From: Liu, Honghu, Ph.D.
To: Kumar, Jayanth@CDPH
Cc: Moss, Mark Eric
Subject: Re: Fluoride-IQ manuscript
Date: Monday, March 7, 2022 12:18:57 PM

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I have the similar feeling---if we do identify one, it will be preliminary with exploratory nature as we do not have enough data from rigorously designed trials.
Best,
H

From: Kumar, Jayanth@CDPH <Jayanth.Kumar@cdph.ca.gov>
Sent: Monday, March 7, 2022 11:49 AM
To: Liu, Honghu, Ph.D. <hhliu@dentistry.ucla.edu>
Cc: Moss, Mark Eric <MOSSM17@ECU.EDU>
Subject: RE: Fluoride-IQ manuscript

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Grandjean calaculated a dose. According to him, there is no safe dose. But he did not consider the Ibarluzea study from Spain. The NAS committee wanted NTP to focus on hazard identification. Based on the unsophisticated quality of the studies, they were skeptical of a dose-response analysis. But one explanation for the heterogeneity in the effect size is dose.

From: Liu, Honghu, Ph.D. <hhliu@dentistry.ucla.edu>
Sent: Monday, March 7, 2022 11:10 AM
To: Kumar, Jayanth@CDPH <Jayanth.Kumar@cdph.ca.gov>
Cc: Moss, Mark Eric <MOSSM17@ECU.EDU>
Subject: Re: Fluoride-IQ manuscript

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Hi Jay,
Yes, I agree that using 9 studies will be consistent with our notion of examining low/normal F level (and the results are in favor of our hypothesis--we can show both the non-linear modeling with restricted cubic spline and the linear approximation).
Zhang Shun is indeed an outlier (it provides among the worst point estimates against our hypothesis with IQ=102 when F is high, but IQ=109 when F is low); given his F high=1.4 and F low=0.63, we will still include his study in analyses; the good thing is his data is not strong enough to turn the hypothesis testing around.

We are in very good shape for the analyses and results for F dosage of low/normal.

I am now working on the next part of the analyses---trying to identify a threshold, if all possible. Given the heterogeneity of the studies available to use and the limited quality for quite some of them, this will be challenging. I think that if we do can identify one, it will unlikely be exactly 1.5 mg/L---it will be somewhere around 1.5. I will let you know the results.

I will do my best to join on Wed.

Have a nice and safe trip.
Best,
Honghu

From: Kumar, Jayanth@CDPH <Jayanth.Kumar@cdph.ca.gov>
Sent: Monday, March 7, 2022 5:29 AM
To: Liu, Honghu, Ph.D. <hhliu@dentistry.ucla.edu>
Cc: Moss, Mark Eric <MOSSM17@ECU.EDU>
Subject: RE: Fluoride-IQ manuscript

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Honghue,

I agree with Mark. This is excellent work. Overall, all the models below 1.5 mg/L are not significant. It makes sense to use 9 studies from non-endemic areas. Figures 2 (SMD- 2. Meta-analysis (linear) -- see Meta_lr_N9.png Wald test: p-value = 0.87); and 3 Absolute IQ (restricted cubic spline) -- see StdIQ_N9.png (Standardized score).

This aligns with our SMD analysis (Normal to lower). Zhang Shun (2015) is an outlier. It changes the heterogeneity from 0% to 69%.

I will send the Zoom link. If possible, please attend.

Jay

From: Liu, Honghu, Ph.D. <hhliu@dentistry.ucla.edu>
Sent: Saturday, March 5, 2022 8:45 PM
To: Kumar, Jayanth@CDPH <Jayanth.Kumar@cdph.ca.gov>
Cc: Moss, Mark Eric <MOSSM17@ECU.EDU>
Subject: Re: Fluoride-IQ manuscript

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hi Jay,

Here are the summarized results. I put them in the attached word file for your convenience. Let me explain how the analyses were done and their implication here below in the email.

(I) <u>low/normal F dosage modeling with the selected 13 studies.</u>

First, let's look at the 13 studies you recommended with Diff in F dosage no more than 1.5 mg/L. Here are a few issues I like to mention:

(1) Among the 13 studies, Xu 2020 used very high IQ value >120 which is significantly higher than most, if not all, other studies so we did analyses with (N=13) and without (N=12) Xu 2020 in the analyses; Also, to evaluate the relationship between absolute F dosage and IQ level, we also looked at the sub-set of studies that have absolute F dosage <1.5mg/L (N=9 studies). Thus, we have analyses results for N=12, 9, and 13 studies, all having Diff in F dosage no more than 1.5 mg/L.

