SUPPLEMENTARY DIGITAL CONTENT

SUPPLEMENTAL METHODS

Population pharmacokinetic modeling

The nonlinear mixed-effects approach using the first order conditional parameter estimation with the interaction option implemented in the software NONMEM version VII (Icon Development Solutions, Hanover, Maryland) was used to simultaneously analyze the pharmacokinetics of phenytoin C_{free} and C_{total}. Inter-subject variability (ISV) was modeled using exponential functions approximating log-normally distributed variability. Residual variability was described with an additive error in the log domain (equivalent to a proportional error when data are in linear scale). Prior to the analysis, the pharmacokinetic observations were logarithmically transformed (natural logarithm).

Model Selection

Selection between the time invariant and time-variant one-compartmental Michaelis-Menten and models was based on visual inspection of goodness of fit plots, the objective function value, and the precision of the parameter estimates. The minimum value of the objective function provided by NONMEM (approximately equal to -2xlog likelihood (-2LL); smaller -2LL is associated with a better model) guided model selection. A decrease in -2LL of 6.63 points for one additional parameter, was regarded as a significant model improvement corresponding to p-value of 0.01 for nested models.

Potential covariates (sex, age, weight, height, hypothermic group, and temperature) that significantly ($p<0.001$) impacted any of the pharmacokinetic parameters were then added to the full model. To determine whether each covariate remained in the final model, each covariate was individually removed from the full model and its effect on the -2LL, diagnostic plots, and

physiological reasonableness of pharmacokinetic parameters was evaluated (backward selection).

Model Evaluation

The final model was evaluated using a nonparametric bootstrap analysis. The precision of the parameter estimates (expressed as 5^{th} to 95^{th} percentiles) were computed from the analysis of 500 bootstrap data sets (sampling with replacement) using Perl-speaks-NONMEM (1). Model performance was evaluated with visual predictive checks. Five hundred simulated studies with the same design characteristics as the original study were generated using NONMEM. At each time point with a measurement, the 5, 50, and 95th percentiles were calculated in every simulated study. The 90% interval from the resultant percentiles was computed and represented over time together with the raw data. The visual predictive checks were developed using MATLAB environment (The Mathworks, MA, United States).

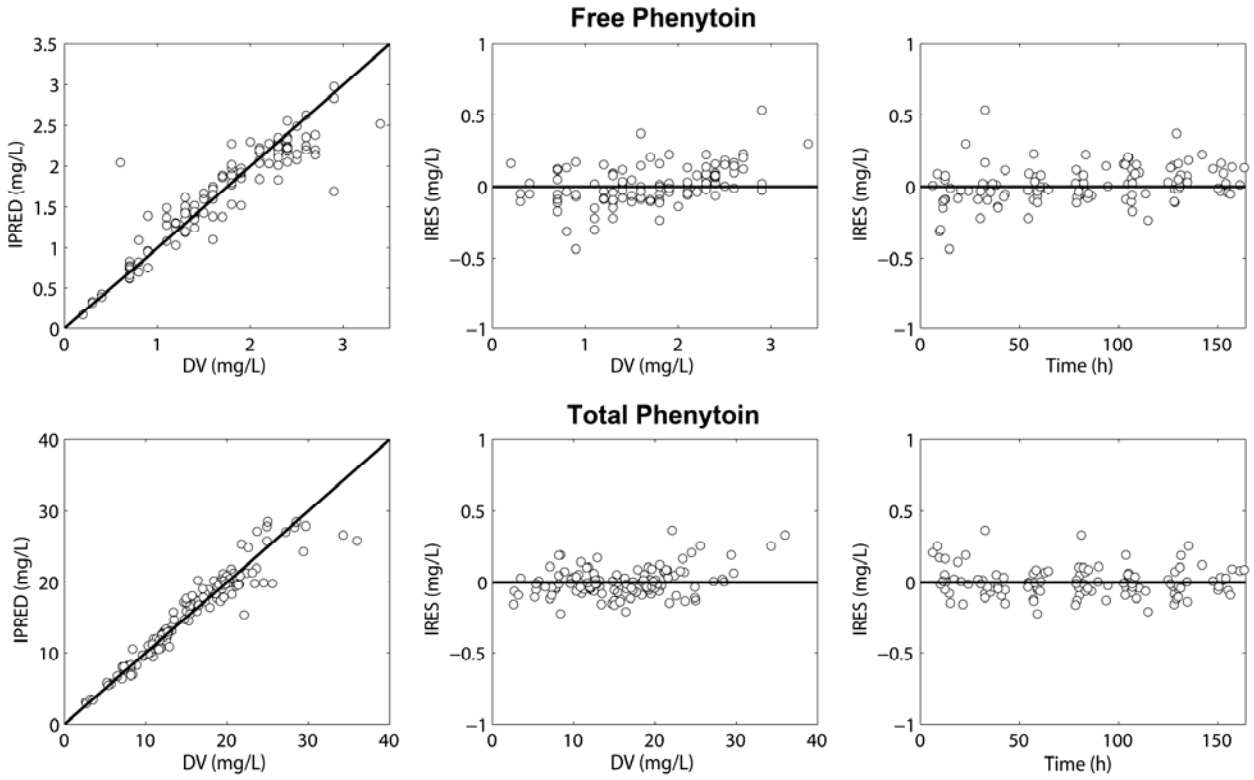
Supplemental Table E1. Bootstrap analysis

