

# PBPK Modeling Through Drug-Induced Liver Injury (DILI) Study by Nevirapine and 12-Hydroxynevirapine Concentrations in Liver Tissue and Blood



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## BACKGROUND

### What is Physiologically based pharmacokinetic (PBPK) Modeling:

- Mechanistic approach predicting drug disposition in specific tissues using physiological parameters.
- Parent-metabolite network modeling assessment of both drug and metabolite tissue exposure.

### Nevirapine & Hepatotoxicity:

- Antiretroviral drug associated with severe drug-induced liver injury (DILI).
- Exhibits complex pharmacokinetic: autoinduction and enterohepatic recirculation.
- Current hepatotoxicity assessment methods inadequately predict liver-specific drug accumulation.

## OBJECTIVE

- Develop parent-metabolite PBPK model for Nevirapine in SD rats.
- Plasma and hepatic concentration data for parent drug and metabolite through *in vivo* experiments.
- Characterize tissue-specific accumulation patterns across different doses and exposure durations.
- Establish framework for future human extrapolation of hepatotoxicity risk assessment

## METHODS

### Study Design and In vivo Experiment:

- Experimental animals: Sprague-Dawley (SD) rats
- Dosage: 18, 36, 72 mg/kg (Human equivalent dose 200, 400, 800 mg)
- Dosing Schedule: Single dose, 1 week to 4 weeks (once daily)

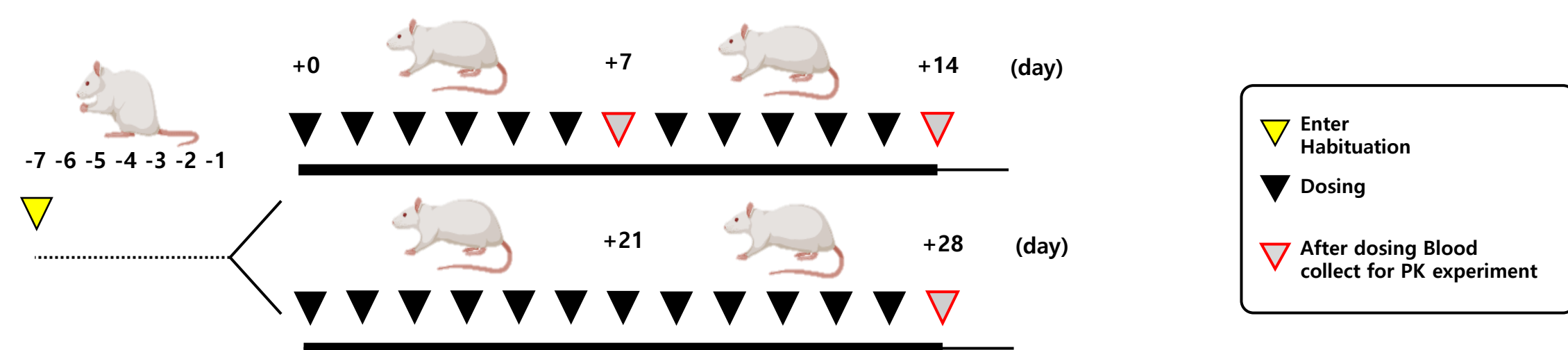


Figure 1. Study design: dosing schedule and blood sampling timepoints

- Sample time:
  - Blood: 0.25, 0.5, 1, 1.5, 2, 4, 6, 8, 12 hours after the last dose
  - Liver tissue: collected at the final time point
- Analysis: Quantification of NVP and 12-OHNVP using LC-MS/MS (Agilent 1290 Infinity-API 4000 Triple Quadrupole MS)
- Sample preparation: Extraction by protein precipitation after plasma or liver tissue homogenization
- Data analysis: Analyst software

