Review of Gelberg 1994, 1995 for

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Gelberg's case---control study of fluoride and osteosarcoma is one of the most important to date, since it used a population---based recruitment design for subjects rather than a hospital---based recruitment design [Gelberg 1994, 1995]. Further, it had a relatively large sample size and obtained lifetime history of fluoride (F) exposures. Although the study found positive associations between water fluoridation and risk of osteosarcoma, Gelberg dismissed these findings. A careful examination of this study suggests that Gelberg's reasons for dismissing the positive findings are unwarranted.

Gelberg's study also had several important limitations which may have led to biased effect estimates. Some of the limitations would have biased her results toward the null (no effect), while others would have biased them away from a positive result, such that the risk of osteosarcoma from fluoride could not just be reduced to null but potentially even beyond so that fluoride would appear to provide a spurious protective effect against osteosarcoma.

Given these issues, a more valid interpretation of her results suggests she did find a positive association with increased water fluoride exposure connected to higher risk of osteosarcoma.

Each of the limitations in Gelberg's study, and their implications for interpreting her study, will be discussed in detail.

1. Only exposure from water fluoride had reliable information. Water fluoride was based on residential history which was not subject to recall bias or poor recall. Other F exposures (F toothpaste, F supplements, F dental office treatments) were only crudely estimated based on questionnaire responses. For example, F toothpaste exposure was based solely on number of brushings per day. No time history of number of brushings, and more importantly, no information was available on how much toothpaste was used per brushing and how much may have been swallowed. In young children, especially, there is a very wide variation in how much is swallowed, which will outweigh differences in exposure based on the number of brushings per day.

Therefore, we focus on the Gelberg's results for water fluoride. All of them show a positive association between estimated lifetime F intake and risk of osteosarcoma.

Some ORs are statistically significant. Yet Gelberg dismissed these findings with the argument that the water F results were not consistent with those from the other sources of F. She thereby ignored the fact that the other sources of F had unreliable exposure estimates.

She also dismissed the positive water F findings by saying they did not show a monotonic increasing dose---response. That is, as lifetime F intake increased, the risk of osteosarcoma did not increase.

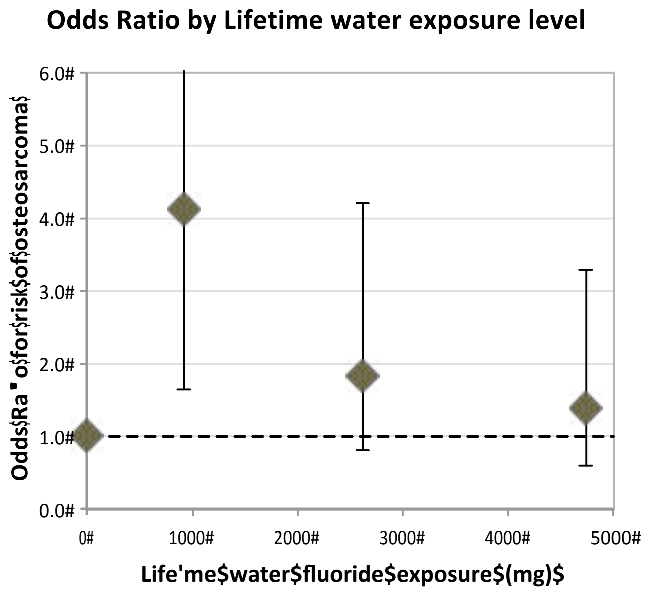
This brings us to the second important issue with Gelberg's study.

2. Gelberg made no adjustments for age in any of her analyses. This caused substantial bias toward the null in her analysis, for two reasons.

2.1. Since age is correlated with F exposure in Gelberg's sample, it is confounding the relationship between F exposure and osteosarcoma risk. The confounding was in the direction to bias the result away from a positive effect. If strong enough, such confounding can even result in a spurious protective effect of F against osteosarcoma.

