Reducing Animal Use in Toxicological Research through Open-Source Individualized (PB)PK/QST Modeling: A Case Study with Phenobarbital in Dogs

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Introduction

As part of ongoing efforts to advance New Approach Methodologies (NAMs), we have previously presented a workflow on building a large virtual population efficiently to capture the overall statistical properties of a target population, and wrapped it around a **DILI QST** model developed in-house [1].

Here, we extend that work with a complementary **virtual-twin** strategy: accurately calibrating a (PB)PK/QST framework to individual longitudinal data. Specifically, we developed a phenobarbital PK model and coupled it to our previously developed DILI QST model to predict ALP dynamics, enabling single-animal fits across IV/PO and chronic dosing phases. The individualized model converts data into a reusable **in-silico proxy** for each animal, enabling head-to-head comparisons of dosing regimens, washout lengths, and sampling schedules so we can pre-select the **minimal, most informative** experimental design. Calibrating complex QST models to individual data is challenging: multi-phase dosing and sparse sampling, simultaneous fitting of exposure and biomarkers, inter-individual heterogeneity, and parameter non-identifiability/computational stiffness. We address these challenges with a principled workflow for virtual twins that complements virtual populations for decision-ready toxicology.

Methods

1. Build a PK model for phenobarbital in dogs fit to data from [1]. Calibrate the model to mean single dose IV and PO data and validate it against mean repeat-dose data (daily wean-off after 28-day administration)

Phase I* – single 12 mg/kg dose*				Phase II – 6 mg/kg PO q24h		Phase III*† – single 12 mg/kg dose			
Dogs 1–4	Administered Orally	28 Day washout	Administered Intravenously	‡28 Day administration	**7 Day washout	Dogs 1–4	Administered Orally	7 Day washout	†Administered Intravenously
Dogs 5–8	Administered Intravenously		Administered Orally			Dogs 5–8	Administered Intravenously		†Administered Orally

2. Use the DILI QST model to predict ALP time course and compare against mean ALP data from [2].
3. Pre-fit the DILI QST model under baseline conditions to reproduce individual steady-state ALP levels.
4. Use Sobol Global Sensitivity Analysis to select PK/PD parameters for optimization.
5. Use a Genetic Algorithm (GA) to fit the model simultaneously to individual PK (IV and PO) and ALP data.
6. For the best individual fit, simulate all dosing phases and report goodness-of-fit.

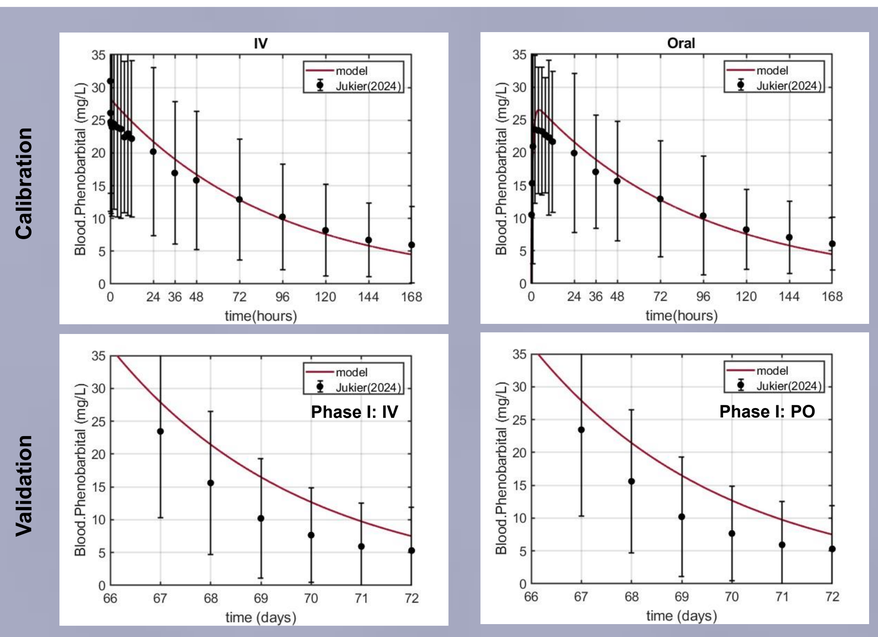

Figure 1. Top row: Phenobarbital PK model calibration to single dose IV and PO data. Bottom row: Phenobarbital PK model validation against repeat-dose data measured during the daily wean-off period following 28-day PO administration.

Results

The phenobarbital PK model reproduced single-dose IV/PO and repeat-dose profiles across study phases. When coupled with the DILI QST model, the model captured the ALP rise over the study timeline.

The GA individualized one parameter set per dog by jointly fitting IV PK, PO PK, and ALP time courses. The GA minimized a composite loss with priors and produced satisfactory goodness-of-fit for each readout. Example readout for Dog 2: PO PK (RMSE = 2.52, MAE = 1.81, R^2 = 0.89), IV PK (RMSE = 4.49, MAE = 3.17, R^2 = 0.55), and ALP (RMSE = 0.41, MAE = 0.28, R^2 = 0.99). Residuals were well-centered with stable patterns over time, indicating good model fit across PK and ALP profiles, with minor deviations in the very early PK phases.