(2) We have done both non-linear modeling with restricted cubic spline (RCS) and linear modeling (piece-wide modeling when necessary) for both SMD IQ and absolute IQ, recognizing that for modeling with absolute values, the two data points within a study are not completely independent, even though the estimates were calculated by different subjects. However, all point estimates of IQ are valid, and the potential non-independence could only lead to an over-estimate of significance to some degree which is not a problem for us here since we are seeking for non-significant impact of F dosage on IQ when F dosage is low/normal (if we do not see significant result with our analyses, it will be even more non-significant, if the data were completely independent.)

(3) For non-linear modeling with RCS, the parameter estimates from regular non-linear modeling with RCS (e.g., using R programming language) are relatively more detail and easier to interpreter than parameter estimates provided from meta analyses with RCS procedure.

For RCS through regular non-linear modeling, the number of cubic terms will equal to the number of knots one selects with two ends being restricted to linear (that is why it is called RCS). The R package generates a truncated power basis for a RCS, which means, the spline is the linear addition of the basis functions across the entire domain. For example, with the spline with absolute IQ and 12 studies (N=12, see page 3 of the word file), each term is called a basis function and together they make up the estimated restricted cubic spline function. There are four cubic terms, in which the values 0.15, 0.63, 1.02, 1.5 are the four knots we specified. Also in the above function, notice that

- when F < 0.15, the fitted function is 98.9959 - 12.38035*Dose
- when F = 1.5, the sum of the coefficients of cubic terms is 14.35524-33.42762+20.21344-1.141062 = -2e-06, approximately equals to zero. So it will be 98.9959 - 12.38035*Dose again as when F<0.15. This will be also true for the quadratic terms (if we expand the fitted model and write out the quadratic terms, we will see this results). This confirms that two ends of the spline are linear. See References about truncated power basis:

https://bmcmedresmethodol.biomedcentral.com/articles/10.1186/s12874-019-0666-3
https://bookdown.org/alecri/thesis/A-sec-rcs-app.html).

For meta analyses for non-linear modeling with RCS, the outputs provided by the software procedure are limited to some degree. For example, for fixed-effects coefficients, it only provides two summarized parameters. These parameters are called regression coefficients for spline. They have some mathematical relationship with the coefficients in the full formula/equation of spline results shown in the regular RCS modeling above. Specifically, the first parameter rcs(dose, knots)dose is associated with linear term in the full formula/equation, and the second parameter rcs(dose, knots)dose' is associated with non-linear terms. Usually people don't interpret these parameters as there is no detailed descriptions available in the software package (even for regular RCS, we normally do not interpret much of the parameter estimates themselves from RCS).

(4) We have obtained p_values for both RCS and linear modeling. For linear modeling, the p-value is simple and associated each line (for its slope; if piece-wide, one p-value for each piece of line); For non-linear with RCS, the p-value is the significance testing result of overall modeling across the multiple spline terms through Wald test that has a Chi-Square distribution. Looking through the p-values across N=12, 9 and 13, we can see that all modeling results both from SMD and absolute IQ are non-significant which support our hypothesis that when F dosage is in normal/low range, there is no significant impact on IQ development among children.

(5) Although not ideal/optimal, the modeling results of both SMD and absolute for F in the low/normal range have each own merit and limitations. For SMD, it uses one summarized data point from each study and standardized the difference in means, and shows the relation between the differences in F dosages in high and low, and the SMD. However, unless we restrict the range of high F dosage to 1.5 mg/L (N=9), even the axis (diff in F) is limited to 1.5mg/L, it is the difference in F dosage between high/low and a little hard to interpret in the context of low/normal range of F (e.g., a low diff in F dosages doesn't necessarily mean low F dosages were used in each arm, and so is true vice versa), since we include studies that use F dosages beyond 1.5 mg/L. For absolute F dosage and IQ, it shows in a straight and bare relationship between levels of F dosage and IQ levels, and easier to understand, but there is a potential non-independent issue which we need to clarify. Although the trends of non-linear curves between SMD (down trend) and absolute F dosage/IQ /F (convex-up) modeling are different, they do not contradict each other, rather they reflect the different ways we use to describe the relationship between F dosage levels and IQ levels. The good news is that no matter which way we analyze the data, we do not see a significant fluctuation in levels of IQ when F dosage is in low/normal range which clearly support our hypothesis----it is safe to drink fluoridated water when F dosage is in low/normal range.

(II) Low/normal and high F dosage modeling with more studies

Second, although we do not have good data when F dosage is high (partially due to low quality of studies in endemic areas with high F dosage), we have done analyses trying to identify a threshold (e.g., around 1.5 mg/L) in hope to see a non-significant fluctuation in IQ before the threshold and significant drop in IQ after the threshold value. Since there is so much variation in IQ metric, F dosage, and study conditions, the analyses are complicated. We have done two modeling with N=33 studies and N=32 studies with *D. Mondal 2015 being excluded* which we cannot standardize its IQ values. Although the results are very sensitive to some single studies (e.g., the N=33 and N=32 piece-wide linear regression results are quite different), the N=33 piece-wide linear regression show some promise (both linear line have down-trend, but the results are opposite to what we hoped for: the line before the threshold of 1.5 mg/L is significant, but the line after the threshold is not significant). There still some more work for this part. Although hard, we can test more models to try to identify a threshold that can lead to a non-significant fluctuation in IQ before the threshold and a significant drop in IQ after the threshold.

