Response to "Comment on 'Bioconcentration Factor Hydrophobicity Cutoff: An Artificial Phenomenon Reconstructed'"

We thank Yang and Zeng for their comments (*1*) on our recent paper in which we experimentally demonstrated that the often-observed bioconcentration factor (BCF) cutoff at logK_{ow} of 5.5 can be ascribed to experimental (third phase and nonequilibrium) artifacts (*2*). In their comment, Yang and Zeng stated that (i) our results do not provide any new insights that have not been obtained by other researchers, and (ii) our conclusion is misleading and may compromise ongoing efforts to investigate the mechanisms behind the BCF hydrophobicity cutoff phenomenon.

The first comment, which would suggest a failure of the three independent reviewers who judged the manuscript's originality for ES&T, unfortunately is not well-founded, as no references were provided to support the criticism. We acknowledge that the BCF hydrophobicity cutoff has been the subject of a scientific debate over the last 20−25 years and that many scientists have studied the phenomenon. Data contradicting the BCF hydrophobicity cutoff at logK_{ow} of 5.5 have been published (see Literature Cited in ref *2*) and several researchers have tried to mechanistically explain (e.g., (*3*)) or invalidate (e.g., (*4*)) the phenomenon. The latter attempts so far, however, concerned modeling approaches (see e.g., *4, 5*). The merit and strength of our paper (*2*) is the direct experimental and conclusive evidence (based on multiple different approaches) for the absence of the cutoff at logK_{ow} of 5.5 and the experimental demonstration of the artifacts that can cause the phenomenon. By applying state-of-the-art analytical approaches (SPME, POM-SPE) and varying exposure conditions we were able to actually reconstruct the cutoff and to validate yet hypothetical explanations. To our knowledge, there have been no previous papers that likewise experimentally reconstructed the hydrophobicity cutoff in a similarly conclusive way. Also note that we developed/used several novel approaches for studying bioaccumulation (addition of cellulose as substrate, in situ and dead worm exposures), thereby contributing to our conceptualization of bioaccumulation (approach 3 vs 5: bioaccumulation is a passive partitioning process) and an improvement of experimental BCF measurement methods.

Our response to Yang and Zeng's second comment (ii) is presented below in the section on the importance of the BCF cutoff in risk assessment. In addition to the above two comments, Yang and Zeng had several detailed comments on our experimental setup, and discussed the implications for risk assessment and alternative explanations for the cutoff. Below, we provide a rebuttal to these comments, following the authors' own section ranging.

Deficits in Experimental Design

Yang and Zeng's comments on our experimental design concern (i) the use of inadequate target analytes, (ii) the contribution of DOC to the cutoff, and (iii) overestimated C_b values obtained with approaches 5 and 6. In the Supporting Information, we provide a detailed rebuttal to all of these comments. Here, we restrict ourselves to briefly mentioning that the first comment probably involves a misunderstanding of our objectives in ref *2*. The second comment is based on an appropriate additional data analysis approach of our data, but Yang and Zeng's analysis is in part misleading and includes an unrealistic assumption for $K_{\text{third phase}}$ values, a too-subjective data interpretation, and a significant calculation error. A recalculation of third phase concentrations following Yang and Zeng's approach revealed realistic data with acceptable variation (see Supporting Information). Finally, overestimation of worm/liposome−water partitioning coefficients as suggested by the third comment does not play a role in our systems as the method applied was validated in previous publications and part of the criticism is not applicable to the materials studied.

Importance of the BCF Cutoff in Risk Assessment

In ref *2* we demonstrated that there is no mechanistic cause for any BCF hydrophobicity cutoff up to logK_{ow} of about 7.5. We, however, stressed that our data do not imply that there is no cutoff at all. In our opinion, there might be a cutoff at higher logK_{ow} values, where chemicals may get too large to penetrate membranes. We questioned though, whether such a cutoff exists within the hydrophobicity range applicable to (anthropogenic) chemicals for which risk assessment is necessary (e.g., brominated flame retardants, dioxins, petrochemicals). In other words, we think the linearity of the logBCF−logK_{ow} relationship might extend to include these chemicals as well. Yang and Zeng labeled this opinion "misleading" and considered it "premature to question the necessity to conduct risk assessment for these known toxins". We think this is a misunderstanding. After all, as mentioned above and in ref *2*, we do not question *risk assessment* for compounds like brominated flame retardants and dioxins; quite the contrary. We think the logBCF−logK_{ow} relationship may still be linear for these very hydrophobic chemicals, which implies that *risk assessment is essential*, because the compounds will have an extremely high bioaccumulation potential.

