A Commentary on Some Epidemiology Data for Chlorpyrifos

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Abstract

- Rauh et al. (2011) claimed statistically significant associations for neurological effects in children at age seven after potential very low exposure to chlorpyrifos (CPF) during pregnancy detected in a single cord blood at birth, with 42% non-detects.

- Although the underlying data have not been made available, we were able to extract data of Figures 1A and 1E in Rauh et al. (2011). Our analyses showed ~35% of the data in Figure 1A and ~15% of data in Figure 1E were missing from 265 children.

- Our analysis of extracted data does not suggest any evidence of an effect on Full-Scale IQ (Figure 1E), and less of a negative association (reduction) in Working Memory Index (Figure 1A).

Background

- Organophosphates are beneficial against Zika virus and malaria.
- Scores of studies suggest that sentinel effect of chlorpyrifos is cholinesterase inhibition at similar dose in animals and humans.
- Rauh et al. (2011) claimed neurological effects in children associated with much lower prenatal levels of chlorpyrifos, suggesting an alternative hypothesis, that the sentinel effect for chlorpyrifos should be based on neurological effects rather than cholinesterase inhibition.
- The purpose of this analysis is to consider this hypothesis by reviewing the underlying epidemiological basis.

Methods

- Figures 1A and 1E of Rauh et al. (2011) were extracted and the results entered into Excel. Consistent Raul et al. (2011, page 1198), ~80% of zero or non-detectable values were assigned a chlorpyrifos level of 0.5 pg/g; ~20% of them assigned 1.0 pg/g.

- The results were then plotted as natural logarithm-adjusted response versus dose (as per Rauh et al., 2011), and as un-adjusted response versus $\log_{10}$ dose using Excel software.

- 4 high dose data points were missing in both graphs; authors' response to EPA states the highest dose "was a highly influential observation (outlier) and drastically impacts inference." One subject had no data; data of two others were not plotted.

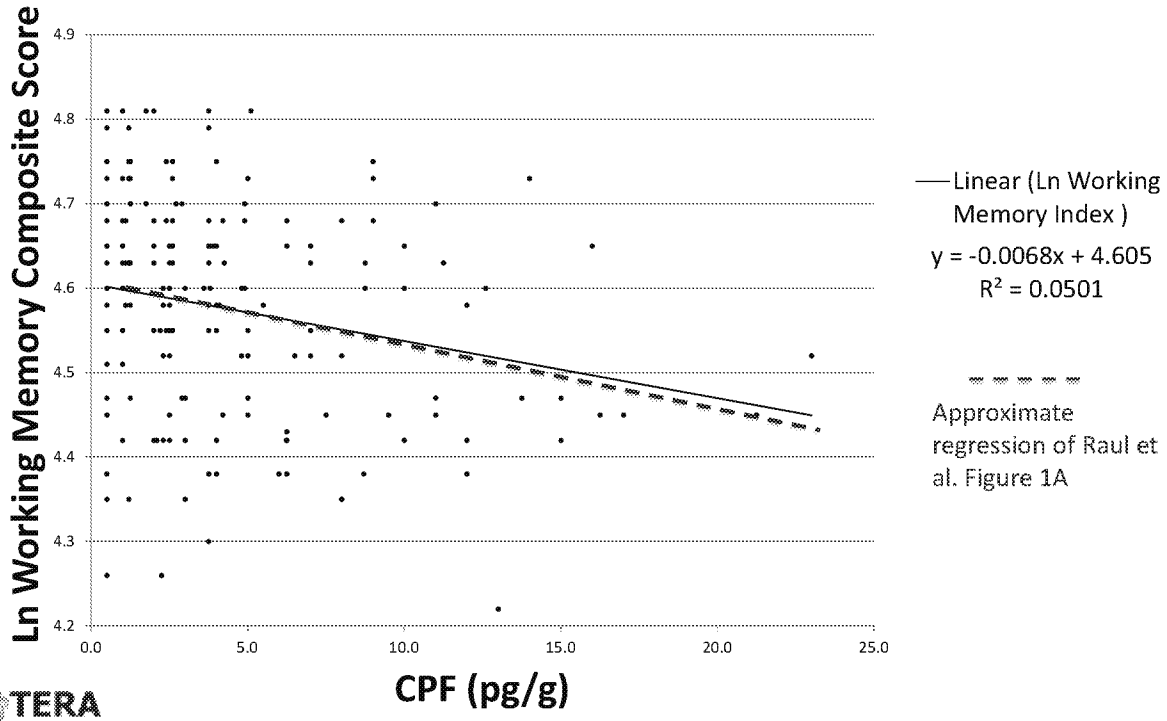
Results

- **Figure 1** is a replication the Rauh et al. (2011) Figure 1A. This replication seems reasonable from a comparison of where the linear regression line lies in relationship to the approximate regression of Rauh et al.