### Metabolic Pathway & Target Analytes:

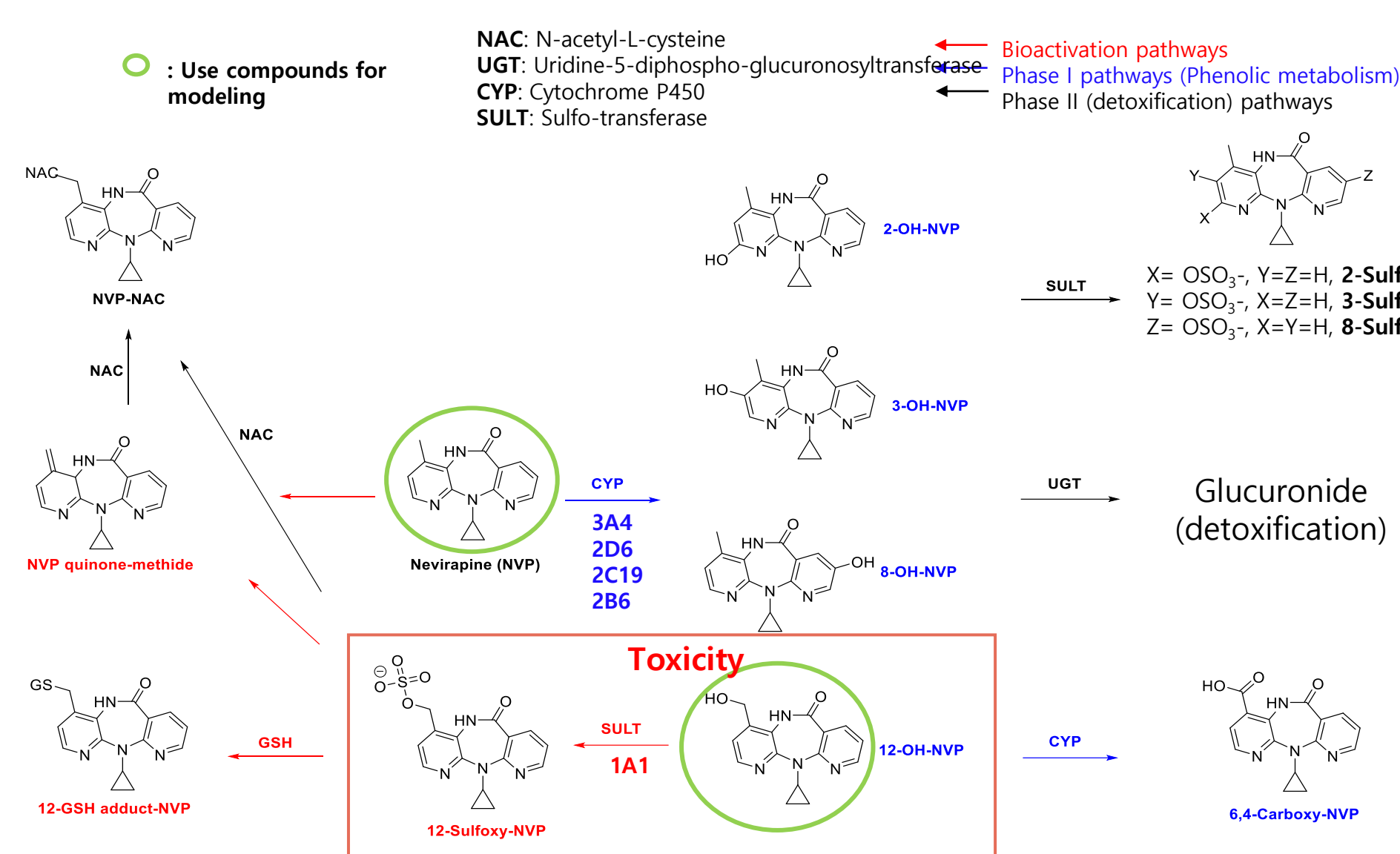


Figure 2. Nevirapine metabolic pathway and hepatotoxic metabolite formation

### PBPK Model Development:

- Software: Berkely Madonna (Ver. 10.6.1)
- Model structure: Parent-metabolite network with enterohepatic circulation and autoinduction
- Physiological parameter for SD rats (organ volumes, blood flows, partition coefficients)
- Compartments: plasma, liver, gut, bile, kidney, NET, HPT
- Key features:
  - Enterohepatic circulation: Bile excretion and gut reabsorption cycle
  - Autoinduction: Time and Concentration-dependent enzyme induction with Hill kinetics
  - Metabolite formation: NVP → 12-OH NVP conversion incorporating formation ration (Rpm)

