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. (2006) provided no information regarding the assessment of unspecific binding and the reported IC_{50} 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. 2006, 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. 2006) were published earlier by Schulte-Oehlmann et al. (2001), yet the originally published data did not incorporate 17α-ethinylestradiol (EE_2) as positive control. Moreover, the EE_2 curve in Figure 1B appears identical to the one on slide 14 from a slide presentation available on Oehlmanns' 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. (2006), 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 EE_2 does not have a monotonic mode of activity in *M. cornuarietis*, but rather appears to stimulate egg laying at 10–25 ng EE_2/L, inhibit egg laying at 50 ng EE_2/L and has no effect at 1 and 100 ng EE_2/L. On the basis of *in vitro* and *in vivo* effects reported by Oehlmann et al. (2006), we question the presence of any estrogen receptor–like interaction. In view of the NTP (2001) definitions and use of controls, the use of EE_2 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. (2006) 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. 2006). 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. 2006). Because 17α-ethinylestradiol (EE_2) 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. 2006), ANOVA confirmed the

ANCOVA results (Figure 2A,2C). A BPA Industry Group–sponsored statistical reevaluation of our raw data (Ecostat 2005) concluded that "at 20°C the mean egg production increased compared to the control in the BPA-exposed females at all applied concentrations (0.25, 0.5, 1 and 5 µg/L), and decreased in the BPA+faslodex- or tamoxifen-exposed females."

We achieved an association for a steady state of specific binding in three independent time-course studies (Oehlmann et al. 2006). We determined nonspecific binding using a 1,000-fold excess of unlabeled ligands resulting in clear specific binding for testosterone and estradiol. At higher concentrations, nonspecific binding was 70%, comparable with findings of Chou and Dietrich (1999), who also performed their experiments in duplicate. This percentage might be due to homogenization of large amounts of tissue with high protein content but a limited degree of specific cytosolic binding sites. In our study (Oehlmann et al. 2006), we did not intend to deliver a complete binding study in which saturation experiments with Scatchard analysis are needed, but to provide indications for the presence of estrogen receptors by a specific binding of ligands to cytosolic extracts (a widely used practice). Tamoxifen was not disqualified as an antiestrogen because it elicited a binding higher than that of BPA. Furthermore, *in vitro* ligand affinities have a limited predictive value for biologic potencies *in viv*o (Kloas et al. 1999). In summary, the binding study was performed appropriately for the desired purpose and provides initial evidence for specific estrogen binding sites with high affinity for BPA.

Data presented in our Figure 1B (Oehlmann et al. 2006) were published in Schulte-Oehlmann et al. (2001) without EE_2 because the focus of that work was comparing responses to BPA in four prosobranch species, including *Marisa*. Because the article was published in German, the distribution was not large enough to bring the issue to a wider audience. In the current article (Oehlmann et al. 2006), EE_2 data were included to demonstrate the masking of BPA effects during the spawning season. Because future BPA industry-sponsored studies intend to investigate BPA effects under conditions maximizing reproduction, the problem of masked effects and an associated loss of sensitivity is of vital importance.

Responses in *Marisa* (ruptured oviducts, increased spawning) are estrogen specific and opposite of androgenic effects (imposex, reduced spawning). This and evidence communicated in our article (Oehlmann et al. 2006) justify the use of EE_2 to demonstrate the responsiveness of organisms. Non-monotonic concentration responses have also been reported for estrogen-regulated end points in EE_2-exposed fish (Pawlowski et al. 2004), supporting our view that estrogen-specific binding sites in *Marisa* may represent functional receptors.

Dietrich et al.'s charges that our "Introduction" and "Discussion" were "imbalanced and indeed alarmist" and that we selectively used literature are unjustified.

We hope that the evidence presented here serves to refute the unjustified claims made by Dietrich et al. We leave it to the readers to make final judgment, but we feel that with the ever-increasing body of evidence showing effects of BPA on reproduction in various animal species, common sense will eventually prevail on this issue.

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Erratum

In the article by Colbert et al. [Environ Health Perspect 113:700–707 (2005)], the authors discovered an error in the units of exposure estimates in the tenth paragraph of the "Discussion." These estimates are expressed in milligrams but should be expressed in micrograms. The corrected sentences are as follows:

> It is estimated that children 1–6 years of age are exposed to 0.167 µg Vz/kg body weight/day …. Given this chronic exposure estimate, a 2-year-old boy who weighs 13 kg … would consume an average of 2.17 µg Vz/day, whereas a 6-year-old with a body weight of 21 kg would consume an average of 3.51 µg Vz/day.