Exploring POD-NAMs for DNT Risk Assessment: Bridging In Vitro Data and Human Exposure Levels

S. Fragki[1], S. Proenca[1], L. Lamon[1], S. Schaller[1], M. Paparella[2], I. Virmani[2], M. Siccardi[1]
1-ESQlabs GmbH, Saterland, Germany
2-Department of Medical Biochemistry, Medical University of Innsbruck, Innsbruck, Austria
*corresponding author: stella.fragki@esqlabs.com

Presenter: Stella (Styliani) Fragki

Intro

Quantitative in vitro to in vivo extrapolation (QIVIVE) links in vitro concentration–effect data to in vivo organ exposure and respective **oral equivalent dose (OED)**, translating as such in vitro readouts into corresponding human doses. The validity of QIVIVE depends on accounting for in vitro kinetics (e.g., protein/lipid binding) and in vivo Absorption, Distribution, Metabolism and Excretion (**ADME**) processes, which require the use of biokinetic modeling. In silico **mass balance models** simulate the in vitro chemical kinetics, while **physiologically based kinetic (PBK)** models can estimate the external dose needed to reach equivalent tissue concentrations. Benchmarking these OEDs against human exposure scenarios enables risk estimation. In this study, QIVIVE was applied to an example selected pesticide, carbaryl, comparing **Points of Departure (PoDs)** from in vitro developmental neurotoxicity (DNT) assays with traditional animal-based PoDs. Reverse dosimetry using PBK models explored different in vitro starting points and in vivo surrogates, aiming to support a tiered, NAM- and NGRA-aligned approach to **DNT risk assessment**.

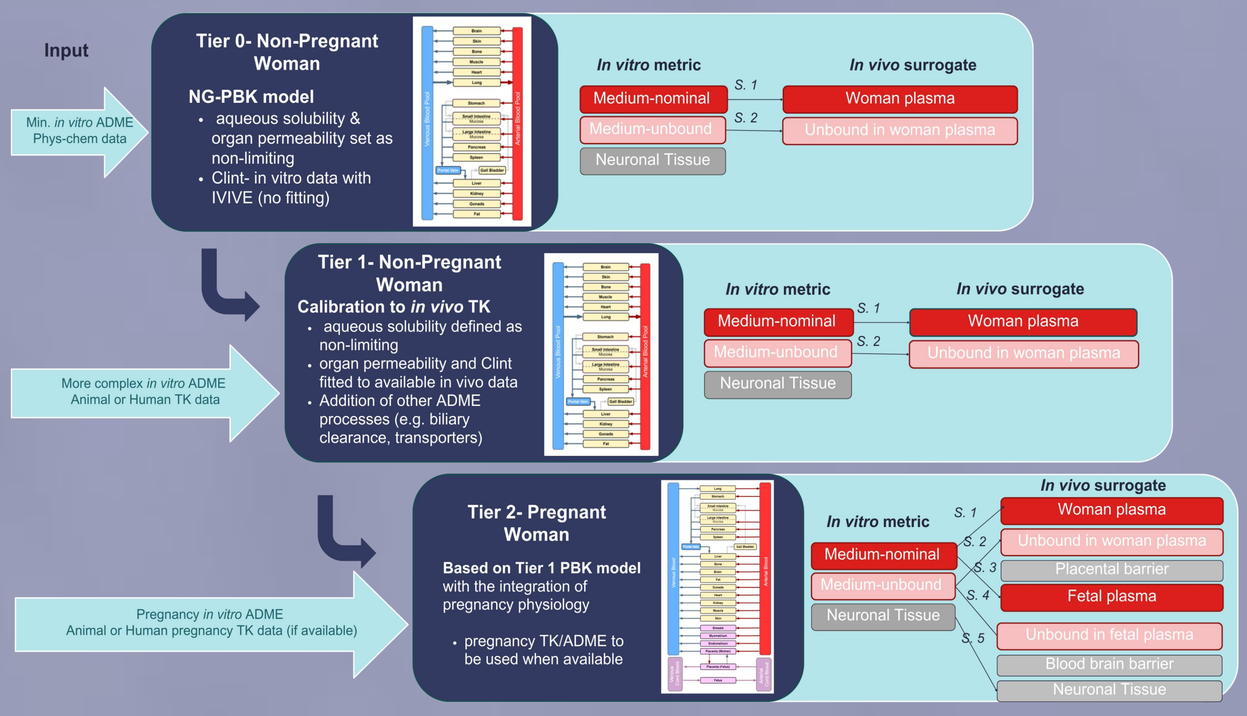
Methods

For the QIVIVE, DNT in vitro data were obtained from [1]. The 5th 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. Carbaryl PBK model simulations were conducted with the Open Systems Pharmacology (OSP) Suite [2], while in vitro mass balance modeling was performed using a model developed at ONTOX [3] specifically for the DNT assays. For reverse dosimetry, peak concentration was applied, as it is most predictive of developmental toxicity. Pregnancy PBK simulations were run for a single oral exposure at gestation week (GW) 16, with additional simulations at GW 36 to estimate fetal brain concentrations.

