas of the United States, rather than within a single state or SEER registry. Also, as in most of the other studies, it did not control for factors such as smoking and socioeconomic level. Nevertheless, Takahashi's results point to the need for more rigorous work in this area

Cohn 1992

In a report published by the New Jersey Department of Health in 1992 Cohn found 4.8 times as many osteosarcoma cases in young white males (95%CI 2.3-8.8) in the fluoridated versus non-fluoridated areas in seven NJ counties. When he looked at a subset of three of these counties, there were 8.0 times as many (95%CI 3.9-15). In neither selection was there a statistically significant difference in the rates among females.

Cohn used several methods which improved the sensitivity of his analysis beyond what other researchers have achieved:

> • **Assessment of fluoride exposure status was based on the town of residence rather than on the much less accurate county of residence.**

- **Within each town Cohn used a narrower definition of "fluoridated" and "non-fluoridated" than other studies to reduce misclassification**. Towns with >85% of the population drinking fluoridated water were defined as "fluoridated". All other ecological studies used lower criteria: Hoover used >60% of a county, Mahoney >56% of a county, and Freni & Gaylor >50% of a nation or a SEER registry area.

- **Controlled for a wider range of confounding variables than many other studies**. Controlled for race, source of drinking water (ground, surface, or mixed), and for air toxics

- **Age ranges examined were narrower than most other ecological studies**. Analyzed by under 20 year olds and 10-19 year olds versus the wider age groupings in other studies: Hoover <20 and 20-39, Freni & Gaylor 10-29

It is striking how frequently the study by Cohn is overlooked - or given short shrift - in commentaries on this issue. However, his reflections on his findings, as quoted below, appear to be have been vindicated by Bassin's work:

> If rapidly growing bone in adolescent males is most susceptible to the development of osteosarcomas (Glass and Fraumeni, 1970), **it is possible that fluoride acts as a cancer promoter during a narrow window of susceptibility**. The interplay of hormonal influences and the intensity of the growth spurts may be potent influences. (Cohn 1992 p. 11)

Yiamouyiannis 1993

Because most studies have found an effect of fluoride on males but not females, Yiamouyiannis used a novel approach in his review of the SEER data. Reasoning that females would act as a control for many factors which might influence osteosarcoma rates other than fluoride, he examined how the difference between male and female osteosarcoma rates varied with fluoridation status. Using this approach Yiamouyiannis reanalyzed the results in Hoover 1991 and found more and stronger correlations than appeared under Hoover's methods.

A potential weakness of this method is that it depends on the assumption that variables other than fluoride influence males and females equally. If there are confounders that influence the sexes unequally then false positive findings could result. It also assumes that fluoride affects the osteosarcoma rate in young males much more than females. To the extent these conditions are met, Yiamouyiannis' method may increase the sensitivity of a study to detect an increase in osteosarcomas caused by fluoride.

2.2 Case-control studies with large sample sizes

Gelberg 1994, 1995, 1997

The case-control study by Gelberg, published first as a PhD dissertation and then later in two peer-reviewed journals, may represent the most substantive study on fluoride/osteosarcoma previous to Bassin's 2001 analysis.

In assessing Gelberg's data, we were at first struck by the existence of several notable errors in both the thesis and papers. In all three documents, Gelberg states that the majority (68%) of her cases were female. In her thesis, Gelberg states this repeatedly and cites 18 studies finding the opposite, that males have higher incidence than females (Gelberg 1994; p. 82, 85, 102). However, if one looks at the data presented in Gelberg's tables, it is clear that 68% of the cases were in fact males, not females. Personal communication with Gelberg confirmed this error. We are not sure how Gelberg got her data confused, and how this error managed to slip by peer review twice.

Another error in Gelberg's paper can be gleaned by comparing Tables 2 and 3 in her published paper with Table 1 (Gelberg 1995). Upon comparing these tables, it is evident that the cases and controls in the "Total Fluoride" and "Toothpaste" categories in Tables 2 and 3 were reversed. Again, we are surprised to see that these errors were not corrected in peer review. These errors do not affect her final results as they were apparently made separately from her statistical analysis for fluoride.

