LETTER TO THE EDITOR

Response to Hunt *et al.*, Invalid Controls Undermine Conclusions of FDA Studies

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Dear Editor,

The comments of Hunt *et al.* on our recent manuscripts (Delclos *et al.* (2014). *Toxicol. Sci.* **139**, 174–197 and Churchwell *et al.* (2014). *Toxicol. Sci.* **139**, 4–20, hereafter Delclos *et al.* and Churchwell *et al.*) raise issues related to both completed and ongoing studies on bisphenol A (BPA) at the National Center for Toxicological Research (NCTR). We address these concerns separately.

Hunt *et al.* suggest that the results of the 90-day BPA subchronic study described in Delclos *et al.* are not interpretable with regard to effects <2700 µg/kg body weight (bw)/day, the study-defined “low dose” region, because of the data reported in the companion manuscript, Churchwell *et al.*, indicating that there was unintended exposure to BPA in the negative controls. As pointed out in Delclos *et al.*, exposure of the negative controls to low levels of the target compounds frequently occurs in the studies of ubiquitous environmental contaminants, with the critical factor being the differential exposure between negative controls and treatments. We concluded that the level of exposure in the negative control animals in our study, as evidenced by the presence of BPA-glucuronide, which can only be produced by inlife exposure, would compromise our ability to interpret any BPA-related treatment effects <8 µg/kg bw/day. However, this was not an issue in the Delclos *et al.*’s study because: (1) there were no effects observed for the endpoints measured in doses up to 2700 µg BPA/kg bw/day versus the negative controls; (2) the reference estrogen had clear effects, even though these animals were equally exposed to the unintended source of environmental BPA; and (3) clinical and histopathological observations in the negative controls were comparable to observations in multigenerational studies conducted at this institution using the same strain of rat fed the same base diet (NTP, 2008, 2010).

Hunt *et al.* are concerned that the detection of BPA-glucuronide in the blood of control animals in the 90-day study indicates that the ongoing chronic toxicity study, which is also providing animals and tissues to multiple academic investigators (Consortium Linking Academic and Regulatory Insights on BPA Toxicity; CLARITY-BPA, Schug *et al.*, 2013), will be of

no use. The considerations discussed above would be applied to the interpretation of data from the chronic/CLARITY-BPA study. However, it should be made clear that the 90-day BPA subchronic study described in our two papers was conceived and implemented prior to the conception or formation of the chronic/CLARITY-BPA study. Although conduct at NCTR is common to both, and the issue of exposure of negative controls became evident only after the start of the chronic/CLARITY-BPA study, it is also the case that there are differences between the studies that significantly limit the potential for background exposure of controls in the ongoing study. The source of the reported unintended exposure in the 90-day study was proposed in Churchwell *et al.* to be related to the use of the broad BPA dose range, including very high doses (100,000 and 300,000 µg BPA/kg bw/day), in the same animal rooms as the control and low BPA doses (0 and 2.5 µg/kg bw/day). Consistent with our hypothesis, unpublished results of analyses of serum from animals in the chronic/CLARITY-BPA study rooms with a high dose of 25,000 µg BPA/kg bw/day indicate that the BPA-glucuronide levels in control rats, the critical measurement in distinguishing inlife exposure from postexposure sample contamination with aglycone BPA, are clearly distinguishable from levels in the lowest BPA dose group, 2.5 µg/kg bw/day. These data will be reported with the first results from the CLARITY-BPA studies.

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REFERENCES

- NTP (2008). Multigenerational reproductive study of genistein (CAS No. 446-72-0) in Sprague-Dawley rats (feed study), Technical report no. TR-539. *Natl. Toxicol. Prog. Tech. Rep. Ser.*, 1–266.
- NTP (2010). Multigenerational reproductive toxicology study of ethinyl estradiol (CAS No. 57-63-6) in Sprague-Dawley rats, Technical report no. TR-547. *Natl. Toxicol. Prog. Tech. Rep. Ser.*, 1–312.
- Schug, T. T., Heindel, J. H., Camacho, L., Delclos, K. B., Howard, P., Johnson, A. F., Aungst, J., Keefe, D., Newbold, R., Walker, N. J., et al. (2013) A new approach to synergize academic and regulatory-compliant research: the CLARITY-BPA research program. *Reprod. Toxicol.* **40**, 35–40.