- ~35% of the data stated in the publication in Rauh et al. (2011, page 1197) are missing in Fig. 1.

- Also not included are high dose data (e.g., see reference to 63 pg/g on Rauh et al. page 1198, column 2, which is not found on Figure 1). Apparently also missing is one child with a value of 32 pg/g (see stated CPF range in Rauh et al., Table 1).

Figure 1. Ln Working Memory Index Versus Dose



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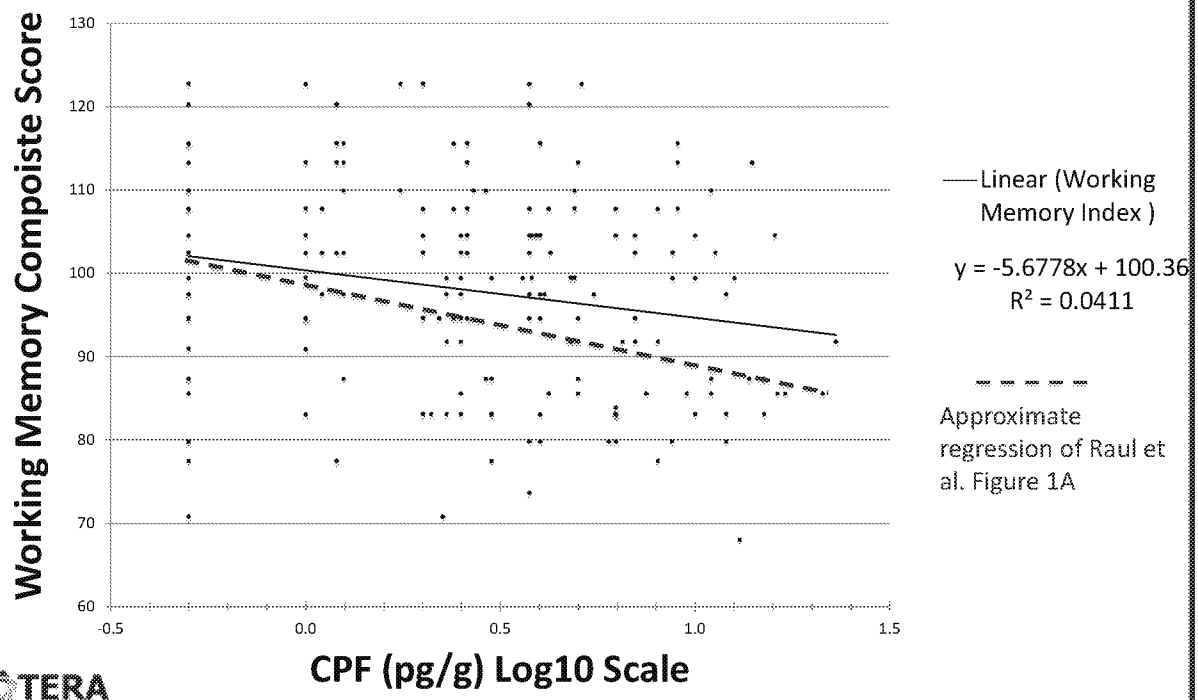
Results Continued

- **Figure 2** reflects the data from Figure 1 plotted with the response unadjusted and the dose in logarithmic units ($\log_{10}$).

- Figure 2 shows a reduced effect on Working Memory Index when compared with Figure 1, found by comparing where the regression lines lie in relationship to high dose data points, indicating that the way data are presented may have an effect on interpretation.

- The R^2 for regression lines in both Figures are very small, which indicates that chlorpyrifos does not explain the data variability (i.e. the scatter in these data).