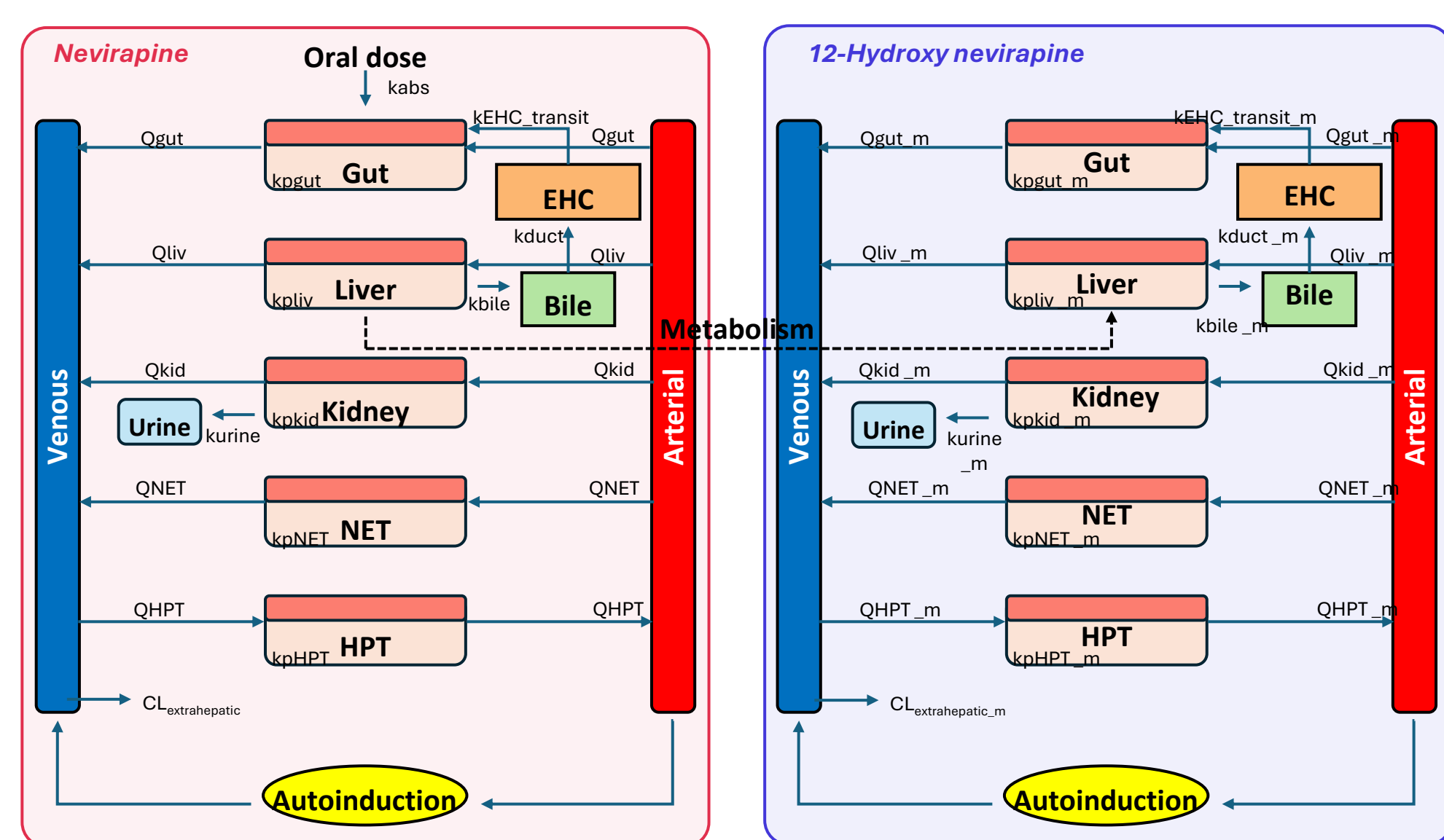


Figure 3. Nevirapine and 12-hydroxy nevirapine PBPK model scheme

- Model Abbreviations: EHC(Enterohepatic circulation), NET(Non-eliminating tissue), HPT(Highly perfused tissue)

