CN-BIO

2021 WEBINAR SERIES

# From Dose to Circulation

## Determination of Drug Oral Bioavailability Using a Gut-Liver Microphysiological System

**A full run down of questions & answers from our November 24th webinar**

# Acronyms & definitions

**CYP** - Cytochrome P450 enzyme

**PHH** - Primary human hepatocytes

**Low clearance compound** - Compounds that have an intrinsic clearance rate of <5 ml/min/kg

**MPS** - Microphysiological system (alternatively known as Organ-on-a-chip)

**MPS TL6 consumable plates** - PhysioMimix™ Transwell®-Liver consumable plate used in multi-organ studies

**Allometric scaling** - Scaling of pharmacokinetic results obtained in animal studies to reflect comparable outcomes in humans, based in relative metabolic rates and size.

**DMPK** - Drug metabolism and pharmacokinetics

**PgP** - P-glycoprotein transporter

**R2 value** - Correlation coefficient

**Phenytoin** - Drug used to treat epilepsy and seizures

First order kinetics - Constant elimination of a drug per unit time.

**Primary cells** - Cells obtained from human tissue (such as patient biopsies)

**Fa** - Dose fraction absorbed

**Fg** - Drug fraction passing through the gut wall un-metabolised

**FaFg** - Fraction of drug available in the portal blood (https://www.jpharmsci.org/article/S0022-3549(15)00120-3/pdf)

**LC-MS analysis** - Analysis using liquid chromatography–mass spectrometry

# Q&A participants

**Dr Yassen Abbas**

Bioengineer

CN Bio

**Dr Audrey Dubourg**

Product Manager PhysioMimix™ OOC,

CN Bio

## Another question?

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# Questions

Q1

**Q:** Why are animals poor predictors of bioavailability?

**A:** From Dr Yassen Abbas, Bioengineer, CN Bio.

Many reasons. Firstly, the repertoire and expression of enzymes that drive human drug metabolism differ compared to those of commonly used animal species. This is also true for transporters that are responsible for the uptake or efflux of drugs through the small intestine. In addition, there are differences in the physiology of certain animal species compared to the human. For example, rats do not have a gall bladder – an important component of the hepatobiliary system.

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Q2

**Q:** Can the gut-liver microphysiological system be used to estimate bioavailability on low clearance compounds?

**A:** From Dr Yassen Abbas, Bioengineer, CN Bio.

Yes, using the gut-liver microphysiological system (MPS) we were able to demonstrate that bioavailability can be estimated for low clearance compounds, which are defined as drugs that have an intrinsic clearance rate of <5 ml/min/kg.

More information can be found in our **Bioavailability Application Note**.

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Q3

**Q:** Have you characterised the liver CYPs in the model, and is their expression the same as in humans?

**A:** From Dr Yassen Abbas, Bioengineer, CN Bio.

We characterise the activity of the CYP3A4 enzyme, one of the main drivers of drug metabolism in humans, for quality control purposes during culture. This ensures that there is sufficient and maintained activity throughout the experiment. This has also been validated in the following study by **Rubiano et al, 2021.**

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Q4

**Q:** How can mathematical modelling be further utilised in these bioavailability experiments?

**A:** From Dr Yassen Abbas, Bioengineer, CN Bio.

We can take data generated from the bioavailability experiments in the **PhysioMimix™ Multi-Organ System**, using the **MPS-TL6 consumable plates**, and conduct parameter fitting to estimate the hepatic clearance rate and permeability of drugs across the intestinal barrier. Here, parameter fitting is the process of computing a model's parameter values from measured values. By utilising mathematical modelling, the data generated from one bioavailability experiment can be maximised.

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Q5

**Q:** Which animals have you compared the liver MPS to in terms of allometric scaling that would be considered the closest to human profiling?

**A:** From Dr Yassen Abbas, Bioengineer, CN Bio.

We have not applied allometric scaling to data generated using either the liver MPS or the gut-liver MPS with a comparison to animal models.

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Q6

**Q:** What was the clearance mechanism of the compounds that you tested? Were any transported substrates included? And would you be willing to share the names of the compounds you tested?

**A:** From Dr Yassen Abbas, Bioengineer, CN Bio.

To assess the model's ability to predict human oral bioavailability, we used well-known compounds with accessible clinical data for drug metabolism and pharmacokinetics (DMPK): acebutolol, phenytoin, naproxen, and methylprednisolone. These compounds are primarily subject to hepatic-mediated metabolism and utilise several transporters (e.g., Pgp) as referenced on **go.drugbank.com**, however, their pharmacological action is still unknown.