Figure 2. Working Memory Index Versus Log10 Dose



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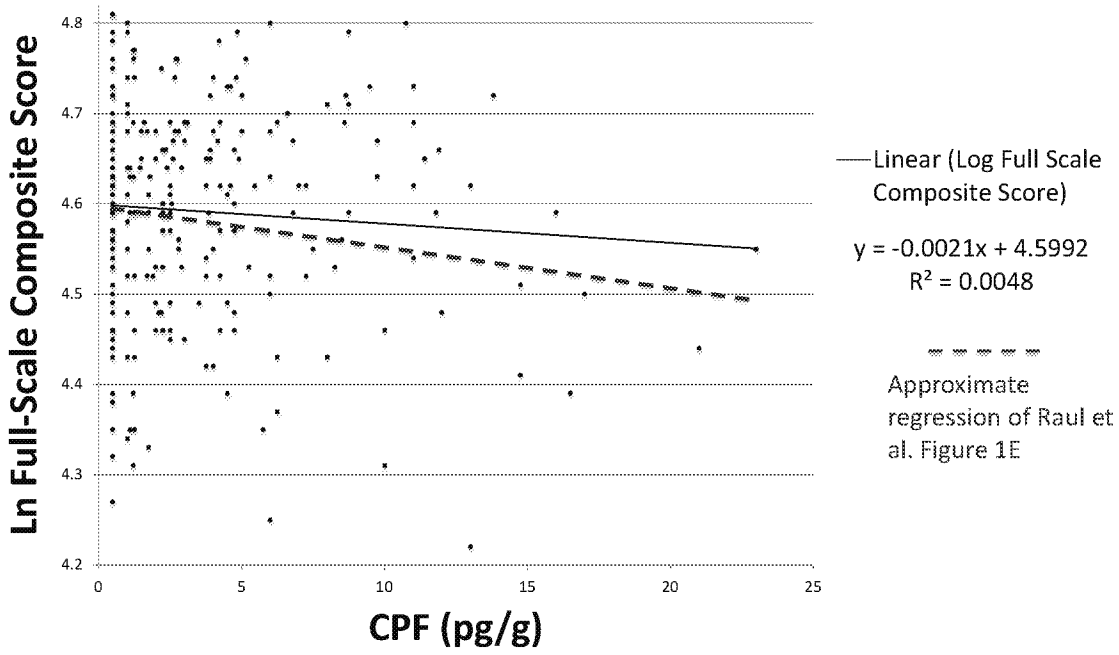
Results Continued

- **Figure 3** attempts to replicate the findings of Rauh et al. (2011), specifically their Figure 1E (Full Scale IQ).
- This replication is not as close as in Figure 1. Again, compare where the regression lines lie in relationship to high dose points.
- As in comparison of Figures 1 and 2, some of the data stated to be available in Rauh et al. (2011) are missing (in this case approximately 15%).

Figure 3. Ln Full Scale IQ Versus Dose



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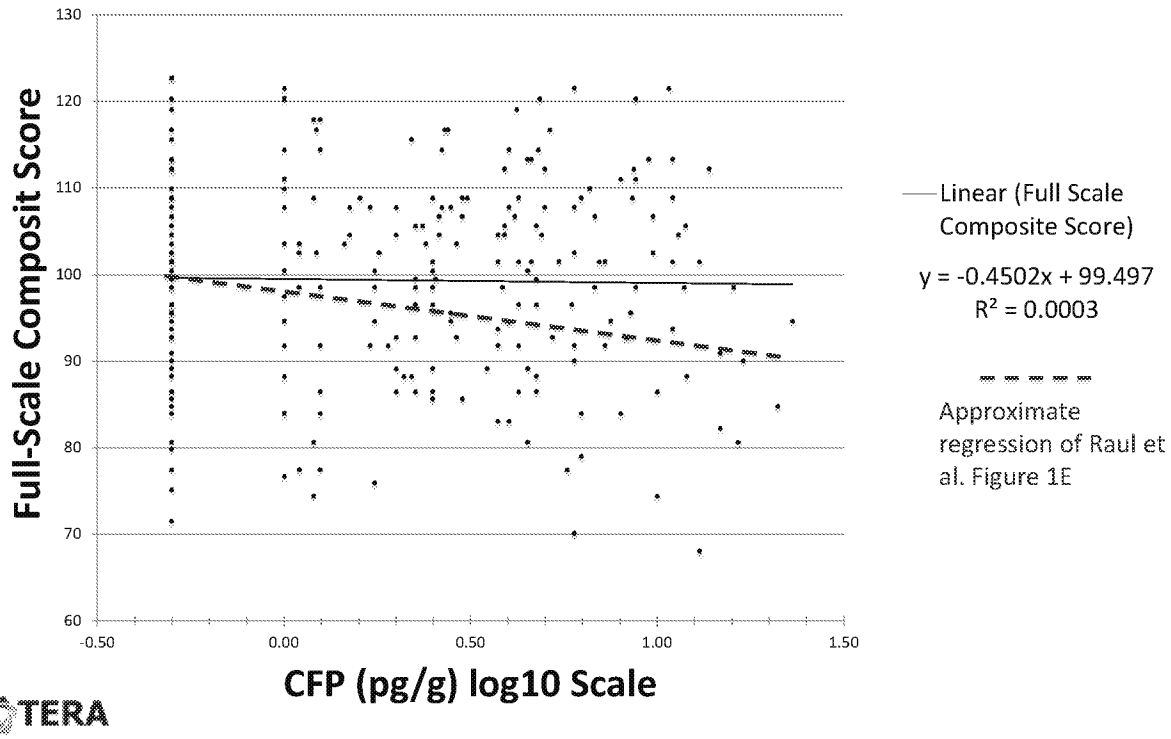
Results Continued

