# Applying PBK Modeling to Support Safety Assessment of Environmental Chemicals: Case Studies for Developmental Neurotoxicity

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## Intro

Developmental neurotoxicity (DNT) testing of environmental contaminants (e.g. pesticides, industrial chemicals) is essential for chemical safety assessment. Current approaches, however, rely on extensive animal studies that are costly, raise ethical concerns, and may poorly reflect human neurodevelopment. To address these issues, new approach methodologies (NAMs) using in vitro and in silico systems are increasingly applied.

A key element of NAMs is quantitative in vitro–in vivo extrapolation (QIVIVE), where in vitro effect concentrations are translated into oral equivalent doses (OEDs). The outcome of QIVIVE depends on factors such as assay relevance, benchmark response, in vitro bioavailability corrections, surrogate tissue selection, and PBK model accuracy. Since full QIVIVE is data- and resource-intensive, a tiered approach is typically adopted, starting with protective prioritization and refined towards predictive accuracy when risks are indicated. Here, we explore suggested tiers through two case-study pesticides: chlorpyrifos and carbaryl.

### Building a Tiered DNT QIVIVE Framework with OSP Suite

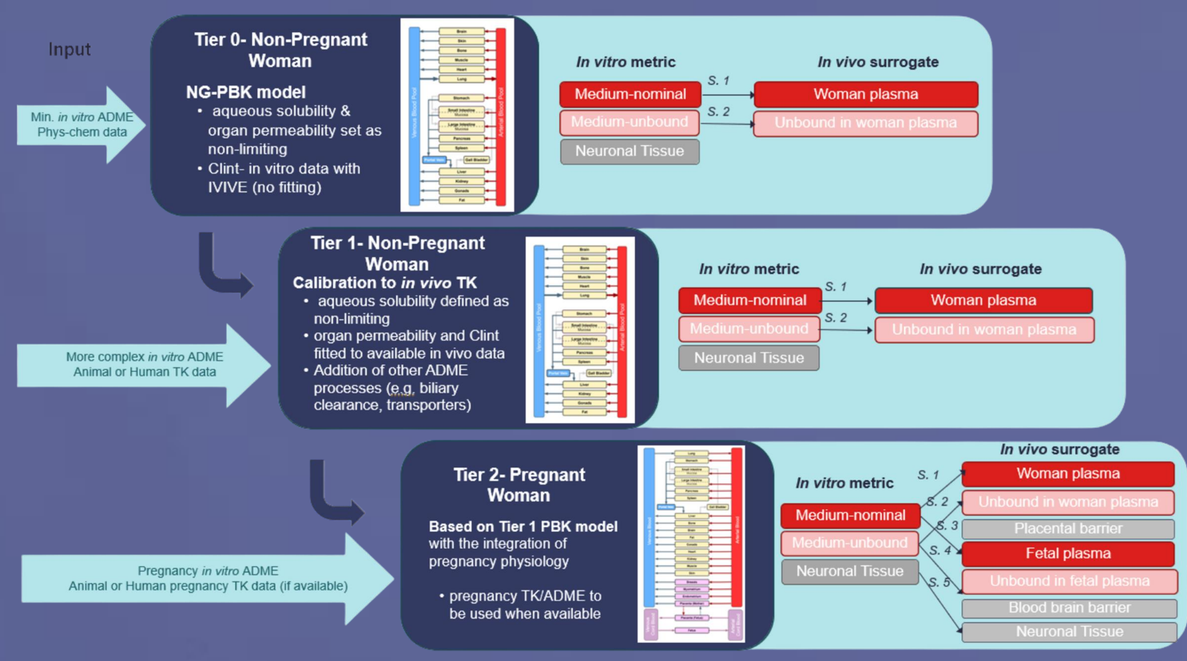


*Figure 1-Tiers and extrapolation scenarios proposed for assessing DNT for pesticides.*

