Author’s response to reviews

Title: Developmental Fluoride Neurotoxicity: An updated review

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Developmental Fluoride Neurotoxicity: An updated review
Response to reviewers

Reviewer #1: The review capably considers recently available information and is highly pertinent to the public health.
Response: Thank you!

Reviewer #2: Peer Review Comments by J. William Hirzy, Ph.D. on Developmental Fluoride Neurotoxicity: An updated review

This is a very important paper that clearly summarizes the latest evidence on this subject. It also states the implications of that evidence for public health in a matter of fact way. In that connection, the author also does an excellent job of noting the latest publications by those who oppose action to lower exposures to fluoride.

He brings to the reader's attention evidence of fluoride's toxic effects on thyroid function, pointing out how these effects may be contributing to neurotoxicity that is manifested in ways other than diminished intelligence.

I was impressed by the author's finding publications involving use of radio-fluoride in cancer treatment that showed fluoride appeared to accumulate in brain areas responsible for memory and learning.

The text on the relationship between maternal serum fluoride levels and umbilical cord serum levels was fascinating, illustrating how measurement of maternal serum levels could be used as indicative of fetal exposure. (In the paper of which I was co-author and is cited in this paper, we postulated that in utero exposures could be a significant issue.) Relative to the parenthesized comment, the paragraph from lines 437-442, particularly the last sentence
indicating the lack of evidence for a post-natal fluoride exposure-related adverse effect (albeit in a very small sample) on IQ was very interesting. But….

The paragraph starting at Line 569 points out the importance of more work to tease out other possible time windows of vulnerability, pointing out how formula-fed neonates may be particularly vulnerable.

Further on this point, the author does a great service in pointing out the +6.2 IQ point advantage to breast fed vs. formula fed infants in the Broadbent et al. study.
Response: These comments are very much appreciated.

I found it a bit hard to grasp exactly what was being conveyed in this excerpt from lines 102-104 In adults, the fasting plasma-fluoride concentration, when expressed in micromoles per liter [µmol/L], is approximately equal to the concentration in the drinking water or in the urine expressed in mg/L. Does this mean, e.g., if the plasma fluoride level is x µmol/L, then the approximate concentration in drinking water or urine is x mg/L? Response: The interpretation is correct, as also indicated by the WHO reference provided.

Toward the lend of Line 455 the word "material" appears, and doesn't seem to fit. Response: We changed the word to “population”.

All in all this is a very impressive and important piece of scientific analysis. Additional Comment.

I would like to publish as a Comment (I would hope in the same Volume/Number) to the above paper when it is published:

One can take the excellent work reflected in Developmental Fluoride Neurotoxicity: An updated review (1) one further step into the realm of risk analysis. This would be to use the BMDLs (the lower one-sided 95% confidence limit of the Benchmark Doses in the linear mode) reported in that study to estimate a Reference Dose (RfD) for fluoride.

As defined by the U.S. Environmental Protection Agency, the RfD for a chemical is the daily dose, within one order of magnitude, that could be experienced for a lifetime without expectation of an adverse effect. Reference Doses are customarily used in standard setting efforts pursuant to various statutes by regulatory agencies.
If one assumes protection should focus on pregnant women (citation of the above publication) and a daily consumption of 2 liters/day of drinking water (2, Table 7.1), using a BMDL of 0.20 mg/L leads to 0.40 mg/day as a Lowest Observed Adverse Effect Level (LOAEL). Using a single Uncertainty Factor of 10 to convert the LOAEL to a No Observed Adverse Effect Level leads to an RfD of 0.04 mg/day.

If one uses the most conservative of the BMDLs reported in (1) viz. 0.13 mg/L, then an RfD of 0.03 mg/day obtains.

Response: I am aware of this possibility, but do not want to take the calculations as far as suggesting actual RfD values, as the RfDs will depend on agency default procedures and preferences, especially the magnitude of uncertainty factors and source attribution.

References


Reviewer #3: The work entitled 'Developmental fluoride neurotoxicity: An updated review' presented by Grandjean P is a good piece of work. In this article author have addressed the recent epidemiological studies of fluorideneurotoxicity in children and tried to highlight that elevated fluoride intake during early development can result in IQ deficits. In my view, presented work can be accepted after addressing following comments:

1. Some of sentences are not clear should be checked and re-written.
Response: These comments are much appreciated. I have updated the text and hope that all sentences are now fully intelligible and grammatically correct.

2. Some of the references have been repeated for instance 63 and 65, check and rectify.

3. Reference are non-uniformly drafted in context to abbreviation of journal name check it through some reliable sites.
Response: This error has been corrected, and the journal name abbreviations have been updated, thank you!
4. Mention the unit for urinary fluoride level in line 429.
Response: The unit (mg/L) has now been inserted in the beginning of the sentence.

