

Tetracycline against *Pseudomonas aeruginosa*: Pharmacokinetic/Pharmacodynamic Modeling of *In Vitro* Time-Kill Curves

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Background & Objectives

- Leveraging *in vitro* time-kill curve data of antibiotics in a model-based approach can lead to more well-informed dose selection for clinical trials.
- Tigecycline and tetracycline hydrochloride (TET) in combination have displayed augmented *in vitro* activity against *Pseudomonas aeruginosa* ATCC® 27853 [1].
- Information regarding the resistance development to an antibiotic or combination should be utilized in model-informed drug development when available in order to account for the potential resistance development with varying exposures.
- The objective of this research is to develop a PK/PD model that describes the observed effects of TET against *P. aeruginosa* in *in vitro* static time-kill experiments incorporating results from resistance testing and accounting for potential drug degradation.

Methods

In Vitro Static Time-Kill Curves [2]

- Varying concentrations of tetracycline (0, 0.25, 0.5, 1, 2, 4, and 8×MIC) added to culture flasks at 0h, 2 hours post-incubation (MIC for *P. aeruginosa* ATCC® 27853 was determined as 16 mg/L)
- Samples diluted and plated on blood agar at 0, 2, 4, 6, 8, 10, 12, 16, and 24 h
- Colonies counted after 16-24 h of incubation post-plating
- Colony-forming units per mL calculated (LOD = 10 CFU/mL)
- Each curve performed in triplicate

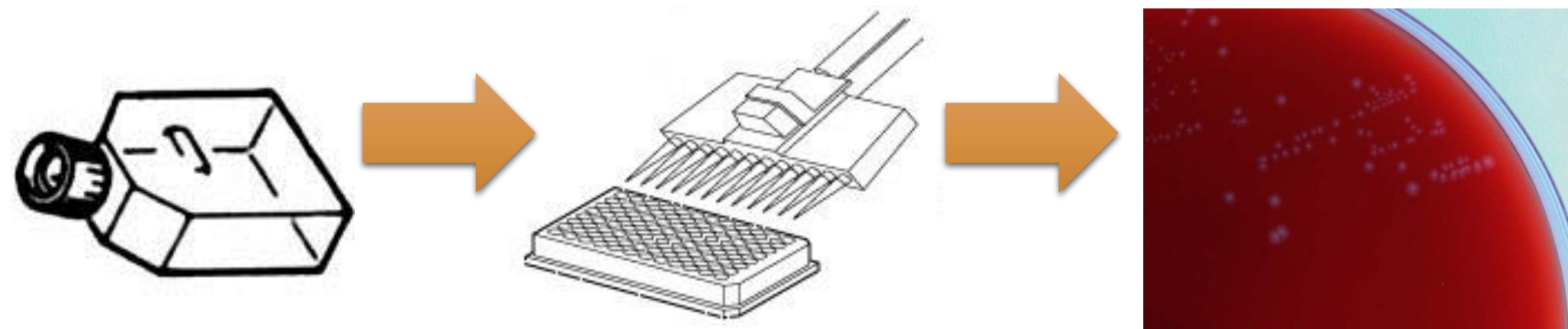


Figure 1 shows the plating process for time-kill curve experiments. A sample is taken from incubated flask at each time point and serial dilutions with normal saline are performed. Select dilutions are plated on blood agar to be incubated and subsequently counted to determine the concentration of bacteria present.

Resistance Testing

- Tetracycline (0.25, 1, and 8×MIC) added at 0h to culture flask (2-hours post-incubation)
- Samples directly plated on 3×MIC tetracycline-containing agar plates at 0 and 24 h; 10- and 100- fold dilutions plated when necessary
- Plates read after 16-24 h of incubation post-plating
- Experiments performed in duplicate

PK/PD Modeling of Time-Kill Curve Data

- Conducted using NONMEM® (version 7.3)
- A logistic growth model was used to fit growth control curves
- The addition of drug effect was incorporated using an Emax model
- Consideration was given to the potential role of
 - One- or two- bacterial subpopulation(s)
 - Delay in drug effect
 - Drug degradation
 - Resistant subpopulations or adaptive resistance, as appropriate per experimental results
- Change in objective function value (OFV), goodness of fit plots, and visual predictive checks (VPCs) were evaluated for final model selection

Conclusions

- The developed model describes the effect of TET against *P. aeruginosa* over time for various drug exposures well.
- This model was informed by resistance testing results.
- A similar approach will be used to investigate and incorporate resistance for TIG and the combination of TIG and TET.
- A final model describing the effects of each drug, alone and in combination, will be developed.
- This final model along with published clinical PK data/models will be used to simulate and evaluate potential dosing regimens for the treatment of *P. aeruginosa* infections.