I will also write out the statistical methods/approaches for these analyses and modeling.

Questions, let me know.

Best,
Honghu

From: Liu, Honghu, Ph.D. <hhliu@dentistry.ucla.edu>
Sent: Friday, March 4, 2022 4:44 PM
To: Kumar, Jayanth@CDPH <Jayanth.Kumar@cdph.ca.gov>
Cc: Moss, Mark Eric <MOSSM17@ECU.EDU>
Subject: Re: Fluoride-IQ manuscript

Hi Jay,
I am organizing and summarizing the model parameter estimates, significance testing, and the smoothed figures using the 13 studies with different sub-sets. I should be able to send you the summary tomorrow Saturday or Sunday.
Talk soon.
Best,
Honghu

From: Kumar, Jayanth@CDPH <Jayanth.Kumar@cdph.ca.gov>
Sent: Thursday, February 17, 2022 10:48 AM
To: Liu, Honghu, Ph.D. <hhliu@dentistry.ucla.edu>; Moss, Mark Eric <MOSSM17@ECU.EDU>
Subject: RE: Fluoride-IQ manuscript

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Honghu,

I was able to find two more studies <1.5 mg/L range. I will check with Mark and send it you by Monday.

Jay

From: Liu, Honghu, Ph.D. <hhliu@dentistry.ucla.edu>
Sent: Sunday, February 13, 2022 1:16 PM
To: Kumar, Jayanth@CDPH <Jayanth.Kumar@cdph.ca.gov>; Moss, Mark Eric <MOSSM17@ECU.EDU>
Subject: Re: Fluoride-IQ manuscript

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Agreed. Specific fitted non-linear models with parameter estimates, statistical tests, and p-values, as well as smoothed figures for visual display will make the paper strong.
Best,
H

From: Kumar, Jayanth@CDPH <Jayanth.Kumar@cdph.ca.gov>
Sent: Sunday, February 13, 2022 10:13 AM
To: Liu, Honghu, Ph.D. <hhliu@dentistry.ucla.edu>; Moss, Mark Eric <MOSSM17@ECU.EDU>
Subject: RE: Fluoride-IQ manuscript

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Thanks. We need to include parameter estimates and test results.

From: Liu, Honghu, Ph.D. <hhliu@dentistry.ucla.edu>
Sent: Saturday, February 12, 2022 1:53 PM
To: Kumar, Jayanth@CDPH <Jayanth.Kumar@cdph.ca.gov>; Moss, Mark Eric <MOSSM17@ECU.EDU>
Subject: Re: Fluoride-IQ manuscript

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Thanks, Jay, for the list of 10 studies.

I checked against my list (see first excel file that was sent to you last Wed 9:52pm and grouped studies into Group 1, Group 1, Group 3) and your 10 listed studies are all in my list. I put group affiliation in the first column in your excel file (second attached excel file) so you can see (G1 (Group 1) or G2 (Group 2)) . I might have used a slightly different calculated F dose for Xu 2020, but will check and re-fit models.

I have thought through more this morning. I will explore further modeling. For example, in addition to the normal range (around <1.5mg/L) modeling, I will try to come up with a parametric test, if possible, to show its fluctuation in IQ is not significant, when F dose is within range of low/normal; Since for high F dose, the quality of studies are not comparable with those with normal/range range, I will try to fit them separately; if possible to come up with another parametric test.

It will be solid/strong and we will get there.

Best,
Honghu

From: Kumar, Jayanth@CDPH <Jayanth.Kumar@cdph.ca.gov>
Sent: Saturday, February 12, 2022 9:51 AM
To: Liu, Honghu, Ph.D. <hhliu@dentistry.ucla.edu>; Moss, Mark Eric <MOSSM17@ECU.EDU>
Subject: RE: Fluoride-IQ manuscript

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I forgot to add the file.

From: Kumar, Jayanth@CDPH
Sent: Saturday, February 12, 2022 9:50 AM
To: Liu, Honghu, Ph.D. <hhliu@dentistry.ucla.edu>; Moss, Mark Eric <MOSSM17@ECU.EDU>
Subject: RE: Fluoride-IQ manuscript

Honghu,

Thank you for that excellent presentation. Regarding more studies <1.5 F difference, I created a separate sheet with 10 studies.

