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Subject: comments on our paper in the prop 65 document on BPA

Your document discusses our BPA paper entitled "Howdeshell KL, Furr J, Lambright CR, Wilson VS, Ryan BC, Gray LE Jr. Gestational and lactational exposure to ethinyl estradiol, but not bisphenol A, decreases androgen-dependent reproductive organ weights and epididymal sperm abundance in the male long evans hooded rat." Toxicol Sci. 2008 Apr;102(2):371-82.

I think some of the comments failed to consider all of the information presented in the paper and the supplemental files.

The CA prop 65 documents mentions that our results show "remarkable" differences in testis weights for the EE2 50 dose among the different blocks.

In fact, this is not the case. If one thoroughly examines the information in the supplemental with the testis weight data it is evident that the block that they authors deemed "remarkable" has only 3 males in it from only 2 litters. In addition, the next block had only one male from this dose group (EE2 50) in it.

The reason for this was that in the first block we found that the EE2 50 dose induce maternal toxicity and poor viability in the F1 pups (Table 1). In the EE2 50 dose group maternal weight gain was reduced by about 50% and the viability of the pups was reduced by about 43%. Since there was little scientific value in continuing to include this dose and because it was causing some overt toxicity in the dams and their pups, we switched from 50 micrograms/kg/d to a lower top dose in the subsequent block, using 15 micrograms/kg/d. Even here there was evidence of toxicity to the dam and offspring (Table 1). When we included "block" as a factor in the analysis it was not statistically significant; not surprising given the limited sample size at this dose in later blocks. Hence, the differences in testis weights are not remarkable. Interpretation of the results of a study should be based upon sound statistical analyses. Furthermore, BPA had no effect at the doses tested on testis weights of the male offspring.

In regards to the discussion of our sperm count data. The data were presented as percent of controls (Fig 3) so they magnitude of the change could be compared to those seen in other male reproductive endpoints that were significantly affected by EE2 but not BPA (Fig 2). Table 3 presents also presents the sperm count data as numbers (millions) of sperm per epididymis as well as % of control. Analysis of the raw data without conversion to % of control does not change the statistical outcome and there are no significant block effects in the study on this endpoint.

I am somewhat surprised to also see the comments on the effects of AGD in the CA prop 65 document. Our data clearly show that BPA at 20 and 200

micrograms/kg/d had no effect on this endpoint (n=136 control males with 40 and 56 males in the BPA 20 and 200 groups, respectively, with over 700 males used in the current study). In addition, EE2 had no effect on male rat AGD. These results are similar to other well done and properly analyzed studies on BPA. In fact, there is little evidence that prenatal or neonatal exposure to any estrogenic chemical affects AGD at low doses levels.

We have been measuring AGD in newborn male rats in our laboratory for over 15 years. We first published on this endpoint in 1994 (Gray et al., vinclozolin) and since then have examined AGD in over 10,000 newborn rats pups and included a description of the methods in a detailed protocol published in Current Protocols in Toxicology (2004; unit 16.8). The effects that we have seen in AGD have been reproducible in other laboratories.

The document states that newborn rats display an AGD of about 5 mm. In fact, male the AGD of newborn male rats is about 3.5 mm, never 5 mm and female rat AGD at birth is about 1.6 mm. Accuracy is not an issue since this is done with the aid of a dissection scope with a micrometer in the lens of the scope. The cited reference for AGD (Vandenbergh and Huggett, 1995) is a review of measurement of AGD only in female mice.

The document states on page 42 that AGD is highly correlated with weight at birth. In fact, the correlation is only about r=0.15 or less and the correction for this does not generally improve the statistical analysis, hence, we include this adjustment and when it is not significant, we delete it from the final analysis.

One of the studies you cite as showing an effect on AGD in rats did not run concurrent controls and others were not analyzed properly, failing to account for litter effects. Therefore, the interpretation of these results in quite uncertain and studies like this were excluded from the CERHR NTP Expert Panel final evaluations for this reason.

Several negative studies have examined AGD in rats and mice that are not included in the summary table on AGD. I do not think an evaluation of all the data from high quality studies is consistent with your conclusion (p 63) that
"However, key findings that have appeared across the range of studies within and between
laboratories when BPA was administered during pregnancy and offspring were evaluated. The
effects of BPA include: .....
ò Effects on gender-differentiated morphology such as AGD. "
In fact, I think the studies fairly clearly indicate the opposite: BPA does not affect AGD or gender -differentiated morphology.

In summary, examination of the data from our paper on F1 male rats and their dams indicates that EE2 doses to the dam during gestation and lactation at levels near those used pharmaceutically produces a profile off estrogenic effects including reduced maternal weight gain, pup viability, permanently reduced body weight, reduced sperm counts, testis weights, and weights of androgen-dependent tissues and induces

histopathological lesions of the testis and sex accessory glands. BPA, in contrast, did not alter any of these endpoints at dose levels used in the current study. BPA was not a developmental toxicant in this study.

Neither EE2 nor BPA affected hormone levels (testosterone, prolactin, corticosterone, thyroid hormone, estradiol and LH; (supplemental tables)), caused obesity, induced reproductive tract malformations (undescended testes, hypospadias or agenesis of sex accessory tissues) in the F1 adult males.

Thank you for your attention

Sincerely

Dr L Earl Gray Jr

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EarthLink Revolves Around You.

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