While these errors do raise questions about the study, our primary concern with Gelberg's work relates to the methods she used to analyze her data.

Gelberg's study population was New York State except New York City and all cases came from the NY Cancer Registry from the years 1978-1988. Unlike most other studies, Gelberg tried to include exposure to fluoride from sources other than from drinking water. From interviews she estimated how often subjects used fluoride supplements, toothpaste, or received fluoride dental treatments. However, she had to use very broad assumptions to assign how much fluoride was ingested from each of these sources. These broad assumptions combined with possible subject recall biases, bring into question the accuracy of these fluoride exposures. For example, between her thesis in 1994 and her published paper in 1995, she apparently revised her methods of assessing toothpaste fluoride exposure so that her assessments for lifetime cumulative exposure went down by a factor of three. No explanation is offered for this change in exposure assignment but it highlights the difficulties in determining fluoride exposures from toothpaste and other non-water sources. We therefore believe that Gelberg's analysis of drinking water fluoride is likely to be the most reliable and will focus on that.

Gelberg, like Hoover 1991, never analyzes her data with subjects divided into a simple two-category model: exposed versus unexposed. Instead, she uses milligrams of total lifetime fluoride exposure as a continuous variable. But then she presents the data broken into "quartiles" of exposure level without ever presenting the outcome of the continuous variable model as an Odds Ratio (OR). So, the reader is left to estimate what such an OR might be from her "quartile" ORs. She then calculates the p-values for the trend of her analysis with a method that assumes linearity of response to the variable. Yet from looking at the ORs for "quartiles" it is apparent that many of the OR trends do not show a linear trend, thereby violating the assumption of her method.

It is still possible, however, to get some feel for the relative risks from drinking water exposure to fluoride. For males the lower "quartile" group shows a borderline statistically significant increased risk OR of 2.8 (95%CI 1.0-8.1). For females the OR is even higher and statistically significant at 10.5 (95%CI 1.2-91). For both males and females in the higher "quartiles" of exposure, the ORs are no longer significant, but the risk for osteosarcoma generally stays above 1.0. If, instead of breaking the data into "quartiles", it had been broken into just "exposed" and "unexposed", it is quite possible the exposed group would have a significantly elevated risk for osteosarcoma compared to the unexposed group.

The best way to resolve this question would be for Gelberg to share her data so that researchers could see whether other methods of analysis might reveal associations that her limited approach failed to uncover. Another benefit of sharing this data is because it is some of the only data for which detailed histories of fluoride exposure are available. This means her data could be reanalyzed looking at age-specific exposure effects as in Bassin's study. Re-using Gelberg's data as a check on Bassin's findings would be much more efficient than the expensive and time-consuming process of collecting new data.

Gelberg 1997; Dental x-rays as possible confounder for osteosarcoma

In looking for other possible risk factors for osteosarcoma, Gelberg (1994) found that a history of exposure to dental x-rays was significantly related to the development of osteosarcoma (OR 4.0; 95%CI 1.3-12) . Dental x-rays were, in fact, one of the few variables Gelberg examined that had an effect reaching statistical significance. While Gelberg does not provide data on the number of cases who had osteosarcoma of the jaw or skull (sites where osteosarcoma is known to develop in humans), it would be interesting to see if there was any correlation between the use of dental x-rays and osteosarcoma of the jaw/skull. If a correlation does in fact exist, it would be important to determine if this correlation impacted

the fluoride analysis. In other words, if the fluoride analysis was limited to only those children with osteosarcoma at other bone sites, would the OR values shift? If so, in what direction?

Whether or not controlling for dental x-rays impacts the fluoride analysis, we believe the finding is of public health significance in and of itself, and are surprised to see that this is the only positive finding from Gelberg's thesis that was not mentioned in her two published papers (Gelberg 1995, 1997).

Bassin 2001

See Part I of our submission for details on Bassin's methods and findings.

2.3 Studies that found no association or inconclusive results

Hrudey 1990

Small sample size coupled with no simultaneous analysis by sex and age means this study would not have sufficient statistical power to reveal a risk that was increased only for young males.

