EXPLORATORY STUDY
Iterative Development and Performance Verification of *In Vitro* Biokinetic Models

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INTRODUCTION

Routinely toxicity endpoints monitored *in vitro* are related to the nominal treatment concentrations applied to the test system. However, it is the concentration within the cell itself that drives the monitored effect in the majority of instances. In WP4 we implemented a steady-state model to predict intracellular concentrations in the monolayer cell culture systems employed within the various case studies of the project. Subsequently, we have gone on to establish a dynamic model framework to better predict intracellular concentrations in repeat exposure assays, where the assumptions of the steady-state model no longer hold true. The developed models expand on previously published approaches however, only a limited amount of performance verification has been conducted for this, and published models. We are undertaking an exploratory case study, drawing on a range of compounds from across the project to experimentally determine intracellular concentrations, and other kinetic parameters. The data generated will allow us to assess, and ultimately refine, the performance of our predictive models.

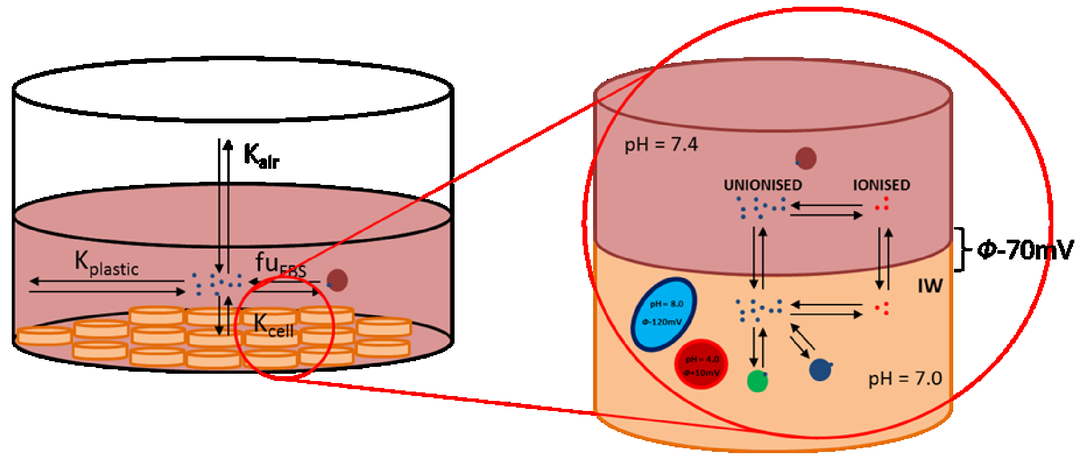
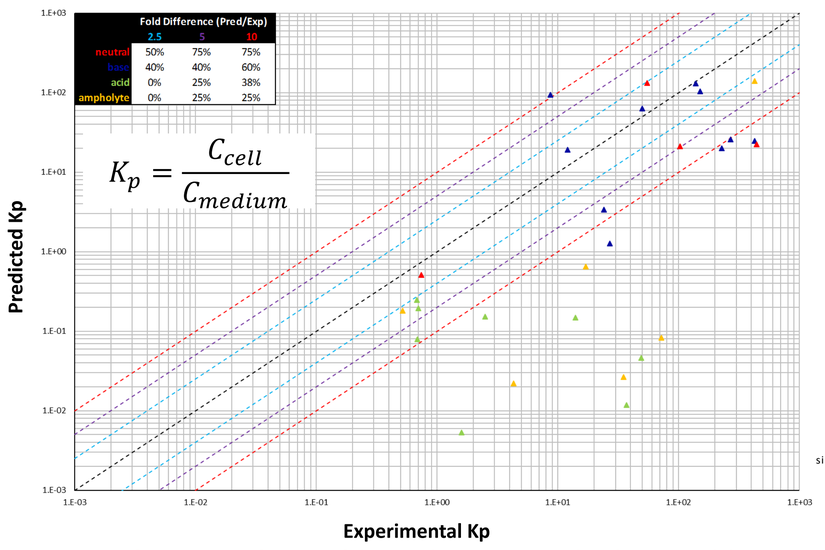
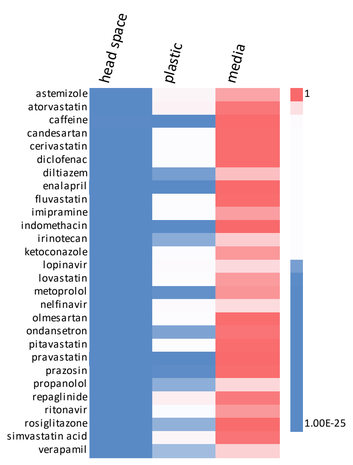