- **Figure 4** reflects the data from Figure 3 plotted with the response unadjusted and the dose in logarithmic units (log_{10}), and shows no effect on Full Scale Composite score when compared with Figure 3.

- As with Figure 2, the y-axis is not compressed in Figure 4, eliminating the subtle visual effect of downward trend due to this compression in the y-axis of Figures 1 & 3.

- The R^2 is for Full Scale IQ is even smaller than for Working Memory, suggesting that chlorpyrifos is not a predictor of the outcome.

Figure 4. Full Scale IQ Versus Log10 Dose



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Results Summary

- The bottom line is that evidence of effect for Full-Scale IQ does not exist when data are presented as in Figure 4.
- Working Memory shows evidence of a negative association (Figure 2), but this evidence is problematic due to missing 35% of the data, including the highest exposed individual that an author states "was a highly influential observation (outlier) and drastically impacts inference" (Whyatt, 2015)
- Data from 3 other high dose individuals are also missing.
- Overall, the lack of raw data from this study makes confirmation of the authors' results impossible.

Discussion

- The most significant challenge, by far, in any reanalysis of the Rauh et al. (2011) study is the absence of data to conduct a credible replication. For example, Rauh et al. (2011) state that:
  - "Of 725 consenting women, 535 were active participants in the ongoing cohort study at the time of this report, and 265 of their children had reached the age of 7 years with complete data on the following: *a*) prenatal maternal interview data, *b*) biomarkers of prenatal CPF exposure level from maternal and/or cord blood samples at delivery, *c*) postnatal covariates, and *d*) neurodevelopmental outcomes."

- However, Rauh et al. (2011) show a series of 5 graphs, two of which (Figures 1A and 1E) show ~35% or ~15% missing data points, respectively. Neither of these graphs include the data points from highest stated cord blood exposures of 32 or 63 pg/g.

Discussion Continued

- Although negative neurological associations are reported in the Rauh et al. (2011) when a plot of Working Memory Composite Scores are normalized by their natural logarithm and plotted with dose, this manner of data display is not the only one possible.

- Another, and more standard approach, would be to plot the unadjusted scores, which are already normally distributed in the human population, against the logarithm of dose.

- When the results of Rauh et al. (2011) are plotted in this way, a reduced association is found. For example, a comparison of Figures 1 and 2 will show that the negative trend of Figure 1 for the Working Memory is less in Figure 2.

Discussion Continued

- When a similar analysis is performed for Full Scale IQ (or Composite Score) the slight negative trend of Figure 3, which is a representation of Rauh et al. (2011) Figure 1E, disappears; compare Figures 3 and 4.

- What about including the missing high dose data?

- Adding the two high dose data points described in Rauh et al. (2011) to figures 2 and 4 and supposing only average responses further decrease the negative slopes, but only slightly (data not shown). This indicates even less of an effect, if any, from chlorpyrifos exposure.

Discussion Continued

- Whether one method of plotting these data is superior to another may be important, but a strong association should not be affected.

- A more appropriate approach to confirm our findings would be to access the underlying data. This would enable us to adjust for confounding factors and discuss our results in more statistical terms, thus allowing a stronger statement on any statistical significant association.

- Underlying data would also allow a refined analysis from a simple linear approach to a manner similar to that shown by Rauh et al. (2011) as a smooth cubic spine curve.

Discussion Continued

- Our analysis from published graphs is a rudimentary way to obtain the data of Rauh et al. (2011), because data points may often overlay one another in published figures. Still, the Rauh et al. (2011) do not plot chlorpyrifos exposures greater than 25 pg/g.