Mahoney 1991

This study of osteosarcoma in New York State employed inaccurate fluoridation status assignment based only on county of residence. Subjects living in three counties containing a large non-fluoridated city were assigned "non-fluoridated", while all other counties in the state were assigned "fluoridated". The counties assigned "fluoridated" status, however, were only on average 56% fluoridated, although a sub-analysis limited to urban counties may have had better precision.

Another possible weakness of this study was the exclusion of all cases from New York City. New York City contains almost half the population of NYS, is 100% fluoridated, and contains a larger proportion of non-whites. Gelberg's 1994 study from the same NY Cancer Registry found that non–whites had significantly higher rates (OR of 5.7, 95%CI 1.7-19) of osteosarcoma. In addition, Freni (1992) found NYC to have the greatest rate increase for young males amongst all cancer registries in the US, Canada, Northern Europe, the UK, Australia, and New Zealand. By excluding NYC, Mahoney deprived this study of a large population, well characterized by fluoridation status. This study also used a large age range category (0-30 years old). From imprecise fluoridation classification and wide age range this study had a high degree of misclassification which limited its statistical power.

McGuire 1991

This case-control study had a very small sample size due to exclusion of many cases because interviews could not be obtained from subjects. Very large age category (0-40 years old) means cases of adult osteosarcoma dilute the juvenile cases. Findings of no statistically significant effect are not surprising given these limitations in study design.

Freni & Gaylor 1992

Despite the title of this paper, and the wording of the abstract, we believe the data compiled by Freni & Gaylor (1992) does not "erode the basis" of the fluoridation/bone cancer relation as the authors claim.

In their analysis of bone cancer trends in Europe and North America, Freni & Gaylor (hereafter F&G) generated several interesting findings, many of which were not appreciated at the time due to a lack of information on water fluoridation status in several of the regions surveyed. In their analysis, F&G found that among all the regions surveyed, the greatest increase in bone cancer incidence occurred among young males and females (ages 10-29) in the United States which has the greatest proportion of fluoridated water of all the regions (US ~60%, Canada ~40%, N. Europe <2%, UK ~10%).

Europe, which unbeknown to the authors does not practice water fluoridation to any significant extent, had considerably lower rates of increase of bone cancer than the U.S., an interesting finding in light of the disparity in fluoridation status between the two regions.

Of interest also, is that while F&G reported considerable inconsistency in the bone cancer trends in the fluoridated versus unfluoridated areas of U.S. and Canada, they did find that "The CR 10-29 (cumulative risk for 10-29 year olds) and CR-74 (cumulative lifetime risk), ... for the United States and Canada, showed more increases in fluoridated registry areas than in the non-fluoridated areas."

Hence, in addition to finding that bone cancer time trends among 10-29 year olds were two to four times greater in the U.S. and Canada (fluoridated) than in Europe (unfluoridated), F&G also found that in the United States and Canada the increase in bone cancer was more frequent, although not consistently so, among the fluoridated areas.

These findings do not prove the fluoridation/osteosarcoma relationship, but they certainly don't "erode the basis" of such a relationship, particularly when considering some of the limitations inherent in the data. Limitations of the data, much of which the authors acknowledged, include:
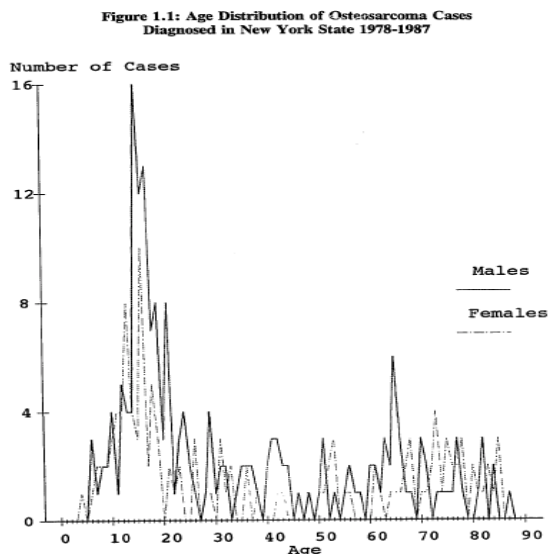
- **No knowledge of fluoridation status for half of their study populations and crude measures of fluoridation status for the rest**. Freni & Gaylor apparently did not know that all the northern Europe and UK countries are minimally fluoridated with less than 10%. For the US and Canada they assigned "fluoridated" status to entire states/provinces or large urban areas if more than 50% of the water in that area was fluoridated. "Non-fluoridated" status was assigned if less than 50% was fluoridated. This is the crudest assignment methodology of any study to date and will lead to widespread misclassification.

