Reviewer’s report

Title: Transgenerational effects of polychlorinated biphenyls: 1. Development and physiology across 3 generations of rats

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Reviewer: Charles Vorhees

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The experiment examined the effects of prenatal exposure to the PCB mixture Aroclor-1221 on several parameters in the F1, F2, and F3 generations in Sprague-Dawley rats. Only a few effects were found in the F2 and F3 generations and most were in the maternal lineage, not the paternal lineage. The effects were on body weight (5-10%) and plasma progesterone in the F2s and progesterone and estrogen in the F3s. No genetic or epigenetic data are presented.

The paper is nicely written, the sample sizes appear reasonable but not necessarily optimal for detecting effects across 3 generations given how subtle they are likely to be and one wonders if a study such as this shouldn’t be as highly powered as feasible. It is not clear that the data were appropriately analyzed.

1. More needs to be said about the choice of E16 and E18 as exposure ages. These ages appear past the peak time of DNA demethylation (erasure) and re-methylation which is closer to E11-12 in mouse (Morgan et al., 2005) which would be approximately E12-13 in rat. This is not the only reference on this process, but please cite references in support of rat E16 and E18 for your exposure period.

2. Statistics: It is stated that Greenhouse-Geisser adjusted F-ratios were used when the data did not meet the sphericity assumption but this correction is only used when the ANOVA is a general linear model, but elsewhere it is stated that Mixed ANOVA models were used. Mixed linear models are maximum likelihood fit models to the data and do not require the same assumption that GLM models do, therefore, this part of the description is unclear. Clarify if the ANOVAs were GLM or mixed linear models.

3. How were the a posteriori tests performed? For example, for body weight the model should have been a 4-way ANOVA (treatment, sex, lineage and week where week was a repeated measure factor). Is this the way the data were analyze or were separate 3-way ANOVAs
done on each weekly body weight? If the latter, this would not be appropriate since it is tantamount to doing many t-tests and that’s a well-known problem. Since body weight measurements across age are correlated, doing separate ANOVAs would inflate the risk of Type I errors. If, on the other hand, a repeated measure ANOVA was used, then the data analysis section needs to be re-written to reflect this.

4. Three males and 3 females were retained per litter but it is unclear if the data from all 3 pairs within each litter were used. How were the data for different pups within a litter handled for body weight? If all the data were used from all the pups within each litter, were the data averaged for the 3 males and 3 females and the average used as the datum for that litter and sex, or was pup a nested factor within litter in a hierarchical ANOVA, or some other approach?

5. Also, litter is an uncontrolled factor (but an important one) and statisticians often recommend it be in the ANOVA as a random factor. We do this, and it works well. I recommend it for these data. I can’t tell if it will help but given the size and length of a study such as this, it would be prudent to use it to find out.

6. If the body weight differences reported as significant were the result of separate ANOVAs done on weight every week, the significance is undoubtedly overestimated. If they are the product of a repeated measure ANOVA in which there were treatment x time interactions, they should be reported as such. Furthermore, a better way to sort interactions is to use Slice-effect ANOVAs because these use the omnibus mean-square error term for each slice (this is SAS terminology, but I’m sure there is an equivalent method in R).

7. Finally, the problem with the data presentation is that the scale in the multi-panel figures (Fig. 2 and 3) are too small to see. For these, the ordinate is more important than the abscissa, therefore, I suggest reorienting these to portrait, narrowing the abscissas and lengthening the ordinates to see if the group differences can be made more visible. Also, try putting labels (such as “Maternal”, “Paternal”) inside the axes (dragging them down) rather than above which wastes space; and push the figure panels closer together. Drag the “Fig. 2” and “Fig. 3” labels closer to the panels. You can also try color to better show the groups (there is no charge for color for the online version) and this would help make the groups more distinctive. Are SEMs shown in Fig. 2 and 3? Maybe they are but are too small to see, but if they are not included they should be. I’d suggest pointing them down for the lower line and up for the upper line so the difference can be seen. These same suggestions apply to Fig. 5. Fig. 6 would be improved by moving the panels closer together, lowering headings closure to the panels and lengthening the ordinates and put the group key inside one of the panels rather than floating below taking up valuable space.
References


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