5. Fluoride mediated toxicity is well known, author should highlight novelty of their work in conclusion. Most of the study has been discussed in light of 2012 study (Choi AL, Sun G, Zhang Y, Grandjean P: Developmental fluorideneurotoxicity: a systematic review and meta-analysis. Environ Health Perspect 2012, 120(10):1362-1368.) Updated findings should be mentioned successively in conclusion section with special emphasis to benefits to society.
Response: Thank you. You are correct that this review represents an update of the 2012 meta-analysis. The Conclusions section has been modified to emphasize the focus on new information. I hesitate to comment in further detail on benefits to society, as the risk management would likely involve a range of issues not covered in this review.

Reviewer #4: This manuscript reports a comprehensive assessment of fluoride related neurotoxicity. However, in its current format it is difficult to read, and needs to be reframed either as a comprehensive review of literature relating to neurotoxic effects of fluoride, or if remaining its current format, revised to clearly frame the recent epidemiological studies.

In the current format, while the abstract lays out a clear flow, the body of the manuscript does not seem to follow the abstract outline. The second paragraph (lines 53-58) lays out a somewhat confusing "roadmap", that should be clarified so that the reader knows the overall goals and structure of the paper. The paper currently lacks a Discussion section, and this should be added, with much of what is currently included in the Background and Results, moved into this section.

Response: Thank you for this suggestion. The manuscript follows the journal instructions and introduces plausibility aspects, such as fluoride toxicokinetics and occupational toxicology, before analyzing the developmental epidemiology data. My preference is to keep the plausibility aspects in the beginning, thereby setting the stage for an integrated assessment of the new epidemiological evidence. The second paragraph of the Background section describes the flow of the manuscript. With additional clarifications in the abstract and in the text, I hope that the roadmap is much less confusing now. The section on Plausibility and implications is intended to serve as a “Discussion” section, although the journal does not require this.

Specific Comments:

Abstract,

It should be clearly stated (if this is the case) that all of the studies reviewed since 2012 found a correlation between fluoride and neurotoxicity; or if otherwise, this should also be stated.
Response: This information has now been added.
Background

Information should be included that will allow the reader to understand the relevance of the continuing review of recent studies, as described in the Methods. This should be done without drawing conclusions; these can be made in the Discussions/Conclusion section. For example, information included in the Background describing how fluoride affects the brain, including learning and memory in animal studies, seems more relevant in a Discussion section where the reasons for changes in IQ might be discussed.

Response: Thank you for this suggestion. While it is a matter of choice, it seems more logical to introduce animal studies and other plausibility information in the beginning of the manuscript before embarking on the epidemiological evidence, thereby allowing a joint consideration of the new evidence from the different types of studies. I have made several changes in the text to guide the reader through the sections. Again, there is no formal Discussion section, but the scientific inference is considered under Plausibility and implications.

line 77, include a second more updated reference, such as the article published by Featherstone et al. in the Journals of the American Dental Association, 2000.
Response: Thank you for this suggestion. I have now added the more recent Featherstone reference.

Consider deleting the last sentence of the paragraph beginning in line 79, as the focus of this review is not to argue the benefits of water fluoridation for caries prevention. It could also possibly be moved to the discussion, where the data is put into context.
Response: Thank you, I agree. In response to this comment, I have moved the whole paragraph to the section on Plausibility and implications.

While information on fluoride crossing the blood brain barrier, and animal studies showing effects on learning and memory can be included in the background as a rationale to further assess neurotoxicity, this, and also most of the section on Experimental Neurotoxicity could be moved to the Discussion, where it adds support for the findings in the review of studies. For example the sentence in line 122, when moved to the discussion, can be used to point out that effects of fluoride, such as those found related to learning and memory in animal studies, could underline some of the findings of fluoride related effects on IQ.

Response: Again, as a Discussion section is not required, I believe that it is logical to include the plausibility background early in the manuscript as part of the justification for carrying out the systematic review. I have revised the text to make this clear and refer in more detail to the plausibility issues in the final section.

The sentence beginning on line 174, stating that most animals studies of subchronic exposure did not include neonatal exposures etc, should be referenced or deleted, as it may not be accurate.

Response: The point is that rodent pups are born at an earlier stage of development than humans and that the pups’ fluoride exposure is substantially decreased during the nursing period. I have now clarified this issue.
Methods

BMD and BMDL are defined and calculated, but then only 2 studies are mentioned? Does this mean that similar measurements are not available for the other studies? Please clarify.
Response: I have now included additional data in the Results section.