- **Broad age brackets (10-29 yrs) for young males.** The broad age brackets may have both 1) blurred the effect for the less than 20 year olds (the target population of greatest concern), and 2) prevented its ability to detect age-specific effects.

- **Data on bone cancer in general, versus osteosarcoma in particular.** Assuming fluoride only increases the incidence of osteosarcoma, the use of bone cancer data may have muted out the relationship between fluoride & osteosarcoma.

- **No means to control for the "diffusion effect" in U.S. and Canada.** Children in unfluoridated regions of Canada and the US frequently consume fluoridated water in the form of processed foods and drinks made in fluoridated regions. This poses a dilemma for all ecological studies on fluoridation in these countries, and may have in turn blurred the differences in F&G's analysis of fluoridated and unfluoridated regions in the U.S/Canada. (The existence of a diffusion effect within the U.S. makes comparisons with unfluoridated nations in Europe of particular interest – although it should be borne in mind that Europe does have a diffusion effect, albeit to a lesser extent than the U.S., due to the presence of fluoridated salt in some regions and widespread use of fluoridated dental products.)

McGuire, Douglass, et al 1995 (abstract only)

This preliminary analysis by McGuire and Douglass et al. (hereafter M&D), is particularly illuminating in light of Bassin's (2001) re-analysis of the data.

M&D conducted a national case-control study (147 cases, 248 controls) and assessed the *non-matched* average fluoride levels in the drinking water of the cases versus controls, finding no significant difference (0.49 ppm versus 0.47 ppm respectively). An additional, *non-matched* analysis of the percentage of cases/controls consuming water with > 0.7 ppm again failed to reveal any significant difference.

We are puzzled that the authors proffered this non-matched comparison for several reasons. 1) By simply taking the average F exposure of all cases and comparing it to the average F exposure of all controls they abandoned any matching between individual case-control pairs. 2) By not controlling for age or sex, it would be difficult to find any meaningful results, since it is now well known that osteosarcoma incidence is very age-dependent and has different rates between males and females as shown in this figure from Gelberg (1994):

Figure 1.1: Age Distribution of Osteosarcoma Cases Diagnosed in New York State 1978-1987

In the abstract, M&D also note that a conditional logistic regression of the data would be forthcoming, however, it has yet to be published. Thus, as it stands, the 1995 abstract remains the only information that we have on Douglass et al.'s ongoing, NIH-sponsored, 13-year research project on fluoride/osteosarcoma other than Bassin's 2001 thesis which has yet to be published and has yet to be made available, despite requests by NRC and others .

Meanwhile, what makes the M&D analysis of 1995 particularly instructive is that Bassin (2001) used this same dataset when conducting her analysis. Since M&D and Bassin reached markedly different conclusions, we believe it is useful to demarcate the difference in methodologies utilized in these two analyses. We believe the Bassin analysis is superior because it examined age-specific exposures which had been postulated by previous researchers based on biological mechanism considerations (Cohn 1992; Lee 1993, 1996). In contrast to M&D, Bassin:

- Maintained matching in her analysis
- Limited her analysis to cases/controls < 20 years old
- Controlled for sex
- Controlled for fluoride exposure as a function of age

Thus, the same data set in the hands of different researchers yielded no association when superficially examined, but yielded a clear association when analyzed with careful control for confounding factors and in a manner that was most sensitive for revealing an age-specific effect.

Moss 1995

In this study, age matching of cases with controls was only to within ±7 years for the youngest age group and only within ±5 years for older categories. This poor matching and control for age will reduce the ability of this study to uncover an association with osteosarcoma of which the incidence rates are highly age dependent. No interviews taken so fluoride exposure assessment was based only on residence at time of diagnosis. In addition, controls were cancer patients with cancers other than osteosarcoma. If fluoride increases the risk for non-bone cancers, this choice of controls might weaken the study. This was the only study of fluoride and osteosarcoma to control for alpha-radiation exposure from drinking water. However, the determination of alpha-radiation exposure was based solely on whether a single public water system measured higher than 9 pCi/l in the county of residence. Such a coarse exposure method will likely lead to extensive miscalssification.