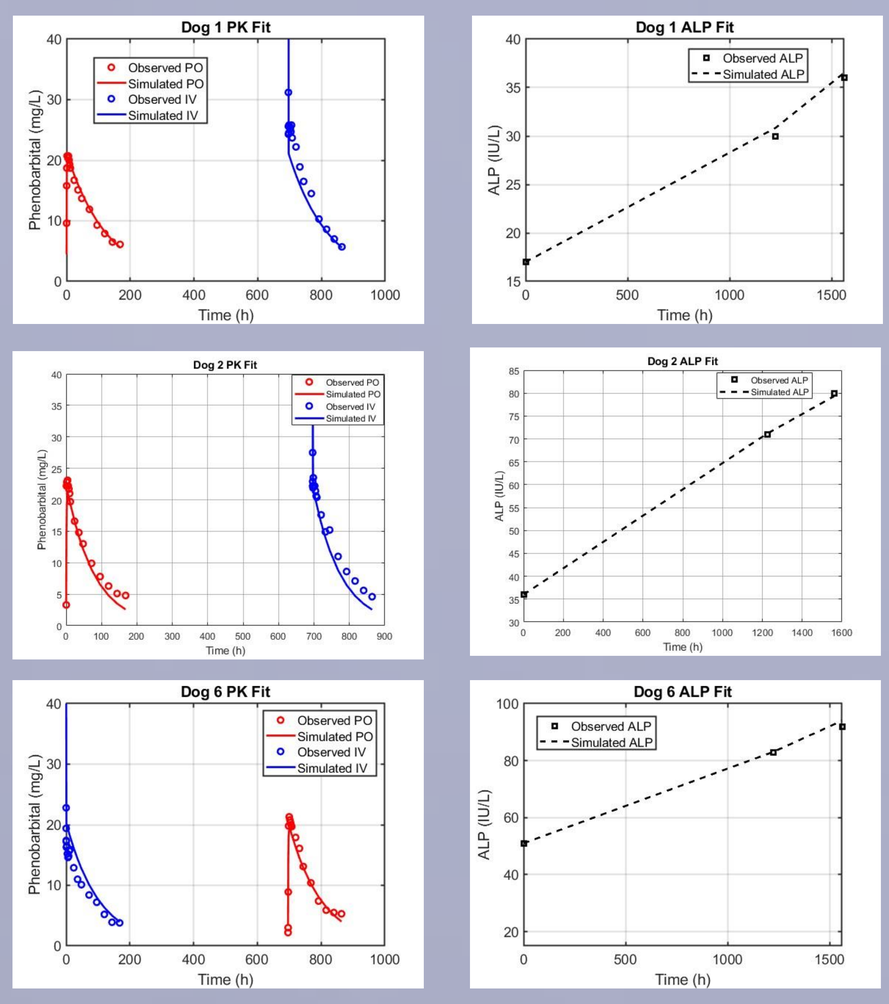

Figure 2. Individualized GA fits for phenobarbital PK and ALP for select study dogs.

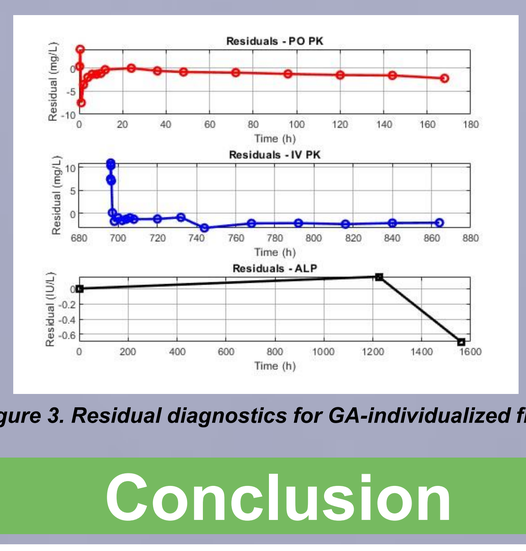

Figure 3. Residual diagnostics for GA-individualized fits.

Conclusion

An individualized (PB)PK/QST workflow accurately reproduced dog-specific phenobarbital exposure and ALP dynamics using genetic-algorithm fits across study phases. The framework enables mechanistic, animal-level predictions and in-silico exploration of dosing strategies to improve study efficiency and reduce animal use in toxicological research.

References

[1] Welcome to the Population Approach Group in Europe. (n.d.). https://www.page-meeting.org/default.asp?abstract=11491
[2] Jukier, T., Gross, A., & Boothe, D. (2024). Pharmacokinetics and tolerability of a veterinary phenobarbital product in healthy dogs. Frontiers in Veterinary Science, 10. https://doi.org/10.3389/fvets.2023.1307888

Modeling

SCAN ME

Coding

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