

newborns were 65–130 times less able to metabolize diazoxon and chlorpyrifos oxon than their mothers (Furlong et al. 2006). To further support the concern for children indicated by our quantitative risk assessment, we cited toxicologic studies establishing that in addition to cholinesterase inhibition, on which the NOAEL for chlorpyrifos is established, chlorpyrifos and chlorpyrifos oxon have other neurodevelopmental toxicity mechanisms (Huff et al. 1994; Qiao et al. 2002). We also noted that cell death has been induced at the reference dose for drinking water (Greenlee et al. 2005).

Peterson argues that the toxicologic studies we cited (Castorina and Woodruff, 2003; Eskenazi et al. 1999; Faustmann et al. 2000; Greenlee et al. 2005; Huff et al. 1994; Qiao et al. 2002) are an insufficient review of the “literature relevant to risk assessment” and that these studies are not appropriate for use in risk assessment. However, in missing the fact that we conducted a quantitative risk assessment, Peterson is misinterpreting our citations as the only basis for our public health concern. We consider it our public health responsibility to at least qualitatively consider recent toxicologic data in addition to a quantitative risk assessment based on established reference values. Others have argued for a complete restructuring of risk assessment for children, including toxicokinetic modeling and assessment of cellular and molecular outcomes over the entire lifespan of experimental subjects (Landrigan et al. 2004).

For many reasons we disagree with the suggestion that the epidemiologic fetal growth and gestational duration findings of Eskenazi et al. (2004) may be used to disregard concern for *in utero* and child organophosphate exposure highlighted by Eskenazi et al. (1999). The associations of reduced gestational duration with dimethyl organophosphate urinary metabolites and cholinesterase inhibition were not clinically significant in the California population studied (recent Mexican immigrants who tend to have very healthy birth outcomes). However, a shortened gestational age of a half-week would represent, for some women, a risk of preterm delivery (Eskenazi et al. 2004). Clearly, this finding and the absence of any adverse association between fetal growth and measures of *in utero* pesticide exposure need to be confirmed or refuted. To be complete, however, we also cited the association found in a New York City population between low birth weight and length and cord plasma levels of chlorpyrifos and diazinon ($n = 314$) (Whyatt et al. 2004). Further, effects of organophosphate pesticide exposure on early child neurodevelopment have been found (Young

et al. 2005) and are continuing to be evaluated in the California and the New York City cohorts. Finally, public health policy is typically developed to protect against a 1 in 1,000, or lower, risk, and the epidemiologic studies cited here are below the sample size necessary to detect such risks.

Peterson notes that a study of children in 10 homes did not demonstrate an association with child urine metabolite levels of chlorpyrifos and ambient air levels following crack and crevice treatment (Hore et al. 2005). Yet, the authors of that study were careful to note a number of study limitations, including the variability and accuracy of the child urinary metabolite readings. We also note that chlorpyrifos oxon, which also breaks down into the measured urinary metabolite, was not measured in air; air concentrations in four of the study homes were not elevated compared to pretreatment levels; and personal air samples were not collected (Hore et al. 2005). Among mothers in New York City ($n = 314$) in another study, 48-hr personal air samples collected during pregnancy were associated with cord and maternal blood levels of chlorpyrifos (Whyatt et al. 2004). This is the same study population within which an association with adverse birth outcomes and pesticide cord blood levels has been demonstrated, and the chlorpyrifos air levels are in the same (average, 15 ng/m³) range, if not lower, as those evaluated in our health risk assessment (Whyatt et al. 2004).

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Effects of BPA in Snails

It is an ethical requirement that new findings be presented in light of and in conjunction with a balanced evaluation of the current knowledge and published literature. We believe that Oehlmann et al. (2005) violated this general principle in several ways. For example, the authors inferred that prosobranch snails have a functional estrogen receptor and therefore a much higher sensitivity to estrogens and endocrine-disrupting compounds (EDCs) than other species previously reported in the literature. We found several other problems in their article:

First, Oehlmann et al. (2005) did not reveal the source of the animals used in their study, thus prohibiting independent repetition of the experiments by others.

Second, the authors stated that male and female *Marisa cornuarietis* cannot be distinguished morphologically without killing the animals. Therefore, the lack of data on the sex distribution of the animals sampled at each time-point leads us to question the stability of the experimental conditions with regard to sex ratios and thus reproductive conditions. Furthermore, the

rapidly changing snail density, and hence the sex distribution at each sampling time point, certainly influenced the remaining animals with respect to mortality and fecundity.

Third, the experimental design and the lack of replication (Experiment 1) did not allow for sound statistical analysis; the statistical methods used were inappropriate, making correct interpretation impossible. Of most concern to us was the analysis of data by analysis of covariance (ANCOVA), mainly because the ANCOVA-inherent assumption of independency of the dependent variable (i.e., total number of eggs) is violated. Thus, small differences among aquaria (treatment groups) might have been propagated over time, resulting in the impression of large differences.