3. Hoover, Douglass, & Whitford: Headed in the Wrong Direction?

While we await with interest the findings of the upcoming paper from Hoover, Douglass and Whitford (Douglass submission to NRC, January 2004), we are nevertheless concerned that their methods may make their analysis incapable of detecting the age-specific effect suggested by Bassin's research.

Based on the abstract, it appears Hoover, Douglass and Whitford are conducting cumulative lifetime fluoride exposure analyses by measuring the fluoride content of bone and toenails from some of their cases and controls. While such an analysis may be an appropriate tool for assessing some chronic health effects from fluoride, it is unlikely to be capable of uncovering effects produced by previous age-specific exposures. Unlike a tree, the bone has no "tree rings" that can allow for a retrospective analysis of when the accumulated fluoride was retained. Thus, barring a separate age-specific analysis, the Hoover, Douglass, and Whitford study will not be able to speak to the central issue raised by Bassin's dissertation. That, in our opinion, would be a step in the wrong direction.

4. Radium as a Possible Confounding Factor

Recent studies suggest that low levels of radium found naturally in some drinking water may cause osteosarcoma (Cohn 2003; Finkelstein 1994, 1996; Moss 1995). The findings from these studies suggest that radium exposure is an important factor that should be controlled for in any study exploring the relationship between fluoride and osteosarcoma. To date, however, only Moss 1995 has tried to control for radium and this therefore represents one of the weaknesses in the research to date, both in the studies finding an effect and in the studies finding no effect.

The converse weakness can also be found in most of the radium papers. Except for Cohn's 2003 paper and Moss' 1995 paper, none of the radium/osteosarcoma studies have controlled for fluoride. This, we believe, is one of the notable strengths of Cohn's 2003 analysis of radium/osteosarcoma: it excluded any town with artificially fluoridated water. Whether this impacted the results in any way is not possible to determine, but it may have increased the sensitivity of the results. Previous papers on radium in drinking water and osteosarcoma did not control for fluoride (Finkelstein 1994, 1996; Guse 2002; Petersen 1966).

The study by Petersen (1966), in particular, which detected a relationship between bone cancer and radium in water in Iowa and Illinois, may have been confounded by fluoride, since a later study by Lynch (1987) specifically reported a significant correlation between naturally occurring radium and naturally occurring fluoride in Iowa water supplies. Whether this correlation between natural radium and natural fluoride holds true for other areas of the country is unclear (Lynch 1987).

Understanding the fluoride/radium relationship seems particularly important in Cohn's studies from New Jersey (Cohn 1992, 2003). In 2003, Cohn reported a significant relationship between radium in water and osteosarcoma in males over the age of 25, but not in females or males under age 25. In light of this finding, it would seem important to determine what kind of overlap, if any, exists between the areas studied in Cohn's 1992 study on fluoride and Cohn's 2003 study on radium. A rough comparison of the counties examined in both studies reveals some overlap on the county level; however, whether this overlap on the county level translates into overlap on the community level can not be determined based on the data presented.

There are, however, several lines of evidence suggesting that the fluoride effect found in the 1992 study was not simply a result of confounding by co-existing radium in the fluoridated water supplies:

- The age range impacted in the fluoride study was different (<20 year olds) from the age range impacted in the radium study (25+ year olds). This is pertinent because the study populations for Cohn's 2003 study and Cohn's 1992 fluoride study were drawn from the same New Jersey Cancer Registry. Hence, there would have been little overlap in actual cases since 25+ aged cases drove the radium findings while <20 aged cases drove the fluoride findings.

- No overlap in *artificially* fluoridated towns exists between the two studies as Cohn 2003 specifically excluded artificially fluoridated towns from his radium analysis.

