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Rebuttal to Correspondence on “Machine Learning-Assisted Identification and Quantification of Hydroxylated Metabolites of Polychlorinated Biphenyls in Animal Samples”

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We received correspondence from Ziegler and colleagues on our recently published paper describing a machine learning-assisted approach for characterizing hydroxylated metabolites of polychlorinated biphenyls (OH-PCBs). Ziegler et al. reemphasize the role of our machine learning-based approach in identifying OH-PCBs by predicting their retention and MS/MS profiles by gas chromatography-tandem mass spectrometry (GC-MS/MS). Because of the limited availability of analytical standards of OH-PCBs and the formation of complex mixtures of OH-PCB congeners by mammalian cytochrome P450 enzymes,[1] the machine learning approach is particularly valuable for predicting the position of chlorine and hydroxyl groups of the detected OH-PCB peaks [analyzed as methylated derivatives (MeO-PCBs)], thus facilitating the targeted synthesis of OH(MeO)-PCB standards for unambiguous identification.

We demonstrated the utility and robustness of our approach using a liver sample derived from a mouse exposed to a PCB mixture containing PCB 28 and 11 other PCB congeners via the maternal diet as a case study. Briefly, we observed three hydroxylated metabolites of PCB 28 in this sample. These metabolites were tentatively identified as 3′-28 (2,4,4′-trichlorobiphenyl-3′-ol), 5-28 (2,4,4′-trichlorobiphenyl-5-ol), and 4-22 (2,3,4′-trichlorobiphenyl-4-ol), a possible 1,2-shift product of PCB 28. The identification of the two major metabolites, 3′-28 and 5-28, was subsequently confirmed with authentic standards (Figure 1). The minor metabolite was not 2′-28 (2,4,4′-trichlorobiphenyl-2′-ol), 3-28 (2,4,4′-trichlorobiphenyl-3-ol), or 4′-25 (2,3′,4-trichlorobiphenyl-4′-ol), as determined using authentic standards of these metabolites. Therefore, on the basis of the candidate ranking scores calculated with experimental and predicted relative retention times and MS/MS profiles, we tentatively identify the minor metabolite as 4-22.

Ziegler and colleagues, in their correspondence, concluded that this minor metabolite is 4-31 (2,4′,5-trichlorobiphenyl-4-ol), another 1,2-shift metabolite of PCB 28. This identification is based on the OH-PCB 28 metabolite profile of a human plasma sample and the elution order of authentic OH-PCB 28 standards on a liquid chromatography-tandem mass spectrometry (LC-MS/MS) system. Their findings appear to disprove our tentative identification of the minor metabolite as 4-22. However, we argue that mice do not necessarily form the same OH-PCB 28 metabolites as humans. For example, 4′-25 appears to be a human metabolite that was present in human

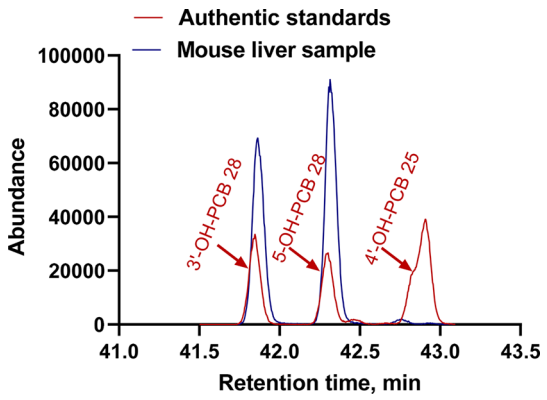

Figure 1. GC-MS/MS chromatograms of the OH-PCB 28 metabolites in an authentic standard solution and a liver sample from a mouse exposed to a PCB mixture containing PCB 28 through the maternal diet. The OH-PCBs were derivatized and analyzed as MeO-PCBs. The concentrations of the authentic standards of MeO-PCBs were approximately 60 ng/mL. Chromatographic separation was achieved with an SPB-Octyl capillary column, as described previously.[5]

samples but was not detected in the mouse liver in our study. Species differences in the metabolism of dioxin-like and non-dioxin-like PCB congeners are well documented in the literature.[2,3] Moreover, the minor OH-PCB 28 metabolite detected by GC-MS/MS versus LC-MS/MS may not be identical because of differences in the elution orders or sensitivities on the respective GC versus LC column. Therefore, the identification of the minor OH-PCB 28 metabolite in our mouse study remains tentative. Further studies using authentic standards of 4-22 and 4-31 are needed to confirm the identification of the minor OH-PCB 28 metabolite observed in mice.

Ziegler and colleagues also recommended using thresholds obtained from receiver operating characteristic curves (ROC curves) to filter the OH-PCB isomers with predicted and

experimental retention times. ROC curves are a performance metric primarily used for binary classification algorithms where the true positive rate (TPR) and false positive rate (FPR) can be readily determined by plotting ROC curves. To plot ROC curves in our multiple-linear regression or random forest regression algorithms, the TPR and FPR need to be calculated from the "positives" or "negatives" defined by retention time tolerances between predicted and experimental values, as described by Osipenko et al.[4] Our major concern is that the threshold obtained from the ROC curve is a compromise between the number of true positives and false positives and that the filtering of OH-PCB candidates with this threshold will inevitably lose true positive candidates with the probability defined by the threshold. In contrast, our strategy is not to filter OH-PCB candidates with predicted retention times and take all candidates to the next step for in silico MS/MS prediction. The final prediction is based on ranking scores of candidates calculated from both retention time and MS/MS predictions. Nevertheless, we agree that filtering candidates with the threshold obtained from ROC curves could be a practical approach for narrowing down the potential candidates with a defined FPR when retention time prediction is the only dimension used.

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Notes

The authors declare no competing financial interest.

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