COMMENTS

Response to the Letter by B. Elswick et al. from the Chemical Industry Institute of Toxicology

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Recently, Elswick, Miller and Welsch from the CIIT in North Carolina wrote a letter to *EBM* expressing concerns with data analysis of my paper, entitled "Reproductive malformation of the male offspring following maternal exposure to estrogenic chemicals" (1). I have given careful consideration to these concerns and have again examined the data very carefully. I have, however, come to the same conclusion that was previously made, i.e., estrogenic chemicals at low dosage produce an effect on the male reproductive development. The details of my responses are described in the following sections.

One of the questions raised by the CIIT scientists is on the post-hoc test. As described, the data were analyzed by ANOVA. Comparisons conducted using the LSD test support the effects reported in the paper. I never mentioned using one-tailed tests and the criticism that this occurred is unfounded. Also, I clearly stated in the data analysis section that there were 15 litters per treatment, and one male from each litter was used on the analysis for organ weights. For anogenital distance (AGD), it is obvious that 3 males per litter, i.e. a total of 45 males per treatment, were measured on postnatal day 3. Out of these 45 males 15 were sacrificed on postnatal day 3, leaving 30 males for subsequent analysis. On postnatal day 21, the remaining 30 males were analyzed for AGD and out of this 15 were sacrificed, leaving 15 males for subsequent analysis on postnatal day 60. The animals were not marked, which requires a toe clip or a tattoo procedure, since these procedures can be stressful. As a result, measurements of AGD at each time point cannot be analyzed using a repeated measure ANOVA.

Regarding the concern that it may be inappropriate to select one animal per litter, I would like to suggest that this is a standard procedure for controlling for litter effects, that is accepted by the NIEHS (2). This standard procedure has been discussed in detail at a recent meeting on endocrine disruptors (2).

Regarding other specific questions, for example for AGD and body weight on day 3, there was a significant correlation between AGD and body weight (Pearson's r = 0.47, n = 225, $P<.001$), and the correlation was significant and very similar for animals within in each treatment group, including controls. I chose to present data as a ratio of AGD to body weight, which is a common practice when the variables are, as shown above, correlated (2). It seems strange to me that this was raised as an issue, since I had pointed out that whether or not the AGD were adjusted for body weight did not alter the conclusions. Thus, whether AGD was analyzed without correction for body weight, by analysis of covariance with body weight as the covariate, or by dividing AGD by body weight, the conclusion did not change. Also, litter was included as nested within treatment in the ANOVA, so adjustment for litter effects occurred. While there was a significant litter effect, this was very small relative to the treatment effect.

Incidentally, I noticed after publication a typographical error in Table III of my paper. The standard deviation for 50pg/ml of bisphenol A and aroclor were reported about 10-fold higher than they really were. The standard deviations were actually 0.024 for BPA and 0.032 for aroclor. These errors in the table made the data set appear to have different variances in the different groups, but in fact this was not the case. What is more, in presenting the level of statistical difference I simply put down that the group differences were at the $p < 0.05$ level, but in fact, the difference between controls and bisphenol A was actually $p < 0.001$.

Thus, it is clear from the data that prenatal exposure to a low level of bisphenol-A, indeed alters male reproductive development. It is interesting to note that the studies that

failed to find an effect of this chemical are funded by the chemical industries (3, 4), whereas, positive findings are reported by independent academic laboratories (1, 5, 6). What is also clear, is that scientists who chose to study a chemical of commercial importance are subjected to intense scrutiny by the chemical industry and by the scientists funded by these industries.

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5. vom Saal FS, Timms BG, Montano MM, Palanza P, Thayer KA, Nagel SC, Minati DD, Ganjam VK, Parmigiani S, Welshons WV. Prostate enlargement in mice due to fetal exposure to low doses of estradiol or diethylstilbestrol and opposite effects at high doses. Proc Natl Acad Sci USA **94**:2056–2061, 1997.
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