## Methods

For the QIVIVE, DNT *in vitro* data were obtained from [1]. The 5$^{th}$ percentile of the geometric mean in vitro benchmark concentrations, calculated across all DNT readouts from four pipelines, was used as the in vitro point of departure. The two pesticides PBK model simulations were conducted with the Open Systems Pharmacology (OSP) Suite [2], while in vitro mass balance modeling was performed using the specific characteristics of the *in vitro* DNT assays, average of logP and linear free energy relationships QSARs [3]. For reverse dosimetry, Cmax after repeated exposure was applied. Pregnancy PBK simulations were run for a gestation week 16. This work aligns with EFSA [4] and OECD [5] initiatives to establish DNT-QIVIVE methodologies for NGRA.

## Results

### Chlorpyrifos and the active metabolite (CPO)

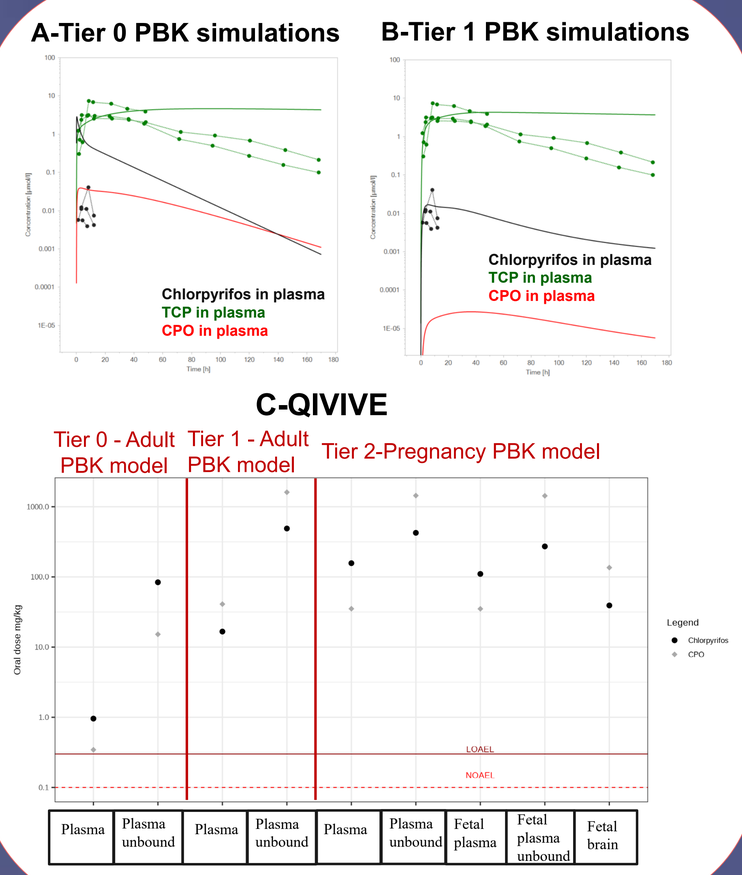


*Figure 2-Predictions and observed plasma concentrations of chlorpyrifos and metabolites, CPO (active metabolite) and TCP (excreted metabolite) in human adults dosed 3 mg/kg BW [6] A-using Tier 0, minimal PBK models and B-using Tier 1 PBK model; model refined with human pharmacokinetics (volunteers and poisoned clinical cases). **C**-OEDs resulting from the different tiers and extrapolation scenarios.*

- Tier 1 improved Cmax predictions for Chlorpyrifos from predictions from 127 fold to 1.29, and CPO predictions from 137 to 0.85. Tier 1 was obtained by fitting intestinal permeability and CPO formation.
- Chlorpyrifos and the CPO metabolite are semi-volatile, causing artifacts in the *in vitro* experiments readouts.
- Predicted OEDs for Tier 2 and Tier 3 are > 10 fold higher that animal LOAELs, but even higher fold differences when plasma unbound is used as surrogate.

