Evaluation of *in vitro* tests to reduce animal testing in drug toxicology studies

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Abstract - **This project (INVITOX), currently at the start of the funding, aims to develop *in vitro* tests for drug toxicology studies. New tests, mimicking more closely animal physiology, will limit the use of animals during formulation development.**

INTRODUCTION

Drug toxicity testing is an essential part of drug development. Formulation development for such studies can be challenging due to the need to achieve exposures which will provide an adequate safety margin for future clinical studies. Prediction of performance, either using *in vitro* dissolution or *in silico* modelling, can be particularly challenging for poorly soluble compounds requiring complex formulations to achieve the desired level of bioenhancement. Toxicokinetic studies in preclinical species are used to define formulation/API solid form selection for regulatory toxicology studies and whilst such studies provide essential pharmacokinetic data, they do not provide mechanistic information on the interplay between formulation performance and absorption. Ideally, we would like to be able to reduce/replace toxicokinetic testing with an *in vitro* test which provides such mechanistic information and is predictive for *in vivo* exposure. This alternative strategy fully aligns with the 3Rs principles (Directive 2010/63/EU) which advocate the development of alternative methods. This INNOVATE UK project gathers experts from a large pharmaceutical company, specialist SME and academia, with the aim to evaluate *in vitro* tests that could reduce or replace animal testing at key stages of preclinical development.

MATERIALS AND METHODS

This project is organised around three main aspects.

- Selection of compounds (broad spectrum of physicochemical properties) and formulations with available *in vivo* data from toxicology and pharmacokinetic studies.
- *In vitro* dissolution testing: biphasic dissolution studies and assessment of characteristics of re-created formulations
- Evaluation of *in vitro* and *in vivo* data and development of biomodelling tools and *in vitro*/*in vivo* correlation (IVIVC).

RESULTS AND DISCUSSION

In order to reduce/replace animal testing, *in vitro* biphasic dissolution experiments will be performed under conditions mimicking the animal physiology. To achieve this goal, a literature review of the gastrointestinal physiology in animals has been conducted and biorelevant gastrointestinal media for animal species will be developed based on the physiological values. Optimisation of the *in vitro* biphasic dissolution test will be based on a Design of Experiments study. The effects of excipients, media and pH on partitioning behaviour will be investigated.

Physicochemical and preclinical pharmacokinetic data will be provided for a number of novel compounds and formulations which have previously progressed through regulatory toxicology testing. This dataset will be augmented with six model compounds (Table 1) and the combined dataset will be used to validate the newly developed *in vitro* method. The selection of compounds has been made based on their physicochemical properties and the availability of pharmacokinetic and toxicokinetic data. *In vitro* dissolution data will be combined with pharmacokinetic data to develop IVIVCs using both traditional and Physiologically Based Pharmacokinetic (PBPK) modelling approaches.

Table 1. Physicochemical properties of model compounds

	ionisation (pKa)			lipophilicity (logP)		solubility		permeability	
	neutral	weak base	weak acid	high (>2)	low	high	low	high	low
Carbamazepine	■			■			■	■	
Indomethacin			■		■		■	■	
Itraconazole		■		■			■	■	
Levetiracetam	■				■	■		■	
Metoprolol tartrate		■			■	■		■	
Paracetamol			■		■	■			■

An example of biphasic output data is provided in Figure 1 for itraconazole. Itraconazole is a poorly soluble weak base with good permeability. The data shows the extent of partitioning of the drug from the formulation vehicle into the lipid sink.

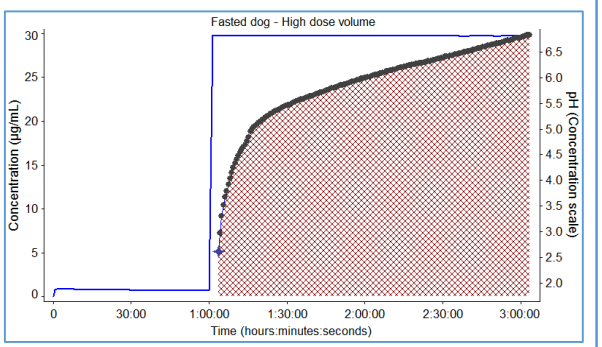

Figure 1. The fasted dog method using simulated animal fluid and a high dose volume. The data shows concentration versus time of itraconazole that partitioned into the organic layer (black circles) and pH (secondary y-axis) versus time (blue solid line).

CONCLUSIONS

The development of new *in vitro* tools which accurately simulate the gastrointestinal environment of preclinical species has the potential to reduce the use of animals in preclinical studies. Such tools will also facilitate the selection of the formulation technologies required to deliver the poorly soluble new chemical entities which dominate current industrial R&D pipelines.

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