Fourth, we believe that carrying out receptor binding experiments only in duplicate and without Scatchard analysis is questionable per se. The number of concentrations tested was extremely limited and consequently cannot allow accurate description of binding curves. Oehlmann et al. (2005) provided no information regarding the assessment of unspecific binding and the reported IC₅₀ values (concentration causing 50% inhibition) are approximately three orders of magnitude higher than what would be expected if this were a real sex-steroid receptor interaction. Because tamoxifen did not elicit a typical and highly specific receptor binding curve (Oehlmann et al. 2005, Figure 3), we question the use of tamoxifen as an “antiestrogen” in this *in vivo* study.

Finally, the data in Figure 1B (Oehlmann et al. 2005) were published earlier by Schulte-Oehlmann et al. (2001), yet the originally published data did not incorporate 17 α -ethinylestradiol (EE₂) as positive control. Moreover, the EE₂ curve in Figure 1B appears identical to the one on slide 14 from a slide presentation available on Oehlmann's website (Schulte-Oehlmann et al. 2006).

The use of a positive control is commendable when the mode of action is known [National Toxicology Program (NTP) 2001]; however, as in the study of Oehlmann et al. (2005), the lack of such knowledge precludes the inclusion of a positive control as proof-of-principle. Slide 14 (Schulte-Oehlmann et al. 2006) demonstrates that EE₂ does not have a monotonic mode of activity in *M. cornuarietis*, but rather appears to stimulate egg laying at 10–25 ng EE₂/L, inhibit egg laying at 50 ng EE₂/L and has no effect at 1 and 100 ng EE₂/L. On the basis of *in vitro* and *in vivo* effects reported by Oehlmann et al. (2005), we question the presence of any estrogen receptor–like interaction. In view of the NTP (2001) definitions and use of con-

trols, the use of EE₂ as a “positive” control, with its nonmonotonic and nonhormetic dose–response curve in comparison with BPA (which has a presumably monotonic response curve), as well as the use of an antiestrogen (tamoxifen), is inappropriate.

In conclusion, the data presented by Oehlmann et al. (2005) are unconvincing. Flaws in the experimental design, data presentation, and interpretation as well as statistical analyses render their findings untenable. Furthermore, the “Introduction” and “Discussion” of their article was written in a way that could be considered highly imbalanced and indeed alarmist. The highly selective inclusion/omission and discussion of previously published research that contradicts the authors' opinion (e.g., Pickford et al. 2003) is particularly disturbing. It is our opinion that our evaluation of the Oehlmann et al. work serves as a useful reminder to scientists that we must constantly strive to formulate clear hypotheses, use sound experimental designs, employ appropriate statistics, and draw conclusions that are supported by the available data and that reflect a balanced assessment of the scientific literature to avoid jumping to erroneous conclusions.

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Effects of BPA in Snails: Oehlmann et al. Respond

We welcome critical appraisals that help to provide balance; however, Dietrich et al. gave an unjustified reproach. We feel that Dietrich's position is severely compromised because he serves as an expert for the bisphenol A (BPA) Industry Group (Brussels, Belgium). We would like to respond to the issues raised by Dietrich et al., as well as to their oversights and inappropriate interpretations of our findings.

The source of test animals was clearly provided in our “Materials and Methods” (Oehlmann et al. 2005). All animals were dissected and sexed; thus, sex distribution was known for each time-point of the experiment. We supposed a 1:1 sex ratio for dead snails, although historical data ($n > 14,000$) indicate a slight prevalence of females (1.13:1); therefore, our assumption was conservative. Egg production was corrected for the number of females in the tanks, and snail densities were equal for all groups at each time-point.

Semistatic designs are widely applied in scientific and regulatory ecotoxicology [Organization for Economic Development and Co-operation (OECD) 1998]. The actual exposure concentrations of BPA were measured and clearly communicated in our Tables 1 and 2 (Oehlmann et al. 2005). Because 17 α -ethinylestradiol (EE₂) is more stable than BPA (Larsson et al. 1999), exposure to the positive control is also guaranteed in our 24-hr renewal test. Interestingly, Dietrich himself coauthored a semistatic study on snails (Czech et al. 2001) with several shortcomings: they used no analytical verification of exposure concentrations, no replicates, and inconsistent group size.

Analysis of covariance (ANCOVA) analyses of fecundity, development, and other cumulative data are widely used (Bochdansky and Bollens 2004; Dziminski and Alford 2005; Schärer and Wedekind 1999). In our experiment 2 with replicates (Oehlmann et al. 2005), ANOVA confirmed the