Figure 1. Schematic representation of the in vitro distribution model

PRELIMINARY VERIFICATION OF THE STEADY-STATE MODEL





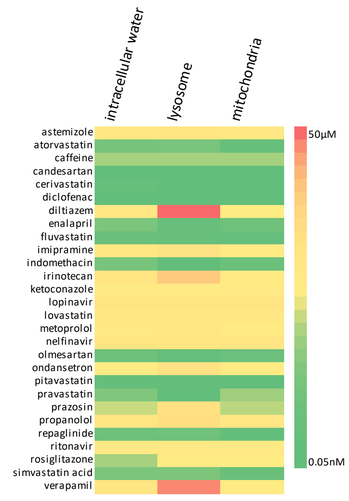
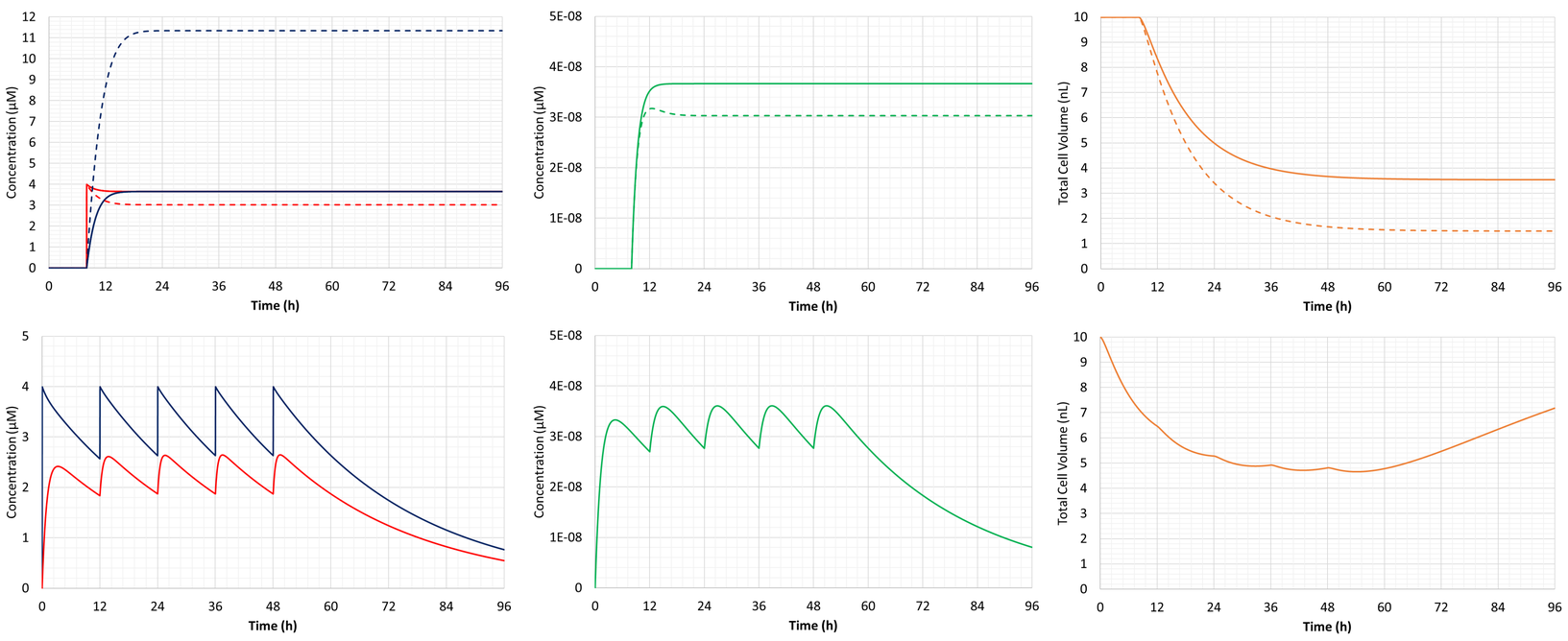


Figure 2. Performance verification of the steady-state in vitro distribution model. Comparison of predicted and published, experimentally determined, Kp values (left), assessment of the extracellular (middle) and intracellular (right) distribution of 28 pharmaceutical compounds predicted using the model.