### Model Performance Assessment:

- Statistical metrics: RMSE(Root Mean Square Error)(1), MAPE(Mean Absolute Percentage Error)(2), MPE(mean Prediction Error)(3), MAE(Mean Absolute Error)(4)

$$RMSE = \sqrt{\frac{1}{n} \sum (predicted - observed)^2} \quad (1)$$

$$MPE = \frac{1}{n} \sum \frac{(predicted - observed)}{predicted} \times 100 \quad (3)$$

$$MAPE = \frac{1}{n} \sum \left| \frac{(predicted - observed)}{predicted} \right| \times 100 \quad (2)$$

$$MAE = \frac{1}{n} \sum |(predicted - observed)| \quad (4)$$

## RESULTS

### Pharmacokinetic Parameters:

- Time-dependent Pharmacokinetic Changes:
  - Peak exposure achieved across all doses (steady state, 1week)
  - Progressive decline in both AUC<sub>last</sub> and C<sub>max</sub> values (2-6-fold)
- Dose-dependent autoinduction:
  - Higher doses show more pronounced reduction
  - 72 mg/kg dose demonstrated up to 3.09-fold reduction in exposure at Week 4
- Dose proportionality:
  - Non-linear pharmacokinetics observed, particularly at higher doses.
  - 4-fold dose increase (18 → 72 mg/kg) resulted in disproportionate exposure changes

### Observed vs Predicted Comparison:

- Parent drug (NVP): Improved prediction accuracy with higher doses (2-fold error: 17.6% → 78.4%)
- Metabolite (12OH NVP): Moderate improvement at higher doses (2-fold error: 43.1% → 54.9%)

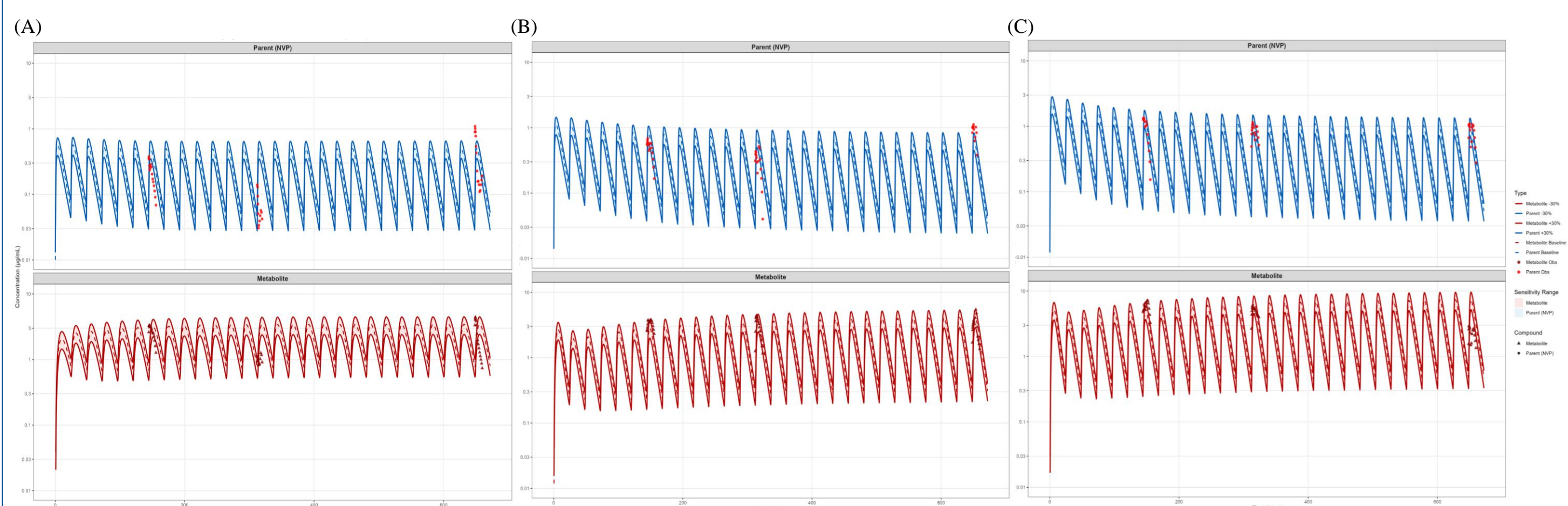


Figure 4. Model Prediction vs Observed Concentration-Time Profiles (Log Scale): (A)18 mg/kg (B)36 mg/kg (C)72 mg/kg

Dosage	RMSE	MAPE	MPE	MAE	% within 2-fold	% within 3-fold	
NVP	18 mg/kg	0.34	330.77	303.40	0.29	17.65	47.06
	36 mg/kg	0.33	88.74	45.23	0.27	61.70	76.60
	72 mg/kg	0.37	39.89	-9.86	0.30	78.43	84.31
12OH NVP	18 mg/kg	2.27	190.34	168.95	1.99	43.14	66.67
	36 mg/kg	1.67	57.90	10.74	1.44	54.90	82.35
	72 mg/kg	3.42	95.84	56.75	2.68	54.90	72.55

Table 1. Model Performance Metrics by Dose Group

- Overall Performance:
  - Model validation showed acceptable accuracy with 52.3%/51% within 2-fold and 69.1%/73.9% within 3-fold criteria for NVP/12OH-NVP, respectively.