	Bootstrap analysis (500 replicates) median (5^{th} - 95^{th})	
Parameter	**Estimate**	**ISV**
V_1 (L) = (weight/40)$^{\gamma wt1}$×θ_{V1}	γ_{wt1} = 0.812 (0.719 - 0.980) θ_{V1} = 437 (410 - 463)	10.2 (5.09 - 14.1)
V_{max0} (mg/h) = θ_{Vmax0}×*exp*[(weight-40)×wt_2]	θ_{Vmax0} = 6.30 (4.23 - 8.79) wt_2 = 0.032 (0.016 - 0.054)	52.3 (26.6 - 71.1)
V_{maxi} (mg/h) = θ_{Vmaxi}×*exp*[(temp-37)×$temp_1$]	θ_{Vmaxi} = 11.5 (2.35 - 95.8) $temp_1$ = 0.376 (0.103 - 0.748)	113 (39.9 - 200)
k_m (mg/L)	0.448 (0.205 - 0.739)	
θ_{prop} (unitless)	8.12 (7.63 - 8.63)	13.8 (6.99 - 19.9)
k_{ind} (h^{-1})	0.005 (0.0004 - 0.0161)	
Log residual error C_{free} (mg/L)	0.033 (0.013 - 0.057)	
Log residual error C_{total} (mg/L)	0.013 (0.0097 - 0.019)	

Median and 90% confidence intervals of the pharmacokinetic estimates generated from the bootstrap analysis of 500 simulated studies were very close to the NONMEM generated estimates (Table 2). ISV = inter-subject variability expressed as coefficient of variation (%); θ terms are the fixed effect parameters, V_1 = apparent volume of distribution; γ_{wt1} = weight effect parameter on V_1; V_{max0} = time-invariant maximum velocity of metabolism at baseline; wt_2 = weight effect parameter on V_{max0}; V_{maxi} = time-dependent velocity defined by the rate constant k_{ind} and time t; $temp_1$ = temperature effect parameter on V_{maxi}; k_m = Michaelis-Menten elimination rate constant; θ_{prop} = proportionality constant for the bound phenytoin; k_{ind} = rate constant for induction of metabolism.