Results

- TET resistance was not observed at any of the tested concentrations at 24 hours
- TET time kill curve data were best described using a model including susceptible and persistent resting subpopulations of bacteria
- A sigmoidal Emax model best described the drug effect
- Delay of drug effect did not improve model fit
- Incorporation of drug degradation allowed for better description of the data

Figure 2. Final model for *in vitro* static time-kill study of tetracycline HCl's (TET) effect on *P. aeruginosa*. k_S : growth rate constant for the susceptible subpopulation, S : susceptible bacterial population, R : resting bacterial population, N_{MAX} : Maximum bacterial population, k_d : death rate constant, k_{SR} : rate constant describing the shift from the susceptible to the resting subpopulation, K_{MAX} : Maximal kill rate constant, C_{TET} : TET concentration, γ : Hill factor, EC_{50} : concentration providing half maximal response, k_{Deg} : drug degradation rate constant

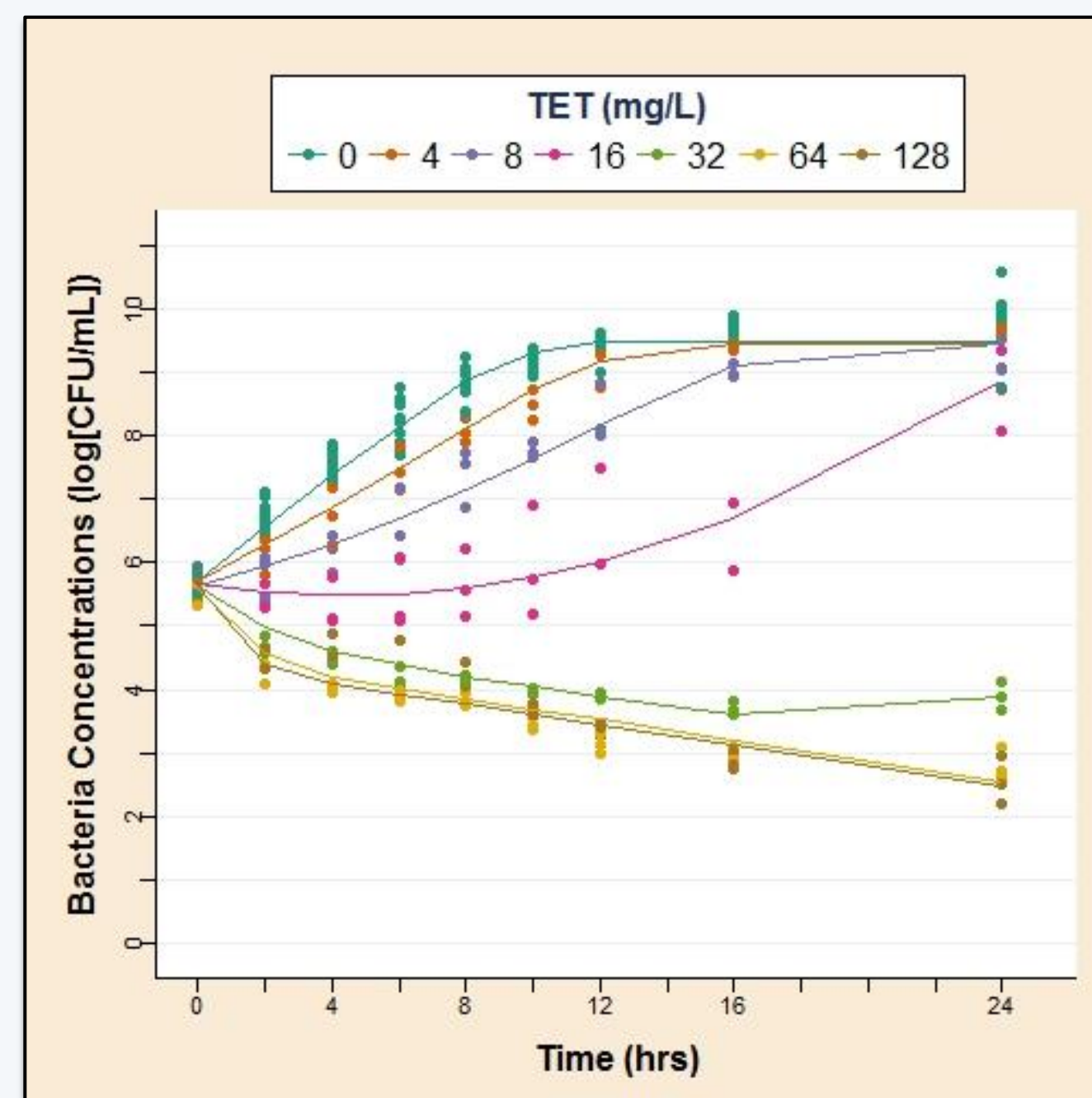
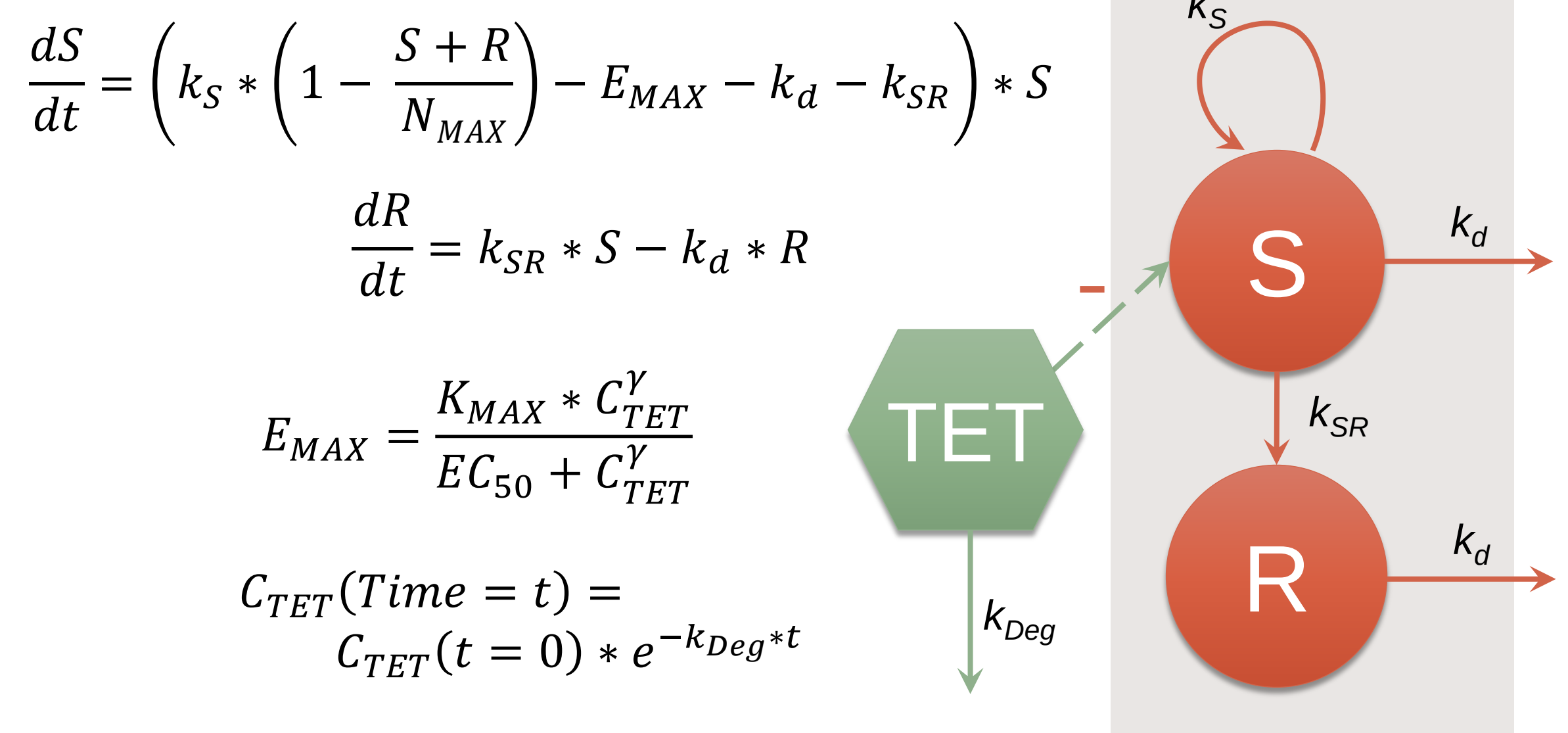


Figure 3. (Above) Model-predicted (lines) and observed (points) bacterial concentrations over 24 hours for various exposures to TET.