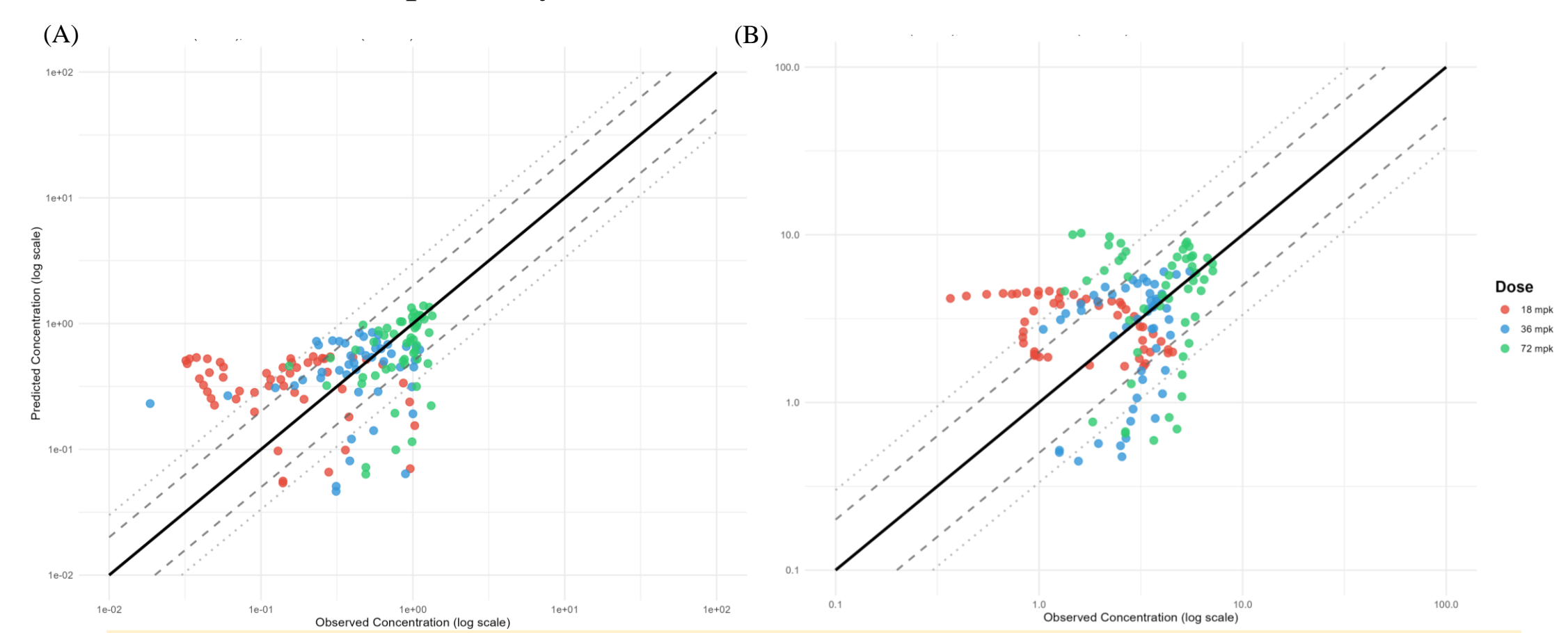


Figure 5. Observed vs Predicted concentration comparison log scale: (A)NVP (B)12OH NVP

### Auto-induction Simulation:

- Simulation confirmed dose-dependent auto-induction of nevirapine clearance.
- Clearance increased over time in a dose-dependent manner:
  - 18 mg/kg showed minimal change (1.2-fold), while 36 mg/kg and 72 mg/kg demonstrated substantial increase (2.3-fold and 2.8-fold, respectively)