### Carbaryl

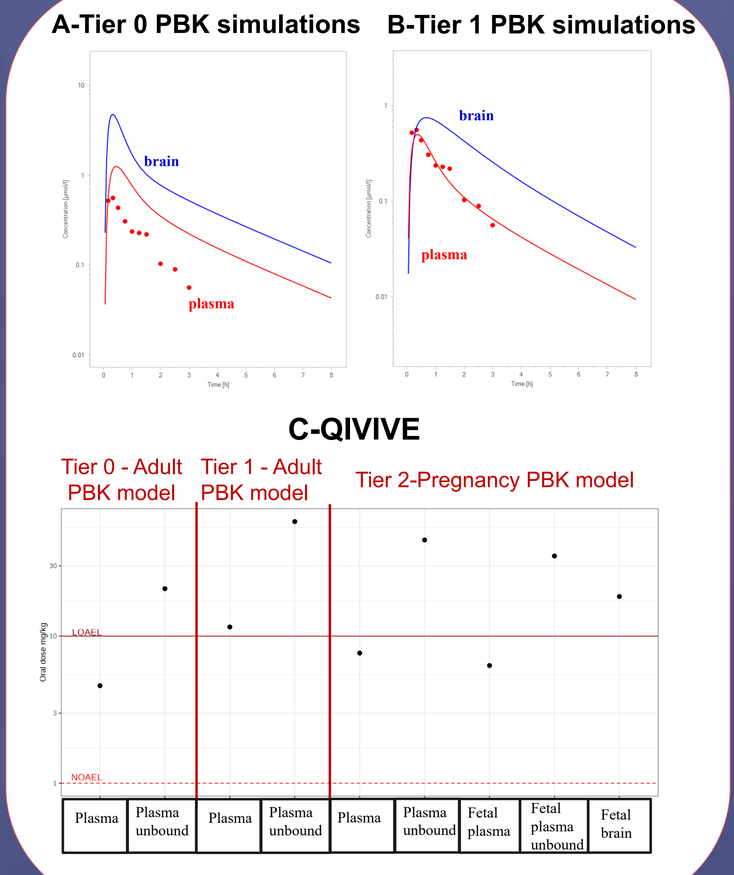


*Figure 3-Predictions and observed plasma and brain concentrations in human adults dosed 1 mg/kg BW [7] A-using Tier 0, minimal PBK models and B-using Tier 1 PBK model; model refined with rat plasma and tissues in vivo kinetics. **C**-OEDs resulting from the different tiers and extrapolation scenarios.*

- Tier 1 improved Cmax predictions from 2.25 fold to 1 by fitting brain permeability, and $Fu_{plasma}$.
- Predicted OEDs differ from LOAELs by less than one order of magnitude. This is reasonable, given that repeated dose assays can vary by two orders [8] and animal DNT data remain highly uncertain [9].

## Conclusion

- ❖ Tier 0 models, though more uncertain in terms of their predictions, provided more conservative OEDs.
- ❖ The pregnancy physiology does not lead to very different predictions from Tier 1. This indicates that uncertainties with highest impact in the QIVIVE approach arise from the chemical's ADME processes.
- ❖ Uncertainties in oral absorption (permeability and solubility) and $Fu_{plasma}$ have the highest impact in predicted OEDs. This suggests that to reduce animal PK experiments more accurate predictions of $Fu_{plasma}$ and oral absorption parameters are needed, even for chemicals with more extreme physicochemical properties.
- ❖ QIVIVE, and especially the in vitro DNT assays need to be tailored to semi-volatile chemicals, which constitute a relatively large portion of DNT chemicals.
- ❖ Future work will include additional case studies, as well as uncertainty and probabilistic analyses.

## References

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[8] Pham et al. 2020; Comput Toxicol. Aug 1;15:1-100126

[9] Paparella et al. 2020; Reprod Toxicol. 2020 Sep:96:327-336

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