To assess model performance, the steady-state *in vitro* distribution model was parameterised to represent HEK293 (human embryonic kidney) cell assay conditions utilised in experimentally determining Kp for 28 pharmaceutical compounds with a range of physicochemical properties (Mateus *et al.*, 2013). Binding to FBS (10% v/v) was predicted and cell composition assumed to be the same as human kidney tissue (Simcyp v16). This first iteration of the steady-state model shows reasonable predictive power compared to experimental data for neutral and basic compounds. However, performance is less robust for acids and ampholytes, possibly due to poor prediction of protein binding in FBS for these compounds. The model allows an assessment of compound distribution across the total *in vitro* assay system. Of the 28 compounds assessed, none showed significant distribution into the headspace, with higher concentrations remaining in the media and binding to plastic (figure 2). Intracellularly, the 28 compounds showed relatively uniform distribution; however, verapamil and diltiazem show high lysosomal concentrations associated with the 'ion-trapping' of weak bases (figure) (Kubo *et al.* , 2016).

IMPLEMENTATION OF A DYNAMIC MODELLING FRAMEWORK



While the steady-state model can provide useful predictions for some cell systems, it does not account for dynamic processes such as metabolism, active transport, cell growth or cytotoxicity. In order to model *in vitro* systems and compounds where these processes are critical to *in vitro* distribution and kinetics we are establishing a dynamic modelling framework. Currently, the implemented model can account for differential ionisation between the media and the intracellular water, as in the steady-state model, but can also account for metabolism, active uptake/efflux, distribution into the headspace, cell turnover, and cell death (figure 3). The modelling of cell growth/turnover is important for predicting kinetics in extended/repeated treatment scenarios which may use proliferating cell lines. Importantly, it also allows the effect of cell death, and possible cell population recovery following compound clearance, to be captured in model simulations.

Figure 3. Simulations of in vitro kinetics using the dynamic distribution model. Concentrations in media (red), cells (blue), headspace air (green) and the total cell volume (orange) are simulated over 96 hours following a single treatment with neutral (solid) and monoprotic base (dashed) compound (top row) after 8 hours in culture. Concentrations and cellular volume are also simulated over 96 hours following repeated treatment with a neutral test compound, every 12 hours, which is a substrate for an uptake transporter expressed in the membrane of cultured cells (bottom).

GENERATING EXPERIMENTAL DATA FOR MODEL VERIFICATION

As part of an iterative model development and refinement strategy, *de novo* experimental data will be generated by Cyprotex using hepatocytes as a baseline test system for model verification; where required other partners will be asked to generate specific data. Key experimental data required are outlined in the adjacent bullet points. Test compounds for evaluating model performance will be selected from across the project case studies with the aim of covering a range of physicochemical properties. Experiments are on-going to determine if there is any significant loss of compound during assay wash steps that could impact on the accurate determination of intracellular concentrations. The results of these experiments will determine whether a multiplex approach can be adopted whereby cell numbers and intracellular concentrations are accurately determined in the same well at multiple time-points over the course of the assay.

Key Experimental Data

- cell counts at time of assay and growth curves
- cell diameter
- fraction unbound in culture media
- Intrinsic clearance values
- transporter kinetics (if relevant)
- intracellular concentrations at multiple time-points
- determination of intracellular loss of compound during assay wash steps

REFERENCES

Kubo Y., Seko N., Usui T., Akanuma S., Hoyosa K. (2016) Biol. Pharm. Bull. 39: 1319-1324
Mateus, A., Matsson P., Artursson, P. (2013) Mol. Pharmaceutics. 10: 2467-2478

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