When Gelberg's results are considered in detail, they show evidence that failure to control for age in fact did result in a bias away from an effect. Gelberg presented results for F exposure stratified into four exposure levels. The lowest level was the reference level and those subjects had zero lifetime water F, according to her method of assigning lifetime F intake. Each succeeding level had higher lifetime water F intake. The trend in odds ratios for each level is counter to what would be expected if there were a monotonic positive dose---response effect of F on osteosarcoma risk. Figure 1 shows the odds ratios for each group.

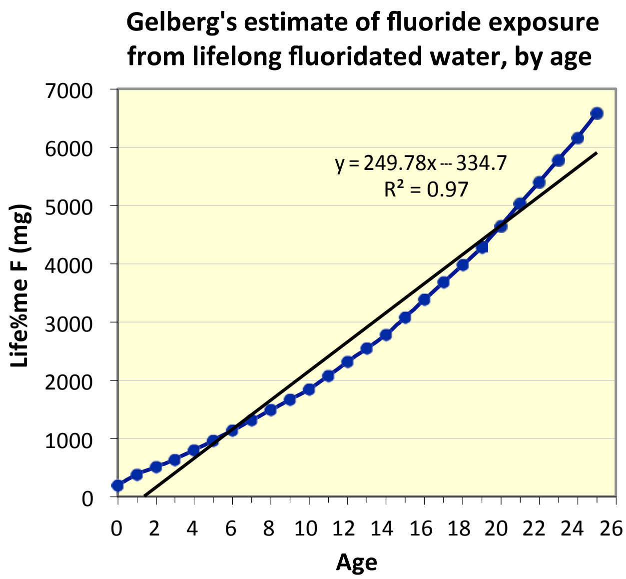
Figure 1. Gelberg ORs by water F exposure level group. Males and females combined.



The OR for all the exposed groups is above 1.0 and the lowest of the exposed groups has an OR that is statistically significant (4.13, 95% CI 1.65, 10.35). This result is for males and females combined.

The unexpected trend of the ORs downward with increasing F exposure may be an artifact of Gelberg's failure to control for age. Gelberg did not report the correlation between age and exposure, but examination of her method of assigning total water F exposure reveals that there is likely to be a strong positive correlation. She assigned total lifetime F by assuming each child had either fluoridated water with a concentration of 1 mg/L F or unfluoridated with a concentration of 0 mg/L F. She then used published values of water intake by year of age to calculate how many milligrams of F would be ingested with water each year. Older ages have about twice the water intake as younger ages. There is a very strong correlation between age and water intake, and thus estimated lifetime water F intake (R^2 = 0.97), as shown in Figure 2 and Appendix 7---B. Gelberg summed the F intake for each year of life to arrive at the lifetime F intake for each subject. With this method of estimating exposure, older children will always have a potential for having higher total lifetime intake than younger children. To illustrate we can look at children who lived their entire lives at fluoridated residences. A 20 year old will have roughly twice as much total F intake as a 10 year old.