- Since *naturally* occurring fluoride has been associated with increased levels of radium (Lynch 1987), there is a possibility that Cohn (1992) found an osteosarcoma effect in some *naturally* fluoridated areas that was actually a result of the radium. However, Cohn (1992) did a separate analysis excluding the one county with *naturally* fluoridated water (Gloucester County), and the Relative Risk (RR) was virtually undiminished.

- The RR values found in the fluoride study were higher than the RR values found in the radium study (maximum RR 8.0 fluoride study in <20 year old white males versus maximum RR 6.2 radium study in 25+ old males).

Thus, while the evidence suggests that radium did not confound Cohn's 1992 fluoride study, we do believe it would be useful to more clearly determine the relationship between fluoride and radium in any osteosarcoma studies, both in Cohn's two analyses and all other analyses.

Towards that end, one possibility which deserves consideration is that fluoride and radium may have interactive/synergistic effects. One such interactive relationship could be a situation where fluoride facilitates the uptake/retention of radium into bone, as hypothesized for fluoride and strontium 90 by Kerwin (1958).

4.2 Silicofluorides & Radium?

It has been suggested by some that one explanation for the observed relationship between water fluoridation and osteosarcoma is the presence of radium in the chemicals used to fluoridate water (hydrofluorosilicic acid and sodium silicofluoride). Upon closer analysis, however, this explanation seems untenable for the following reasons:

- Recent analyses of the radium content in the undiluted fluoridation chemicals have failed to detect radium levels above 4pCi/L (NSF 2000). This is the concentration of radium *before* dilution of the silicofluorides into the water supply.

- If we assume a dilution factor of between 150,000 and 200,000, we can expect a post-dilution concentration of five orders of magnitude lower than 4 pCi/L. To put this figure in perspective, the

studies detecting an increased risk of osteosarcoma from radium in water have been based on radium levels exceeding 1 pCi/L.

- Thus, barring any major deficiency in the analyses of radium in the silicofluoride product, the relationship between the radium in silicofluorides and osteosarcoma is unlikely.

4.3 Sex-specific effect observed in radium studies

It is interesting to find in the recent studies on radium/osteosarcoma that a sex-specific effect has been reported in most studies. The studies by Finkelstein (1994, 1996) and Cohn (2003) found an increased risk of osteosarcoma in radium-exposed males, but not in radium-exposed females.

These findings suggest that a sex-specific effect for chemical-induced osteosarcoma is not unusual. As such, they may bolster the significance of the consistent sex-specific effect found in the fluoride/osteosarcoma studies (NTP 1990; Hoover 1991; Cohn 1992; Bassin 2001) where males seem to be the sex at greatest risk.

4.4 Duration-dependent effects in radium studies

Another interesting finding on radium is the reported absence of a duration-dependent effect in the Ontario studies by Finkelstein (1994; 1996). According to Finkelstein and Kreiger (1996):

> "A previous investigation in Ontario found an association between low level exposure to radium in birthplace drinking water and an increased risk of death from bone sarcoma (Finkelstein 1994). The study we report here supports the earlier findings in relation to birthplace exposure, but the results of the analyses with lifetime exposure histories are not significant, and do not show a trend with increasing dose. Absence of a dose-response trend would tend to argue against causality. Our findings are thus compatible with the presence of a threshold and the absence of a risk at low doses, but they might also reflect inadequate statistical power to measure a true risk at environmental exposure levels."

Finkelstein's findings are interesting and indicate the possible existence of an age-specific effect from radium during the early years of life. If such an age-specific effect does in fact exist for early-life exposure to radium, Finkelstein's analysis would underscore the limitations of using a dose dependent analysis (e.g. Hoover 1991; Gelberg 1994, 1995) for detecting the risk of a chemical with an age-specific effect.

It should be noted, meanwhile, that in Cohn's 2003 study, an increased risk was found from cumulative radium exposure in males > 25 years of age, thus suggesting that, in addition to a possible age-specific effect for childhood osteosarcoma, it may have a cumulative effect for adult osteosarcoma as well.