Figure 4. (Right) Individual fits (lines) from the final model (Figure 2) overlaying observed time-kill curve data (points). Note: Not all growth control curves are shown.

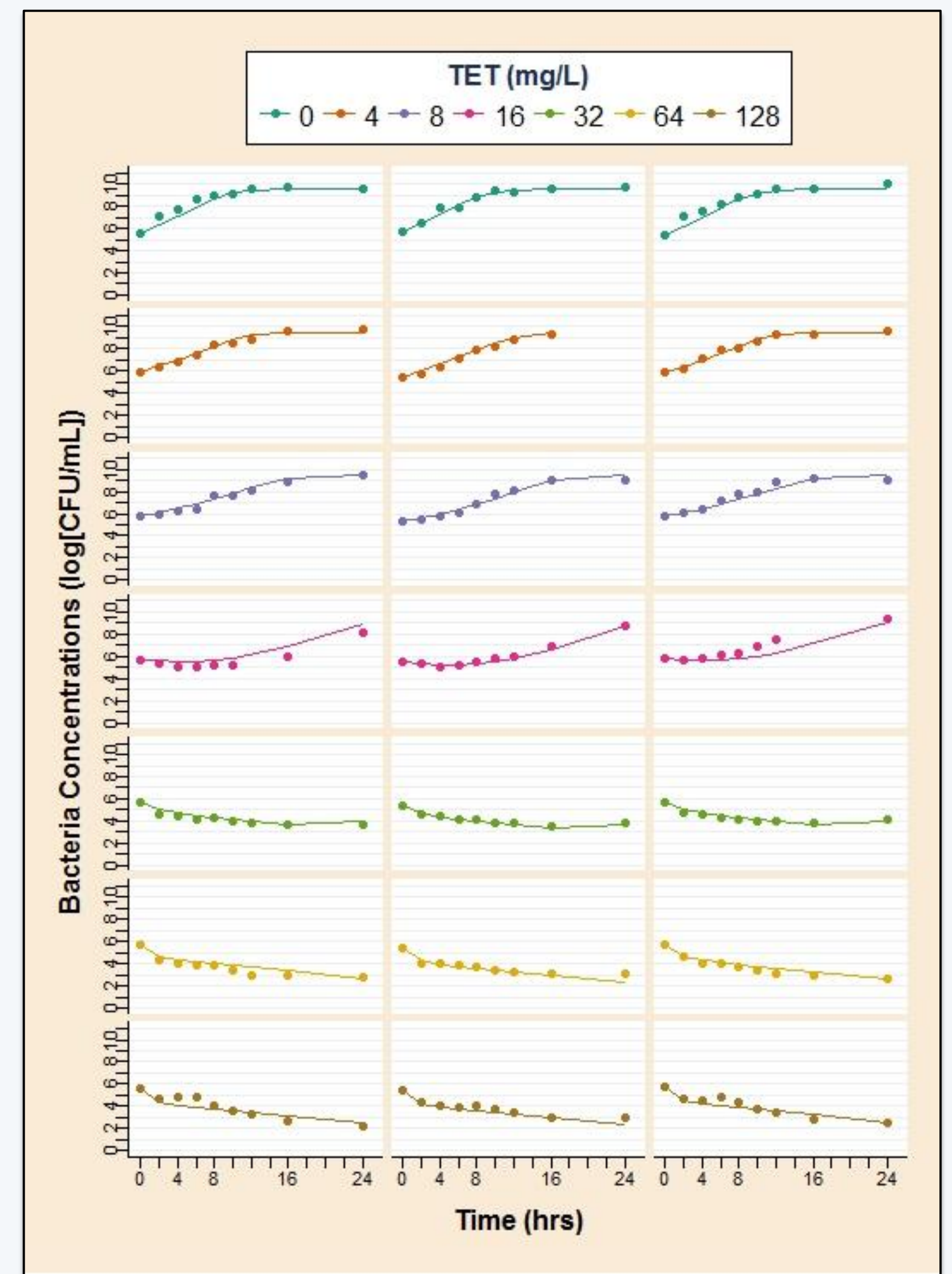
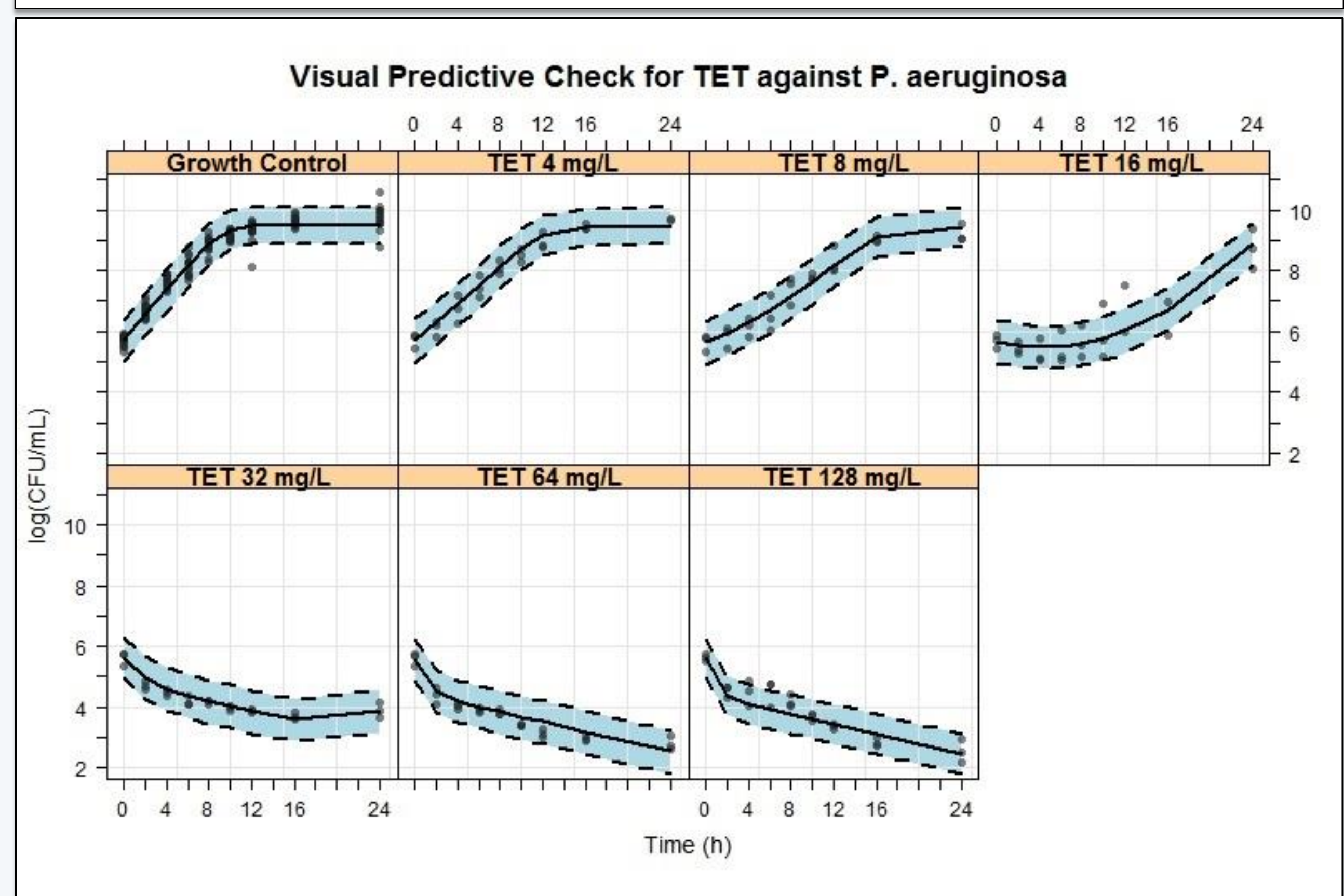


Figure 5. Visual Predictive Checks (VPCs) stratified by tetracycline HCl (TET) concentration for static time-kill curve data with *P. aeruginosa*.



References

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