Letter to the Editor

Dear Editor,

Recent discussions in publications and at meetings, on the presence (or not) of effects from low oral doses of BPA, have focused on the sensitivity, specificity, validity, and relevance of published studies on BPA. Most of them are small, exploratory/basic research studies with unique designs, non-oral routes of administration, few groups, few animals/group, and novel (nonvalidated) endpoints. The relatively few regulatory testing guidelines of large multigeneration reproductive toxicology studies are under governmental guidelines and GLPs, with oral routes of administration, a larger number of groups (minimum 4), large numbers of animals/group, and validated endpoints (e.g., Myers *et al.*, 2008; Tyl, 2009). The dietary BPA multigeneration studies (under EPA and OECD testing guidelines and GLPs) in CD (SD) rats (Tyl *et al.*, 2002) and CD-1 (Swiss) mice (Tyl *et al.*, 2008c) have been used in safety and risk assessments by the FDA (presented in an open meeting in Washington, DC on September 16, 2008), CERHR (2007), NTP (2008), EFSA (2006), and in weight-of-evidence assessments by Gray *et al.* (2004), Goodman *et al.* (2006, 2008 and 2009), Willhite *et al.* (2008), and others. The Tyl studies reported no systemic effects at or below 5 mg/kg/day (down to less than 1 µg/kg/day) and no reproductive/developmental effects at or below 50 mg/kg/day in either species. There were systemic effects (reduced body and organ weights, renal and hepatic histopathology) at and above 50 mg/kg/day, and reproductive and developmental effects at 500 (rat)/600 (mouse) mg/kg/day, including delayed acquisition of puberty in both sexes in rats and in male mice, and reduced weanling testis weights and delayed inguinal-scrotal testis descent in mice. There were no non-monotonic, dose-response curves for any parameter at any dose in either species. The authors concluded that BPA was not a selective reproductive/developmental toxicant in rats or mice.

The dietary BPA (Tyl *et al.*, 2002, 2008c) and E2 studies (Tyl *et al.*, 2008a, b) have been specifically and harshly criticized (e.g., Myers *et al.*, 2008) and the criticisms refuted (Tyl, 2009). The most recent concern with the Tyl studies is about the ages of the male mice at termination, since there were questions about terminal prostate wet weights in our males versus prostate weights in males at termination in some other laboratories. However, there were no statistically or biologically significant prostate weight differences across control and BPA groups for either rats or mice, for a given generation (F0, F1, F2), or within age. Critics have intimated that we do not track or know the ages of our animals in our multigener-

ation reproductive toxicity studies. Since our BPA work was published in *Toxicological Sciences* (Tyl *et al.*, 2002, 2008c), as well as other two-generation studies from our laboratory, I wanted to provide an explanation of the ages of our males.

The Tyl mouse study (under OECD Testing Guideline 416) included 9 groups: 2 vehicle control groups (both 0.0 ppm), 6 dietary BPA groups (from 0.018 to 3,500 ppm; 0.003-600 mg/kg/day), and 1 positive control group (0.5 ppm E2; ~80 µg/kg/day), with 28 F0 animals/sex/group to start, plus sentinels. A total of 280 males and 280 females at 5 weeks of age arrived at RTI and were held for a 1-week quarantine period. The mice were then distributed into the 9 groups (28/sex/group), stratified by body weight, and began their 8-week prebreed exposure period. At the completion of the prebreed exposure, the animals were paired 1:1 within groups and cohabited (with exposures continuing) for up to 2 weeks, with daily checking for evidence of copulation. On the day of evidence of mating (designated gestational day [gd] 0), the F0 female was removed from the male's cage and housed for her gestation (approximately 19 days, with dietary exposure continuing). The date of birth of the F1 offspring was designated postnatal day (pnd) 0, and the F0 female and her F1 pups remained together for the 3-week lactation period (with dietary exposure continuing). After mating, each F0 male was singly housed, with exposure continuing during gestation and lactation for the F0 dams. The F0 males were terminated 1 week prior to the first pnd 21 for the F0 females (to assure that there was no need to rebreed the males). Therefore, the F0 males' age at termination was 5 (arrival) + 1 (quarantine) + 8 (prebreed) + 3 (gestation) + 2 (lactation) + 3 days (necropsy) = ~19 weeks.

The F1 offspring stayed with their dams for the 3-week lactation, were weaned on pnd 21, with a 0-2 week holding period until the last F0 female weaned her F1 litter, then subjected to an 8-week prebreed period, and retained through F1 gestation of F2 litters (3 weeks) and 2 weeks of lactation, and then terminated 1 week prior to the first pnd 21 for the F1 dams (to assure that there was no need to rebreed the males) over 1 week. Therefore, the F1 males' age at termination was 3 (lactation) + 0-2 (holding) + 8 (prebreed) + 3 (gestation) + 2 (2/3 lactation) + 0-1 (necropsy) = 16-19 weeks. In most multigeneration studies, the F2 offspring are terminated at weaning (i.e., at 3 weeks of age).

PLEASE NOTE that the age of the males at demise, will depend on the following:

1. The start of the formal 8-week prebreed period, relative to when the F0 animals mated and delivered, since

the formal prebreed period begins after the last pregnant female weans her litter, so that all F1 offspring animals will be available for pairing at the end of the formal 8-week prebreed exposure period in all groups; and

2. The specific day during the necropsy when a given animal is terminated; we necropsy representatives of all groups on each day (if possible), and we cannot and do not necropsy more than a given number per day, typically 25-30/day. Technicians are “blind” for dose during all in-life and necropsy activities; dose groups are identified only by an Rx code and a color code.

Therefore, the F0 parental males were terminated when they were approximately 19 weeks of age (~4.75 months), and the F1 parental males were terminated when they were approximately 16-19 weeks of age (~4.0-4.75 months). Obviously, the study records required to be maintained in GLP studies have the exact ages of each male and female on study, from birth (or arrival at RTI) to demise.

There were no “errors” in the ages of our animals; the study reports and archived records are thorough and detailed. The publications used “approximate” ages to prevent having to provide a detailed explanation (presented here), and the author was not aware of the concern about the F0 and F1 male ages at necropsy at that time.

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