5. More on biological plausibility

In Part 1 of our submission, we discussed the biological plausibility of a fluoride-osteosarcoma connection, especially for young males. We argued that it was plausible because: 1) the bone is the site where fluoride accumulates, with the accumulation rate higher during periods of bone formation; 2) fluoride is known to stimulate bone growth; 3) unscheduled or accelerated growth in tissues can lead to cancer, especially if it is accompanied by genetic damage in the dividing cells; and 4) *in vitro* research has established that fluoride can cause genetic damage.

Whenever cells divide there is a risk that mutations may occur, and mutations in the sensitive spots (such as tumor suppressor genes) can lead to uncontrolled cell growth and then to cancer. This risk would be increased if the agent that stimulates cell division also causes damage to the genetic material. There is a growing body of evidence that fluoride does this.

In 1996, Mihashi and Tsutsui were able to demonstrate that fluoride caused chromosomal aberrations in a time and dose dependent manner in cultured cells derived from the vertebral bones of the same strain of rats (F344/N) used in the NTP rat-osteosarcoma study (Mihashi & Tsutsui 1996). Effects were observed at 4.3 ppm fluoride, a level which may be reached in key microenvironments in bone *in vivo*. They argued that their results, "…demonstrate that NaF is clastogenic to rat vertebral body-derived cells, providing a mechanistic basis for NaF to induce osteosarcomas in NaF-treated rats."

Mihashi and Tsutsui's results are consistent with the majority of *in vitro* studies on fluoride's genotoxicity. According to the National Toxicology Program,

> "In summary, sodium fluoride is mutagenic in cultured mammalian cells and produces transformation of Syrian hamster cells *in vitro*. The reports of *in vitro* cytogenetic studies are mixed, but **the preponderance of evidence indicates that sodium fluoride can induce chromosome aberrations and sister chromatid exchanges in cultured mammalian cells**" (NTP 1990; p. 20)

As noted by Freni & Gaylor (1992):

> "[T]he carcinogenicity of fluoride is consistent with growth stimulation of osteoblasts, unscheduled DNA synthesis by human fibroblasts, and transformation of embryonal hamster fibroblasts into transplantable sarcoma cells. Osteoblasts are differentiated fibroblasts, and fluoride is accumulated in the skeleton. Therefore, osteosarcoma would be the natural target effect to look for in a cancer bioassay of fluoride, and an excess of osteosarcoma in rats exposed to fluoride in drinking water clearly confirms an *a priori* hypothesis."

6. Fluoride and other cancers

As for the possibility that fluoride may cause other types of cancer, it should be noted that Battelle Laboratories, the NTP contractor for the rat studies, initially reported a dose-dependent increase in hepatocholangiocarcinoma (a rare liver cancer) in both sexes of the fluoride-treated mice, but these were later re-classified by a separate panel convened by the NTP. Scientists at the EPA strongly objected to this re-classification, particularly since Dr. Melvin Reuber, who first discovered hepatocholangiocarcinoma as a separate entity, reviewed the slides and concurred that they were the correct diagnosis.

Because of the importance of NTP's tumor slides regarding fluoride's potential as a carcinogen, and in light of recent findings of an association between fluoride and a variety of non-bone cancer sites (Tohyama 1996; Takahashi 2001; and Grandjean 2004), we would urge the NRC panel to request that these slides be made available for an independent review.

The possibility that fluoride can cause cancer in humans is supported also by recent findings of fluoride genotoxicity in humans. While there was virtually no research on this subject before 1993, several research teams in India and China (Sheth 1994; Meng 1995, 1997; Wu 1995; Joseph 2000) have reported an increased incidence of mutagenic effects (e.g. increased sister chromatid exchange in peripheral blood lympochytes) in fluoride-exposed *humans*. These results are interesting when considering the *in vitro* research from Kishi (1993) who has shown that primate cells (great apes and humans) are more sensitive to fluoride's genotoxic effects than rodents. Kishi's work may help explain why human cells – exposed to considerably less fluoride than typically used in *in vitro* research –

exhibited genotoxic effects. Other studies, however, have failed to replicate these findings in humans (Li 1995; Jackson 1997).

7. Discussion and conclusions

7.1 Bassin found a statistically significant relationship between osteosarcoma in young males and exposure to fluoride. The critical ages for exposure appear to be their 6th, 7th, and 8th years, i.e. during a window of vulnerability when boys' mid-childhood bone growth spurt is taking place.