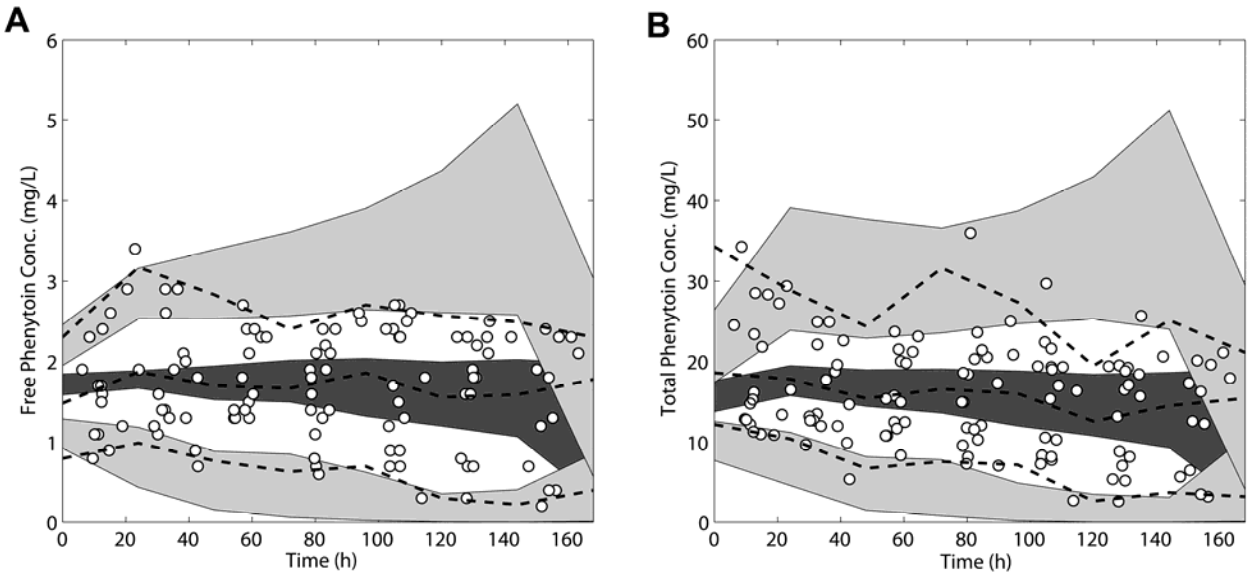
FIGURES

Supplemental Figure E1



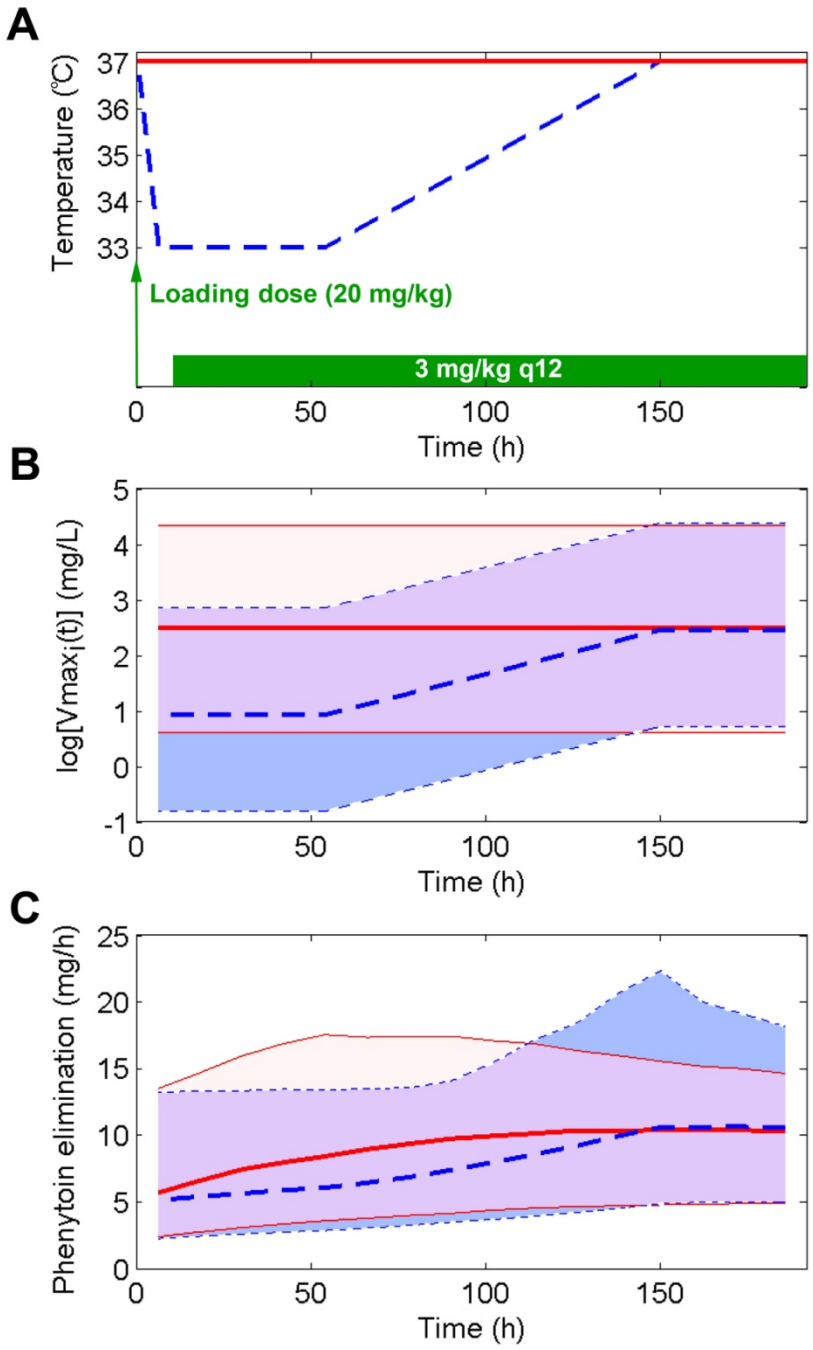
Goodness-of-fit plots demonstrate free (top) and total phenytoin (bottom) concentrations are well described by the final model. IPREDs = individual model predictions; DV = observed concentrations; IRES = individual residuals. Solid black lines are lines of identity (first column) and individual weighted residuals = 0 (second and third columns). The η-shrinkage of 31% (V_1), 10.35% (V_{max0}), 14.12% (θ_{prop}) and 23.29% (V_{maxi}); and ε-shrinkage of 7.15 % (C_{free}) and 14.56 % (C_{total}) shows that these goodness of fit plots are reliable. ε-shrinkage is calculated as 1-SD(IWRES) where IWRES is an individual weighted residual (weight=1). η-shrinkage is calculated as 1-SD(η)/ω where η are the ISV terms and ω is the population model estimate of the standard deviation in η. Both terms can vary from 0-100% with lower percentages a more informative model.