- Not surprisingly, as study co-sponsors, EPA asked Rauh et al. (2011)for these data–twice. Such requests seem reasonable since:
  - "This study was supported by the National Institute of Environmental Health Sciences (grants 5P01ES09600, P50ES015905, and 5R01ES08977), the U.S. Environmental Protection Agency (grants R827027, 8260901, and RR00645), the Educational Foundation of America, the John and Wendy Neu Family Foundation, the New York Community Trust, and the Trustees of the Blanchette Hooker Rockefeller Fund."

Discussion Continued

- A number of other items about this study are noted:
  - Rauh et al. (2011) Figure 1 E has more data points than Rauh et al. (2011) Figure 1A, but the former depends on the latter.
  - Rauh et al. (2011) did not collect cord blood samples in 12% of the population. Blood leads collected for only 34% of mothers and children.
  - The hypothesis from Rauh et al. (2011), that neurological effects occur at doses lower than cholinesterase inhibition, has been tested in experimental animals and is not supported.
  - The metabolite causing toxicity irreversibly binds to cholinesterase in the blood. So bound, it is not expected to affect fetal neurological development at levels lower than those not causing blood inhibition.
  - Systemic availability or brain cholinesterase inhibition has not been seen at levels comparable to safe doses (Marty et al., 2012).
  - The specific results described by Rauh et al. (2011) are not reproduced in other epidemiology studies (Burns, 2018).
  - Scientists with USDA, EPA's SAP, and OMB also have substantive questions about the Rauh et al. (2011) work.
  - The IQs in the control and exposed groups of Rauh et al. (2011) both approximated 99, and were not statistical significantly different.

Conclusion

- Rauh et al. (2011), has been cited as showing a statistical association between CPF exposure and intelligence.
- An analysis of the published figures shows that up to 35% of the data appear to be missing, including high dose data, inclusion of which would appear to mollify or obviate the effect as stated by the authors.
- The associations are lessened or no longer apparent when different logarithmic assumptions were used in the reanalysis.
- The data, generated in part by public funds, have not been made available for independent review.
- These shortcomings make it difficult to use this study in any risk assessment for chlorpyrifos.

Funding

- The origin of this work came about from briefings of MLD as a U.S. Environmental Protection Agency senior advisor in 2017.
- Afterwards, DowAgro Sciences funded, in part, an independently developed report by Toxicology Excellence for Risk Assessment (TERA), which confirmed the findings of EPA staff.
- The development and presentation of this poster has been supported by the Internal Development Reserve funds of TERA.

ED_002962_00011804-00021

References & Notes

- Rauh et al. (2011). Seven-Year Neurodevelopmental Scores and Prenatal Exposure to Chlorpyrifos, a Common Agricultural Pesticide, Environmental Health Perspectives, Volume 119: 1196-1201.
- Memo to: Carrol Christensen, Ph.D; From: Robin M. Whyatt, DrPH; Date: April 9, 2015:
  - Re: July 2011 letter to Deborah Smegal, M.P.H.: [Full memo available as Appendix A]
  - **EPA comment:** In Figure 1 page 29, the upper bound of the x axis (chlorpyrifos) is shown to be 25 pg/gm. However, in the second paragraph of page 11 it was reported that the maximum CPF exposure is 63 pg/g. It was not clear to us why in Figure 1 the range of CPF was truncated.
  - **CCCEH response:** The maximum CPF exposure in the sample was indeed 63 pg/g. The number of children with CPF levels above 25 pg/g were 4. The x-axis was truncated at 25 pg/gm for the following reasons:
    - One of the subjects did not have the outcomes measured.
    - The subject with 63 pg/g was a highly influential observation (outlier) and drastically impacts inference. This was confirmed based on residual analysis in most analyses. Where appropriate, this observation was removed from the analysis. This influence was observed in the spline plots as well and this lone outlier at the extreme end of the exposure made the plot unstable and uninformative.
    - With just two observation left in this range, the data were too sparse and the splines too unstable in this region.
- It is essentially impossible that all of the missing data points in the Rauh et al. (2011) Figures 1A and 1E are underneath the other points. There are 265 children in the study, but only approximately 170 data points observable in Figure 1A. Rather it appears that many of these data points weren't added.
- US EPA. 2014. Chlorpyrifos: Revised Human Health Risk Assessment for Registration Review. Appendix 6, page 384 and Addendum, page 394. December 29.
- M.S.Marty, et al., Cholinesterase inhibition and toxicokinetics in immature and adult rats after acute or repeated exposures to chlorpyrifos or chlorpyrifos–oxon. Reg. Toxicol. Pharmacol. 63: 209-224.
- Burns, C.J. 2018. Reproducibility is critical for determining scientific validity. Sanford, MI. June 6.