7.2 Bassin found statistically significant increases in osteosarcoma risk among children exposed to as little as 0.3 to 1 ppm, thus suggesting that, if there's a threshold for fluoride-induced osteosarcoma, it lies below 1 ppm.

7.3 Several reasons can explain why older studies may have missed the relationship that Bassin found. This would have occurred if they: 1) failed to control for age of exposure to fluoride; 2) included in their controls people exposed to fluoride between 0.3 and 1 ppm fluoride; 3) had insufficient sample size 4) had a high degree of exposure misclassification or 5) didn't control for important covariates.

7.4 Several previous studies which concluded they could not find an effect, may in fact, upon closer analysis, show an effect (Maurer & FDA 1990, Hoover 1991, Freni & Gaylor 1992, and maybe Gelberg 1994, 1995).

7.5 Past, ongoing and future studies need to utilize age-specific analyses. We hope, in particular, that Gelberg et al. share their data for a re-analysis.

7.6 Combining Bassin's findings with: 1) the acknowledged biologic plausibility of a fluoride-osteosarcoma link; 2) the NTP animal findings, and 3) a re-interpretation of other epidemiological studies based on Bassin's research, the conclusion must be that the preponderance of evidence indicates a probable relationship between osteosarcoma in young men and exposure to fluoride.

7.7 We note that Bassin's work has not been made public for 4 years since its completion. Douglass, despite over 8 years of NIH grants, has never published anything (outside of the 1995 abstract), regarding his team's osteosarcoma research. Moreover, he has yet to publicly mention Bassin's findings, despite being her thesis advisor and the grant Principal Investigator under which her work was performed, and despite submissions given to the NRC and the Royal College of Physicians in London where he has specifically discussed his research of osteosarcoma. We recommend that Douglass et al. explain why they have chosen to ignore Bassin's findings.

7.8 The summary of Douglass and Hoover's current research provided to the NRC describes a new methodology, claimed to be the best to date, but which will be even less amenable to the needed age-specific analysis. Bone F levels and toenail F levels can not be used to assess exposure during specific ages of life. We ask that the NRC recommend that Douglass and Hoover share their data with others so that age-specific (or other approaches) can be independently assessed.

7.9 In light of Sheth (1994), Wu (1995), Tohyama (1996), Joseph (2000), Takahashi (2001), Grandjean (2004), and possibly Hoover (1991), the possibility that fluoride may cause other cancers in addition to osteosarcoma should be further explored. In this context, we would also ask the NRC panel to request the slides of hepatocholangiocarcinoma and other cancer slides from the NTP (1990) animal study so that they may be reviewed by an independent panel.

7.10 In light of recent studies implicating low levels of radium with osteosarcoma, it will be important for research on fluoride/osteosarcoma to control for radium exposure, and vice versa. An analysis of the two studies from New Jersey (Cohn 1992, 2003) indicates however, that radium was most likely not a major confounding factor in the fluoride study.

7.11 The possibility that the radium content in silicofluorides may play a role in the fluoridation/osteosarcoma relationship seems unlikely based on the concentrations of radium that would be expected after dilution into water.

Postscript: Additional studies

In researching the papers on the possible connection between fluoride and osteosarcoma, we came across an abstract for an interesting study from Kenya. Bovill et al. (1985) published their findings on 251 cases of osteosarcoma reported between 1968 and 1978 in Kenya. They reported a much higher than expected incidence in the Eastern province, whereas the Nyanza Province and the adjacent Western Province showed a lower than predicted incidence. They concluded that there was no genetic variation which could explain this difference but suggested some "geomedical variable" might. While waiting for a full copy of this paper to see if they offered any suggestions as to what this "geomedical variable" might be, we sought information on what the fluoride exposure might be in these different provinces. We found a paper by Chibole (1987) on dental fluorosis rates in Kenya. The high osteosarcoma Eastern province had high dental fluorosis rates (the second highest county in the country) affecting 47.1 % of the population, whereas the low osteosarcoma Nyanza and Western provinces had much lower dental fluorosis rates, affecting 20.2% and 11.7% respectively.

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