Results:

It is confusing to begin this section with additional background. The results section should begin at line 332 (?) with the total number of cross sectional and prospective studies that were identified for review, as outlined in the Methods?
Response: I understand, but these epidemiological studies were also identified through the search described in the Methods. I would not want to delete this material and can’t think of any better place to include this information. I have edited the text to clarify the line of thought.

It seems that the section "Occupational and Endemic area studies" should be moved in part to the Background section, and in part to the Discussion.
Response: Please see my above response.

Likewise, some the section on cross-sectional studies in exposed communities could be moved to the Methods and Discussion
Response: I don’t think that this part belongs in the Methods section. As the references cited were identified as described in the Methods, my preference is to keep this section in the sequence where it is now.

Line 300, the previous 2012 meta-analysis published by this author, included in this Results results is confusing. These were previous results not current results? Likewise, in line 326, the discussion of the more recent 2018 published meta-analyses from China, should also be included in the discussion, rather than in the Results section.

Same question as to why discussion of prospective studies from New Zealand in prior to 2012, is included in the Results section?
Response: As this is a review paper, I would prefer to keep all the childhood epidemiology references in the Results section.

Discussion:

There does not currently appear to be a Discussion section; only a short Conclusion section.
Response: The instructions do not require a Discussion section, but the Plausibility and implications section can be considered as a “Discussion” section, as discussed above.

In summary, this is a comprehensive review of the literature that is included in both a Background and Results section of a paper reviewing recent epidemiological studies assessing an
association between fluoride and neurotoxicity. While comprehensive, as currently organized, the flow of the manuscript is difficult to follow and read. A reassessment of the focus and goals of this manuscript, and editing to reformat to meet these, should be done.

Response: Thank you for the detailed comments, which have inspired much revision to sharpen and clarify the sequence and line of thinking, while respecting the journal instructions that do not require a Discussion in review manuscripts.

Reviewer #5: This is a very timely review given the number of studies that have been published in recent years. The review is well-written and organized. It integrates relevant information, such as the importance of examining critical windows of exposure and the evidence that fluoride (at levels that are realistic for human exposure) crosses the BBB in the developing fetus and adult. I also appreciated the nuanced discussion of some of the variability in results and the direct comparison of serum concentrations associated with neurotoxicity for lead versus fluoride. Finally, the calculation of the benchmark dose provides a new way of presenting the data. I have some minor comments and suggestions:
Response: Thank you, much appreciated.

1. Line 59: change to "Potential sources of fluoride exposure"
Response: Thank you, I agree and have made the change.

2. Line 66: It would be more accurate to state: "For adults in the U.S., fluoride in water and beverages contributes an average of about 80% of the daily total fluoride intake (estimated to average 2.91 mg) in fluoridated communities." (fluoride intake only from water is estimated at 60%).
Response: Thank you, the change has been made.

3. Line 91. The pineal gland is a structure that is not protected by the blood-brain barrier (https://www.ncbi.nlm.nih.gov/pubmed/28177105 ). Thus, calcification of this structure should not be interpreted as evidence of fluoride crossing the BBB.
Response: Thank you, I have made the correction.

4. Line 163: change to "...NTP focused on fluoride neurotoxicity in regard to learning and memory."
Response: I have made this change, thank you.

Response: Correction made.
6. Line 434: Minor typo: (CI, 4:12; -0:59) should use decimals (not ";").
Response: Thank you, correction made.

7. Benchmark calculations:

a. Table 3. The title for this table is "adjusted differences in IQ per mg fluoride per liter maternal urine during pregnancy". However, the estimate that is provided in the table for the ELEMENT study is per 0.5 mg/L. In contrast, the estimate that is provided for the MIREC study is per 1.0 mg/L. Units should be consistent.
Response: I can confirm that the calculations took this into regard and multiplied the results from the ELEMENT study by 2.

b. The estimates in Table 3 are shown for the ELEMENT cohort using the WASI outcome (FSIQ) with the sample of 211 mother-child pairs. Why show this outcome as opposed to the McCarthy scale outcome (GCI)? The outcome on the McCarthy scale has a larger N and is more similar in age at testing (4 year olds) with the MIREC cohort.
Response: Thank you, the table has been expanded as suggested.

8. Line 480 and in Acknowledgements: typo: ELEMENTS (should read ELEMENT).
Response: The correction has been made.

9. Line 567: delete "the" in "as the thyroid hormone is crucial.."
Response: The suggested change has been made.

10. Conclusion. I am not clear why the evidence today "may well underestimate the true extent of the fluoridotoxicity". With non-differential measurement error of the exposure, there can also be an overestimate of the true effect.
Response: As the exposure parameter is an independent variable assumed to be free of error, the impact of imprecision will on average tend toward an underestimation. I have now explained this further and provided a reference.