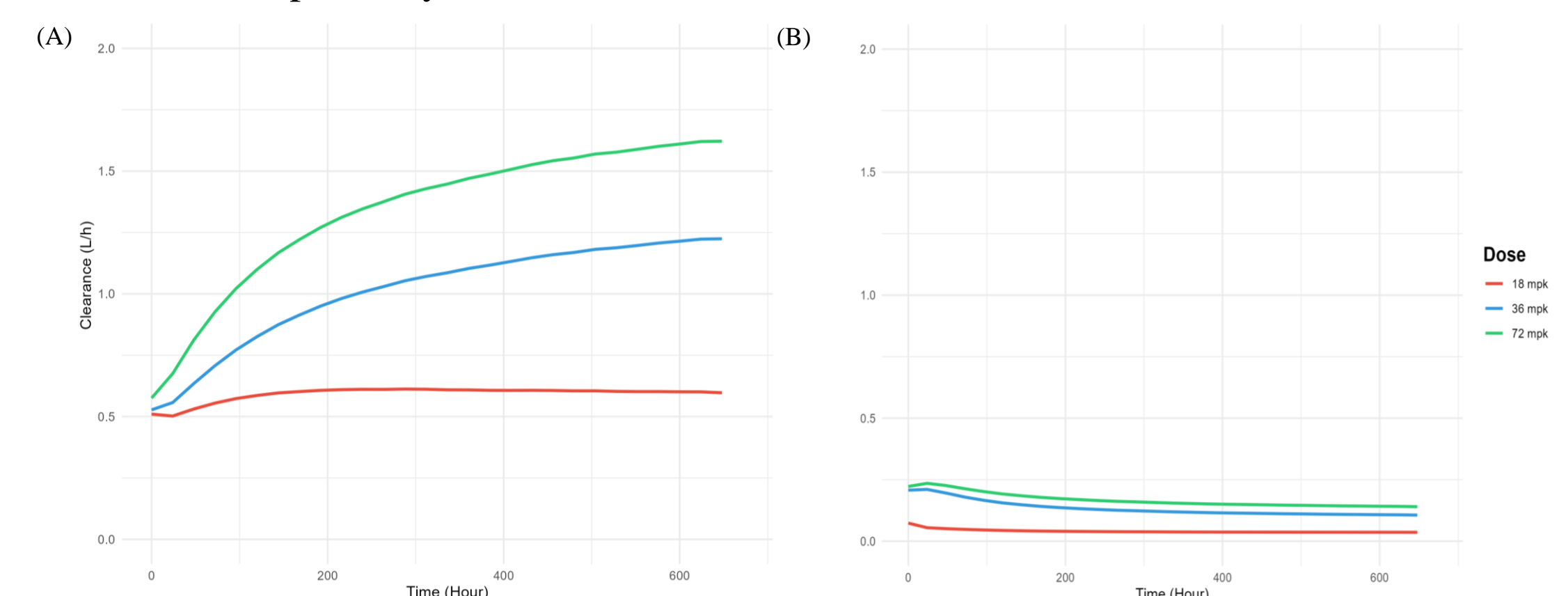


Figure 6. PBPK model-predicted clearance profiles over time; (A) NVP (B) 12OH NVP

### Exposure Analysis:

- HepG2 cell-based analysis revealed distinct toxicity thresholds for NVP and 12OH-NVP.
- 12OH-NVP showed Nrf2/ARE pathway activation at 2.82 μg/mL, while NVP activation occurred at 133.15 μg/mL.
- ROS generation was observed at 14.12 μg/mL for 12OH-NVP and 13.31 μg/mL for NVP.
- The metabolite demonstrated a 47-fold lower threshold for Nrf2/ARE activation compared to the parent compound. (Data not shown)

## CONCLUSION & DISCUSSION

- This study successfully demonstrated dose-dependent increases in nevirapine oral clearance in rats, consistent with clinical observations where oral clearance increases 1.5-2 fold during chronic dosing (200-400 mg/day over 2-4 weeks).
- Simulated hepatic nevirapine concentrations at 12 hours post-dose (~1 μg/mL) fell below our analytical detection limit (LLOQ: 1.5 μg/mL), but the predicted hepatic profiles showed similar patterns to plasma kinetics, confirming appropriate validation of our tissue distribution model.
- While direct comparison of *in vitro* cytotoxicity data to *in vivo* hepatic concentrations has limitations due to protein binding and cellular uptake differences, the proximity of model-predicted metabolite concentrations to cytotoxic thresholds suggested potential dose-dependent hepatotoxic risk.
- These findings highlighted the importance of careful safety evaluation in chronic nevirapine therapy and supported the need for extended dosing studies to better understand hepatotoxicity mechanisms.

## ACKNOWLEDGEMENT

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