Name chemicals	MW	logP	Fu plasma	pKa	Absorption	Distribution	Clearance
Carbaryl	201.22	2.36	0.16	neutral	PK-Sim QSAR for oral permeability and water solubility depending on the Tier. Gut compartments are described with different pHs.	Berezhkovskiy algorithm; organ permeability: Tier 0: 100 cm/min; Tier 1: 0.5 cm/min, fitted to rat brain data.	Eliminated via metabolism; Tier 0: 27.27 µl/min/million hepatocytes (ToxCast database [4]); Tier 1: 19.78 1/min (fitted).

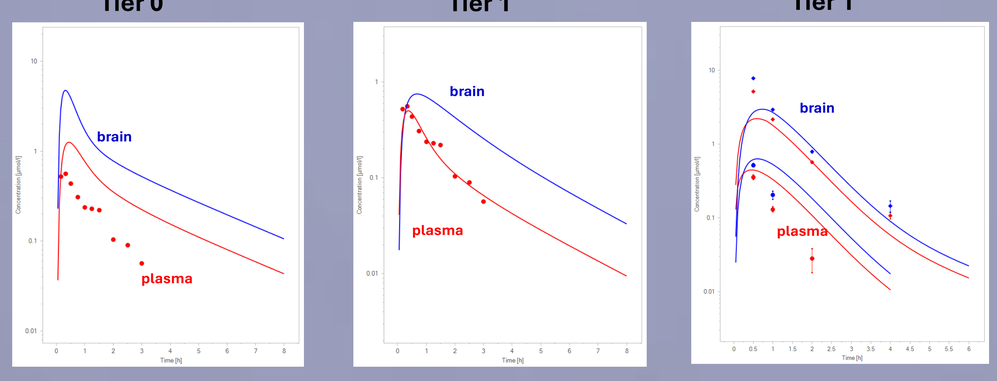
Results

Building a Tiered DNT QIVIVE Framework with OSP Suite

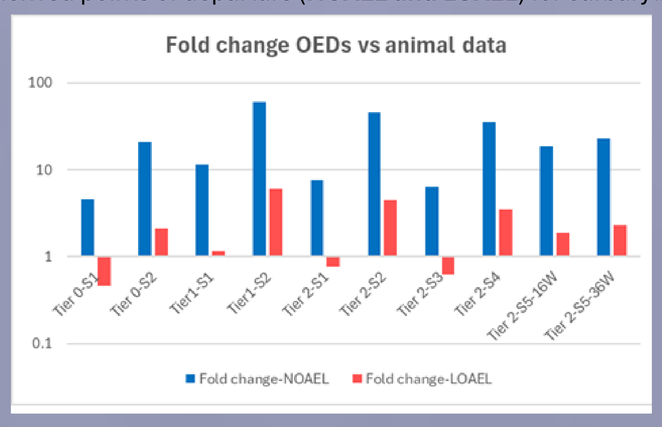


Tier 0 (Non-pregnant, Next Generation-PBK): minimal in vitro ADME/physicochemical inputs, assumes non-limiting solubility/permeability, and applies IVIVE of clearance (Clint) without parameter fitting. **Tier 1** (Non-pregnant, calibrated PBK): calibrated to in vivo TK data with adjusted permeability/clearance and added ADME mechanisms (e.g., transport, biliary clearance). **Tier 2** (Pregnancy-PBK): integrates pregnancy physiology and, when available, pregnancy-specific TK/ADME data to capture maternal–fetal kinetics. Across tiers, **in vitro metrics** (medium–nominal, medium–unbound) are quantitatively linked to **in vivo surrogates** (maternal plasma, unbound plasma, fetal plasma, fetal unbound plasma, fetal brain). **Scenarios S1–S5** represent specific in vitro–in vivo pairings used to estimate OEDs.

Carbaryl concentrations in plasma and brain simulated with Tier 0 (left) and Tier 1 (middle & right) PBK models. Left, middle : humans, oral, 1 mg/kg bw [5]; right: rats, oral 3 (diamonds) and 15 (circles) mg/kg bw [6].



Ratios of predicted OEDs from all PBK tier–scenario combinations relative to animal-derived points of departure (**NOAEL and LOAEL**) for carbaryl.



Conclusion

- This works explores a DNT-QIVIVE framework with PBK and in vitro mass balance modeling, attempting to bridge in vitro DNT data to human OEDs.
- In the carbaryl case study, most tier–scenario combinations predicted OEDs higher than animal NOAELs/LOAELs.
- Predicted OEDs differ from LOAELs by less than one order of magnitude, a good outcome given that repeated dose assays can vary by two orders [8] and animal DNT data remain highly uncertain [9].
- Tier 0 models, though more uncertain in terms of their predictions, provided more conservative OEDs.
- Scenarios 1-3 yielded lower OEDs than Scenarios 2-4, as carbaryl fu in vitro is higher than in vivo.
- Future work will include additional case studies, as well as uncertainty and probabilistic analyses.
- This work aligns with EFSA and OECD [7] initiatives to establish DNT-QIVIVE methodologies for NGRA

References

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