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We thank Dr. Rayne for the comments on our publication. It should be stated at the outset that none of the comments Dr. Rayne makes challenges the validity of the experimental data indicating that 25 of the 28 hydroxylated PCB's tested bind to the calf uterine estrogen receptor with measurable affinity. The objectives as set out in our publication were achieved.

Regarding the applicability of modeling the protonated form, the literature tells us that this is the form of 17β-estradiol that specifically binds to the estrogen receptor with the phenolic proton forming a key hydrogen bond (Tannenbaum et al., 1998). Thus, the molecular properties of the phenolic forms of the hydroxylated PCB's were modeled and examined for relationships with binding affinity. It is true that the anionic form may dominate in bulk solution for a subset of these molecules. However, receptor binding occurs between one receptor and one ligand and that reaction does not occur in bulk solution (it is a desolvating process). The binding pocket of the receptor is hydrophobic. So, bulk properties that may dominate in solution do not necessarily dominate at the level of the individual receptor and ligand binding reactions. Thus, it is appropriate to model the properties of the protonated form for purposes of examining relationships of molecular properties with receptor binding. The molecular modeling of the phenolic structure reflected the ionizability of the oxygen atom as measured by the OCHARGE parameter. So, that parameter does contain information on the potential ionization state of the oxygen atom. The binding data also incorporates the ionization state that was actually achieved in the buffer system used. The ionization potential, which is related to pKa, was incorporated in the QSAR and was discussed in the publication.

The bulk solution equilibrium influences the frequency of encounters between protonated ligand and receptor. However, the affinity with which the protonated ligand binds to the receptor is not affected by the bulk solution equilibrium since this is an entirely separate chemical interaction (Eq. (1)).

\[
[\text{ER–OH–PCB}] = K_{eq} \text{ER} + \text{OH–PCB} \\
\text{OH–PCB} + H_2O \leftrightarrow O\text{–PCB} + H_3O^+ 
\]

Examination of this series of equations indicates that the equilibrium concentration of OH–PCB is affected by both the pH in the dissociation reaction and the concentration of estrogen receptor (ER) to which the OH–PCB may become bound. Therefore, whereas the affinity \((K_{eq})\) with which binding occurs is separate from the dissociation reaction, the measured IC50 is affected by the experimental conditions including temperature, receptor concentration and pH of the reaction medium. Two phenolic molecules with theoretically identical ligand binding affinity may have different IC50's if one is present at equilibrium at 1% versus 99% for the other. It may be anticipated that the IC50's in this theoretical case would differ by two orders of magnitude. Experimentally, this effect could be demonstrated by altering either the pH and/or the receptor concentration. In the experiments described in our publication, pH and receptor concentration were held constant.

The model was optimized in a way to minimize susceptibility to bias. The method for optimization is clearly described in the paper. It is not a conventional method for optimization which is usually focused on a single measure of “goodness of fit”. We did not choose to apply an arbitrary p value to the selection of parameters for the multiple regression model because 1) any particular p value is arbitrary, 2) the parameter may be contributing information to the overall model even if the p value is not less than 0.05. The fact that the model predicts IC50 to within one order of magnitude for all of the OH–PCB's that had measureable binding suggests some level of general applicability of the model to this set of chemicals. Is the model perfect? Clearly the model is not perfect, as reflected in residual plot provided by Dr. Rayne. No model is perfect. Do the percent ionization predictions prepared by Dr. Rayne invalidate the model? No. This question may be answered by calculating an adjustment to the predicted IC50's taking into account, as a worst case, the potential removal of the anionic species from the binding reaction. This
adjustment then predicts what would the predicted IC50 have been if all of the anionic species were unavailable for binding, hence shifting the predicted IC50 to a higher concentration. This is an unrealistic, worst case assumption because the dissociation reaction is a reversible reaction and will reach an equilibrium with the receptor binding reaction. Therefore, these adjustments are maximum deviations from expectation. As shown by Dr. Rayne calculations, a significant shift in predicted IC50 would be expected for 7 out of the 28 OH-PCB’s examined (those with predicted ionizations greater than 50% at pH = 7.4). None of the OH-PCB’s with low IC50’s less than 10^4 nM (hence stronger binding affinities) are significantly affected by these ionization calculations. Adjustment of the predicted IC50’s for percent ionization has little overall effect on the model (Fig. 1), though there is an improvement in the distribution of the residuals (Fig. 2).

Advanced receptor modeling, including docking models, may improve predictions of binding of hydroxylated PCB’s to the estrogen receptor (Yang et al., 2010).

**Conflict of interest**

The authors of this response declare that there is no conflict of interest in the preparation of this response.

**References**


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