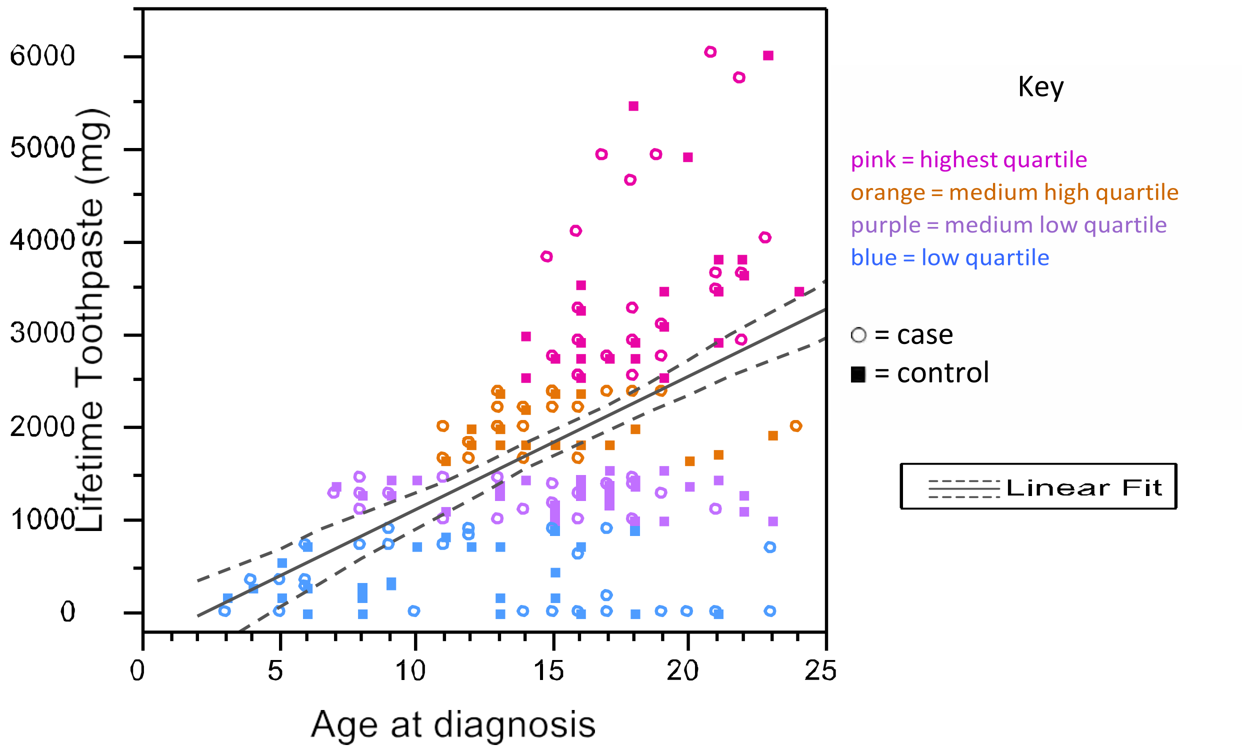
Figure 2.



We were able to check that Gelberg's method of assigning exposure would result in a positive correlation between age and exposure in her subjects, using Gelberg's own data. Gelberg provided part of her data to us. We received individual subject age and toothpaste exposure data, but not water F data. Gelberg assigned lifetime toothpaste F intake similarly to how she assigned lifetime water F intake so it can be a proxy for water F in our analyses. Gelberg used questionnaire responses on the ages the child used fluoridated toothpaste and the frequency of tooth brushing, together with literature estimates of the average amount of toothpaste swallowed at each age, she calculated a lifetime F exposure in milligrams. Similar to her water F estimation method, older children will have the opportunity to achieve higher lifetime F intake than younger children.

Examining the relationship between age and lifetime toothpaste F intake, we found a strong correlation in Gelberg's original data, shown in Figure 3.

Figure 3. Correlation between lifetime toothpaste F and age in Gelberg's subjects.



The linear regression of age and lifetime toothpaste F is highly statistically significant ($p<0.0001$) and has a moderately strong correlation coefficient (adjusted $R^2 = 0.27$). As can be seen, the highest quartile of toothpaste F can only be achieved by those older than 13. The next highest quartile can only be achieved by those over age 10. But the lowest quartile can include all ages.

Therefore, the absence of adjustment for age in Gelberg's analysis will create the strongest bias toward the null in the highest exposure group. Bias will decrease as exposure decreases, reaching no bias in those with zero exposure.

The correlation between lifetime water F and age is likely to be similar to that of lifetime toothpaste F, and may thus explain Gelberg's decrease of OR with increasing lifetime water F exposure as an artifact of her method of estimating water F exposure and her failure to adjust for age. The true relationship may be an increasing risk with increasing water F exposure.