Alternative Explanations for a Curvilinear logBCF−logK_{ow} Relationship

In this section, Yang and Zeng provided alternative explanations for the hydrophobicity cutoff. We are not sure, however, what their exact aim was. Did the authors (i) question our conclusions in ref *2* (the cutoff at logK_{ow} of 5.5 is caused by third phase and nonequilibrium artifacts) and therefore provided alternative explanations to our observations, or (ii) list additional explanations for any BCF cutoff, for instance at higher logK_{ow} values? In the first case, we refer to ref *2*, where in our opinion we provided conclusive evidence for third phase and nonequilibrium effects being the causative explanations for the cutoff at logK_{ow} of 5.5. Elimination via feces does not apply in ref *2*, as worms in approaches 1 and 2 were gut-purged prior to the experiments and the organisms were not fed during exposure; metabolism of PAHs hardly occurs in *L. variegatus* (and if, then predominantly for the low-molecular-weight PAHs and not for the very hydrophobic ones (*6, 7*); restricted membrane permeability was not observed in approaches 3 and 4 and thus cannot explain the cutoff in approach 1; and octanol being a poor surrogate for lipids would not have allowed the linear relationships to be observed in approaches 3−6. A detailed discussion of the alternative explanations for any cutoff provided by others and listed by Yang and Zeng (second case) was beyond the scope of ref *2* and thus of this response. Note again that in ref *2* we focused on the cutoff at logK_{ow} of 5.5. In the Supporting Information however, some brief considerations with respect to this issue are provided.

In conclusion, we cannot agree with any of the comments from Yang and Zeng. In our view, we did provide insights in

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ref *2* that were not provided by others before, our experimental design was robust and in accordance with our objectives, and our conclusions are supported by our data and cannot be considered misleading.

Supporting Information Available

Additional text and references. This material is available free of charge via the Internet at http://pubs.acs.org.

Literature Cited

(1) Yang, Z-Y; Zeng, E. Y. Comment on "Bioconcentration factor hydrophobicity cutoff: An artificial phenomenon reconstructed". *Environ. Sci. Technol.* **2008**, *42*, 9449–9450.

(2) Jonker, M. T. O.; Van der Heijden, S. A. Bioconcentration factor hydrophobicity cutoff: An artificial phenomenon reconstructed. *Environ. Sci. Technol.* **2007**, *41*, 7363–7369.

(3) Opperhuizen, A.; Van de Velde, E. W.; Gobas, F. A. P. C.; Liem, D. A. K.; Van der Steen, J. M. D. Relationship between bioconcentration in fish and steric factors of hydrophobic chemicals. *Chemosphere* **1985**, *14*, 1871–1896.

(4) Gobas, F.; Clark, K. E.; Shiu, W. Y.; Mackay, D. Bioconcentration of polybrominated benzenes and biphenyls and related superhydrophobic chemicals in fish - role of bioavailability and elimination into the feces. *Environ. Toxicol. Chem.* **1989**, *8*, 231–245.

(5) Verhaar, H. J. M.; De Jongh, J.; Hermens, J. L. M. Modeling the bioconcentration of organic compounds by fish: A novel approach. *Environ. Sci. Technol.* **1999**, *33*, 4069–4072.

(6) Leppanen, M. T.; Kukkonen, J. V. K. Fate of sediment-associated pyrene and benzo[a]pyrene in the freshwater oligochaete *Lumbriculus variegatus* (Muller). *Aquat. Toxicol.* **2000**, *49*, 199–212.

(7) Schuler, L. J.; Wheeler, M.; Bailer, A. J.; Lydy, M. J. Toxicokinetics of sediment-sorbed benzo[a]pyrene and hexachlorobiphenyl using the freshwater invertebrates *Hyalella azteca*, *Chironomus tentans*, and *Lumbriculus variegatus*. *Environ. Toxicol. Chem.* **2003**, *22*, 439–449.

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