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Q7

**Q:** What is the $R^2$ value of the model *vs* in human you showed for the animal model *vs* human?

**A:** From Dr Yassen Abbas, Bioengineer, CN Bio.

The $R^2$ value of the animal model *vs* human bioavailability was taken from a publication by **Musther *et al*, (2014)**. For 184 drugs investigated, their $R^2$ value was 0.34, demonstrating that animals are a poor predictor of human bioavailability.

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Q8

**Q:** You used first-order differential equations. Were they modelling the rates kinetics? Phenytoin shows saturation kinetics, so what limitations exist on using the modelling in this context?

**A:** From Dr Yassen Abbas, Bioengineer, CN Bio.

First order differential equations are used to model the concentration of a compound in each of the compartments of the **MPS-TL6 plate**. The intrinsic clearance rate and permeability across an intestinal barrier, which are obtained from literature, are the two main parameters used to generate plots of concentration *vs* time. Drugs that are slowly metabolized (such as phenytoin) will have a low intrinsic clearance value (<5 ml/min/kg) and may indicate saturation when modelled.

The modelling we did was based on first-order kinetics, which describe the concentration of these compounds within the system. Essentially what we do is extract information such as hepatic clearance rate and the permeability for an intestinal layer. Those two parameters are used within our model to describe the concentration within each of the compartments. We can then take that one step further and determine bioavailability predictions.

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Q9

**Q:** If these are primary cells, how many donors have you used to account for genetic variability within the study?

**A:** From Dr Yassen Abbas, Bioengineer, CN Bio.

From our experiments, we generally see similar CYP enzyme expression profiles, which we continually measure (for quality control purposes) throughout experiments. It is well known that the repertoire and expression of enzymes that drive metabolism

differ with genetic diversity. In this experiment only used one donor, however, one of the benefits of our MPS is that we could incorporate multiple donors to account for genetic diversity. This is not possible to model using animal studies.

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## Q10

**Q:** Does the system allow for a calculation of the fraction absorbed (Fa) and the intestinal availability (Fg), and the product of Fa and Fg (FaFg)?

**A:** From Dr Yassen Abbas, Bioengineer, CN Bio.

Yes, we can take samples in the gut apical compartment as well as in the systematic circulation of the plate to determine the fraction absorbed of a drug added and the amount available to the hepatocytes. This will require slight adjustments to the design of the experiment to calculate Fa and FaFg.

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## Q11

**Q:** Do you think this technology could replace the need for animals when it comes to bioavailability studies?

**A:** From Dr Yassen Abbas, Bioengineer, CN Bio and Dr Audrey Dubourg, Product Manager PhysioMimix™ OOC, CN Bio.

That is a good question. In the short-term we do not expect an overnight mass-adoption of MPS to replace animal models, however, we do believe that the combination of MPS and animal studies can be used, right now, to de-risk drug development. With respect to bioavailability, evidence demonstrates how poorly animals predict this key parameter **Musther *et al*, (2014)**. This presents a good opportunity for the gut-liver MPS to address a workflow limitation by providing human-relevant insights.

The technology is still relatively new so getting key stakeholders, such as MPS suppliers, drug developers and regulators on board has been our first priority. If you compare the current MPS market to where we were 10 years ago, there's now much greater acceptance and adoption.

Collaborating with regulators, notably the **US Food and Drug Administration (FDA)**, over the past few years to address standardisation and reliability concerns has played a key part in accelerating MPS adoption. Through this collaboration the capabilities our Liver MPS, (or liver-on-a-chip) has been highlighted and its potential to fill the limitations of current gold-standard

preclinical methods showcased. To learn more about the assessment of MPS technologies in the hands of the regulators, please read our latest co-publication with the FDA: **Rubiano *et al*, 2021**; or watch our on-demand webinar: "**A regulators viewpoint**".

The fact that both the US Congress and the **European parliament** are also looking at alternative methods to gradually replace animal models will help drive the implementation of MPS into drug development faster.

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## Q12

**Q:** With this system, can we also estimate bioavailability of a combination study?

**A:** From Dr Yassen Abbas, Bioengineer, CN Bio.

Combination dosing a gut-liver MPS is not a regimen that we have tried but where LC-MS analysis is able to differentiate between compounds, the bioavailability of drug combinations could be estimated using a modified version of this approach. Compared to animal studies, the benefit of MPS is that many more dosing regimens and concentrations can be tested to finely tune estimations.

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## Q13

**Q:** Is there any serum in the culture medium in this bioavailability study?

**A:** From Dr Yassen Abbas, Bioengineer, CN Bio.

Serum-free media is used during the bioavailability experiment to limit drug binding with proteins.

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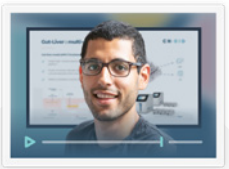

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