Supplemental Figure E2



Visual predictive checks of the pharmacokinetic model. Data dynamics, as well as their dispersion, are well-captured by the model, although the 95th percentile was overpredicted. Shaded area corresponds to the 90% prediction interval of the median, and 50th and 95th percentiles. Dashed lines represent the median, and 5th and 95th percentiles of the raw data profiles. **A.** Free phenytoin concentrations **B.** Total phenytoin concentrations.

Supplemental Figure E3



The magnitude and timing of the effects of temperature on phenytoin elimination. Simulations of 1000 children receiving either therapeutic hypothermia or controlled normothermia. Solid lines/lightest(red) shading represents the normothermic group while dashed lines/darkest(blue) shading represents the hypothermic group. **A.** Design of the simulation. The cooling protocol was as follows: start at 37 °C, hypothermia induction over 6 h to 33 °C, hold at 33 °C for 48 h, and then rewarm to 37 °C at 1 °C per 24 h (over 96 h total). Controlled normothermia patients were fixed at 37 °C. The arrow and shading indicate the fosphenytoin dosing schedule (20 mg/kg IV loading dose followed by 6 mg/kg/day divided every

12 h). All individuals were simulated with a weight of 40 kg. **B.** The time-dependent velocity of metabolism (V_{maxi}) on the natural log scale is decreased by lower temperatures in patients receiving the hypothermia protocol versus normothermia as shown by the population predicted median (lines) and 90% confidence interval (shading) over time. **C.** This results in an extended period of reduced phenytoin elimination [$(V_{max(t)} \times C_{free})/(K_m+C_{free})$] in patients receiving hypothermia versus normothermia as shown by the population predicted median (lines) and 90% confidence interval (shading) over time.

REFERENCES

1. Lindbom L, Ribbing J, Jonsson EN. Perl-speaks-NONMEM (PsN)--a Perl module for NONMEM related programming. *Comput Methods Programs Biomed* 2004;75(2):85-94