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Dear Mike,

I hope things are going well for you. I am writing briefly in response to the recent draft interim report of the review of bisphenol A (BPA) toxicity conducted on behalf of NTP's CERHR, and specifically to address the review of my work published in Endocrinology (Zoeller RT, Bansal R, Parris C 2005. Bisphenol-A, an environmental contaminant that acts as a thyroid hormone receptor antagonist in vitro, increases serum thyroxine, and alters RC3/neurogranin expression in the developing rat brain. Endocrinology 146:607-612). I believe that there are some important observations in this paper that appeared to be discounted for reasons that are not apparent to me.

The most important issue is that BPA produced a profile of effects that were consistent with the interpretation that BPA acts as a *selective* indirect antagonist on the beta thyroid hormone receptor (TRβ). These profiles include:

1. BPA increases serum total T_4 specifically on postnatal day 15. We measured the effect of maternal exposure to BPA on neonatal serum T_4 levels on postnatal day 4, 8, 15, and 35. We observed an effect only on postnatal day 15. There are two reasons this observation is consistent with the interpretation that BPA is blocking the action of the TRβ receptor. First, BPA exposure *increased* serum total T_4 in the pups on P15. If BPA blocks the negative feedback effect of thyroid hormone on the TR, and the TRβ receptor mediates negative feedback, then one would predict that BPA would increase serum T_4 (i.e., it takes more T_4 to exert a negative feedback effect in the presence of BPA). Second, if BPA is blocking negative feedback, then BPA should increase serum total T_4 when negative feedback is operative. In the rat, negative feedback does not begin to function until about postnatal day 7 (as discussed in the manuscript). Thus, the developmental timing and the direction of change of serum T_4 are consistent with the interpretation.

2. Effect of BPA on serum total T_4 is not "dose-dependent". BPA was given to the dams at concentrations of 0, 1, 10, and 50 mg/kg-day. Serum total T_4 was increased in animals given 1, 10 and 50 mg/kg-day, but the dose-response was not broad. In fact, this kind of "flat" dose-response is characteristic of indirect antagonists like RU-486. As discussed in the paper, both

RU-486 and BPA have been shown to bind to receptors (PR or TR) and stabilize the interaction of the receptor with a co-repressor. This mechanism of action appears to produce this kind of flat dose-response. Thus, the flat shape of the dose-response is more consistent with the proposed mechanism of action than if the dose-response had been more "traditional".

3. *There was no effect of BPA on serum TSH.* If BPA acts as a TRβ antagonist, then it should increase serum total T_4 in the face of normal TSH. This profile would reflect a greater amount of T_4 required for suppression of TSH (i.e., maintain normal values) and is what was observed.

4. *RC3 expression in the dentate gyrus was increased in BPA-treated pups.* RC3 is a gene that is directly regulated by thyroid hormone. Moreover, in the dentate gyrus, RC3 is regulated by the TRα receptor because TRβ is not expressed there. The observation that RC3 expression was elevated in dentate gyrus in pups of mothers treated with BPA indicates that the TR is not being opposed by BPA in this brain region. Although we do not have proof of this concept, this interpretation is fully consistent with the data.

5. *There was no positive control used.* This highly curious comment reveals that the reviewer didn't recognize that there ARE NO similar compounds; thus, there is no positive control. That is, no study has ever before used an indirect antagonist to evaluate effects on the HPT axis *in vivo*.

Finally, the draft interim report states that, "this study is inadequate based on inappropriate statistics. This statement was wholly unjustified; that is, there was no justification provided. Moreover, it is unlikely that this statement can be justified. We used one pup from each dam in four treatment groups and used one-way analysis of variance to evaluate the results.

I hope you find these comments helpful. Please let me know if I can provide additional information.

Sincerely,

Tom