1. Xu 1994 has data comparing normal (control group) vs. low.
2. Bashah 2007. The authors provide the range of CUF (0.18 to 2.8). Then they provide the 25^{th} and 75^{th} percentile values (0.54, 1.01). I used these for lower and higher values.
3. Xu 2020 has average CUF. The difference is 1.19.

From: Liu, Honghu, Ph.D. <hhliu@dentistry.ucla.edu>
Sent: Wednesday, February 9, 2022 9:52 PM
To: Kumar, Jayanth@CDPH <Jayanth.Kumar@cdph.ca.gov>; Moss, Mark Eric <MOSSM17@ECU.EDU>
Subject: Re: Fluoride-IQ manuscript

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Hi Jay,

I summarize here the nonlinear modeling with restricted cubic spline results examining the relationship between fluoride dosage and IQ level through meta-analyses using SMD and through our own meta-analyses with regular spline procedure using observed IQ scores under varying conditions of weighted/unweighted for precisions, standardize/non-standardized IQ, absolute/difference in F dose on x-axis, etc.

First, the excel file summarizes the 7 studies we use in the current draft paper, and the 26 studies used in Duan's paper, including the year of the study, country, and intelligence assessment methods each one used. I divide all studies into Group 1-3:

- Group 1: the 7 studies in our draft
- Group 2: studies from Duan's paper with F dosage<= 5.2
- Group 3: studies from Duan's paper with F dosage > 5.2

1. Standardization of the IQ score.

One of the issues is to try to standardize the IQ scores, if possible, which has been challenging due to limited information available, but we have tried and standardized for most of them except two studies so far.

We found that most of the IQ scales yield standard scores, i.e.,
- Wechsler test: Mean = 100, SD = 15
- Stanford-Binet test: Mean = 100, SD = 16
- Catell test: Mean = 100, SD = 24
- McCarthy Scales of Children's Abilities: Mean = 100, SD = 16.

Besides, the Raven test and Chinese standardized Raven test use the same methodology as the Wechsler Intelligence Scale.
So we converted IQ scores from other tests to the Wechsler test (Mean = 100, SD = 15):
NewIQ = ((OldIQ - 100) / OldTestSD) * 15 + 100.

However, there are two studies we cannot standardize so far (still searching ways to do so):

1. No.8 (Zhang JW et al.) used the Draw-a-Person test standardized by a Japanese researcher. We couldn't find detailed information about this test.
2. No.25 (D. Mondal et al.) reported the raw Raven score. We need the corresponding distribution in Wechsler scale for raw Raven score conversion, but it is not available.

We are still looking, and hope can find a solution.

(II)non-linear cubic spline results: the attached zipped file contains 14 fitted restricted cubic spline models with figures:

1. SMD_Meta figures. These 6 figures were fitted using R language meta nonlinear cubic spline procedure with SMD or log SMD as the y-axis. The SMD_Meta 4, SMD_Meta 5, and SMD_Spline yield pretty good results.
2. AbsoluteIQ_Spline. These 3 figures were fitted with absolute IQ values (for each study, the two arms contribute two data points in the figure). We can see that the Spline 2 which is weighted by precision of point estimates (the size of the circles is proportional to the level of precision), gives quite good results.
3. StandardizedIQ_Spline3. These 5 figures were fitted with standardized IQ using Wechsler metric as the norm. The StandardizedIQ_All Data gives pretty good results except ((i)an initial acute drop, (ii)two studies are not standardized yet.)

You can tell which is which by checking the labels/legend/title of each figure, but I will walk you through when we meet on Friday.

It is very reasonable to hypothesize that the change in IQ with early low F dose is ignorable (decrease/increase/near flat), and then it is possible to drop once the F dose exceeds certain threshold. Our results are getting closer to support this, and some additional work is still

needed to reach that conclusion. I have some ideas on the next step and will explain to you when we meet.

Regards,
Honghu

From: Kumar, Jayanth@CDPH <Jayanth.Kumar@cdph.ca.gov>
Sent: Monday, February 7, 2022 3:12 PM
To: Moss, Mark Eric <MOSSM17@ECU.EDU>; Liu, Honghu, Ph.D. <hhliu@dentistry.ucla.edu>
Subject: Fluoride-IQ manuscript

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Mark,

I am virtually introducing you to Dr. Honghu Liu, Professor of Statistics at UCLA. I scheduled a Zoom meeting this Friday at 2 PM PST to discuss the population dose-response analysis to explain the heterogeneity found in the SMD meta-analysis.

Honghu,

Please meet Mark Moss, DDS, PhD. Mark and I have coauthored many papers on fluoridation and its health effects. In addition, we have worked together on the draft manuscript. Before moving to California, we had developed the Fluoride Science website to educate and inform dental and medical professionals.

Looking forward to the presentation.

Thanks.

Jay

Jayanth Kumar, DDS, MPH
State Dental Director
Office of Oral Health, Center for Healthy Communities
California Department of Public Health

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