2.2. The second result of failure to control for age arises in Gelberg's analyses that included matching on age (conditional logistic regression of matched cases and controls). When matching on a variable is included in a regression model, that variable must be controlled. Rothman explains why failure to control for matching variables will lead to bias of the results to the null [Rothman 1998].

Matching forces the control distribution to be similar to the case distribution. When the matched variable is associated with the outcome, this can result in severe selection bias. The OR will become 1.0 if age and exposure are perfectly correlated and age is not controlled. Furthermore, the better the correlation between age and exposure, the closer to 1.0 the crude result will be. In Section 2.1 above, we showed that age and lifetime water F exposure are likely to be relatively strongly correlated.

The more closely the ages are matched, the greater the bias toward the null, when there is no adjustment for age.

Gelberg's controls were age---matched to her cases by year of birth, so that all controls had an age within 1 year of the case to which they were matched. This is tight age matching and can result in a large bias toward the null. For comparison Bassin's study used age matching within 5 years of age, which resulted in a 10---year span. Furthermore, Bassin did control for age in her analyses, so her study did not suffer from the error of neglecting to control for age in matched analyses.

3. Gelberg's study may have suffered from selection bias because her cases were not drawn from the identical population as her controls and the two populations differed in lifetime exposure to fluoridated water. Gelberg's cases were recruited from the New York (NY) State cancer registry, and were restricted to those diagnosed when they lived in NY State but outside of NY City. In contrast, controls were recruited from birth records of those born in NY State but outside of NY City. By the age when matched to cases, the controls could have been living in NY City, but the cases could not.

NY City is 100% fluoridated whereas NY State outside of NY City is only about 50% fluoridated. Controls would have zero chance of being born in NY City because of Gelberg's exclusion criteria. Controls would also be less likely to have lived their early years in NY City than cases. In contrast, cases could have lived their early years in NY City since the only geographic exclusion criteria was being diagnosed with osteosarcoma while resident in NY City.

With Gelberg's method of exposure assignment, early years of life contribute substantially less to lifetime water F exposure than later years of exposure. This is because younger children drink less water than older children do, as explained in Section 2.1 above.

Therefore, the control group, who had a lower chance of living their early years in 100% fluoridated NYC and a higher chance of living their later years in NYC, would have biased lifetime water F intake upward relative to the cases.

This would result in effect estimates (ORs) being biased away from a positive effect.

There is no indication Gelberg considered or tried to control for this potential selection bias.

4. Gelberg did not conduct any age---specific exposure analyses, although she had information to do so. If Bassin's [2006] age---specific exposure analyses findings are true, then failure to conduct age---specific exposure analyses will reduce the power of the study to detect an effect, since only exposures during a narrow developmental period of life (ages 6---8) contributed strongly to the risk of osteosarcoma from fluoride. Rothman and the EPA explain why age---specific exposure analysis is more likely to detect an effect [Rothman 1981, EPA 2005].

5. Gelberg made several errors in interpreting and reporting her data. She switched males with females and did not recognize this error. In both her 1994 dissertation and in her 1995 published paper she states there were more females than males. This is highly unlikely based on extensive literature where males always have higher rates of childhood osteosarcoma than females [Ottaviani 2010]. Yet Gelberg discussed this apparent contradiction between her data and the existing literature in her dissertation, without recognizing the contradiction was due to her error in switching males with females.

She also switches cases and controls in one section of her result tables [Gelberg 1995, Table 2, the numbers of cases and controls in the last section under "Total fluoride, mg" are reversed].

Gelberg stated that neither of these mix---ups carried through to her final analyses and results (personal communication from Gelberg). The numbers of males and females reported in her published data tables show there were in fact more males than females [Gelberg 1995] and the OR reported for the switched cases and controls does appear to have used them in their proper roles. However, these errors have never been corrected in any errata, and can confuse those reading her papers. We therefore felt it was important to point them out to the NTP reviewers.

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